ORIGINAL RESEARCH

Molecular Detection and Characterization of Newcastle Disease Virus from Chickens in Mid-Rift Valley and Central Part of Ethiopia

Esubalew Endale Alemu[1], Bayeta Senbata[2], Melaku Sombo[2], Chala Guyassa [2],
Dawit Hailu Alemayehu[3], Eleni Kidane[4], Adane Mihret[3], Andargachew Mulu[3], Hunduma Dinka[1]

[1]Department of Applied Biology, Adama Science and Technology University, Adama, Ethiopia; [2]Molecular Biology Laboratory, Animal Health Institute (AHI), Sebeta, Ethiopia; [3]Biotechnology and Bioinformatics Research Directorate, Armauer Hansen Research Institute (AHRI), Addis Ababa, Ethiopia; [4]TB and HIV/AIDS Disease Research Directorate, Ethiopian Public Health Institute (EPHI), Addis Ababa, Ethiopia

Correspondence: Hunduma Dinka; Bayeta Senbata, Email dinkahu@gmail.com; didigabruma@gmail.com

Background: Newcastle disease (ND) is a highly infectious poultry disease that causes major economic losses worldwide. The disease is caused by Newcastle Disease Virus (NDV) and early detection and identification of the viral strain is essential. Having knowledge of the NDV strain genotype that circulates in some regions would help in designing an effective vaccine to control the disease. In this regard, there is little information on NDV strain in chickens in mid Rift Valley and the central part of Ethiopia. Therefore, the purpose of this study was to detect and characterize NDV strain genotype from chickens in mid-Rift Valley and the central part of Ethiopia and test whether this NDV strain genotype matches the vaccine strain currently used in the study area.
Methods: A total of 98 samples: 78 (tracheal and cloacal) swabs from chicken pools of five and 20 tissue samples were collected. To detect NDV strain, conserved region of the virus Matrix (M) gene was amplified by qRT-PCR. To characterize NDV strain genotypes, M-gene positive samples were specifically re-amplified by conventional PCR targeting the Fusion (F) gene region and sequenced by Sanger method.
Results: 13.26% of tested samples were positive for NDV strain in the study area with statistically significant difference (P<0.05) among the study sites. Further characterization of the F genes from NDV strain isolates by phylogenetic analysis indicated that one field isolate clustered with genotype VII whereas three of the isolates clustered to genotype I, II, and III. The isolate of the current NDV strain vaccine in use in the study area clustered with genotype II.
Conclusion: The current study indicates the existence of different NDV strain genotype from that of the vaccine strain currently used. Even though large-scale characterization of several isolates is required at national level, the current study laid baseline information for the existence of variations between field NDV strain genotype and vaccine strain currently used against ND in the country.
Keywords: APMV-1 strain genotype, F gene, mid rift valley, phylogenetic tree, qRT-PCR

Background

Newcastle disease is an infectious disease in avian species including wild and domestic avian species,[1] in both sexes and in all age groups.[2] The disease is caused by Newcastle Disease Virus (NDV) or avian paramyxovirus type-1 (APMV-1), which is one of the most important poultry diseases that highly affects the poultry sector in terms of production and productivity at domestic and international level.[3,4] The impact of Newcastle Disease (ND) has been estimated to cause great economic losses through many epidemics associated with very high mortality and morbidity, and many other production-related losses.[5]

The causative agent of Newcastle Disease is grouped under Phylum Negarnaviricota, Order Mononegavirales, Family Paramyxoviridae, Genus Avulavirus, and Species Avian paramyxovirus type 1 (APMV-1) or NDV.[6] It is an RNA virus and negative-stranded, whose genome size is about 15.2 kb, in which the genome is non-segmented and organized into six genes translating six structural proteins, namely: RNA-dependent RNA polymerase (L), nucleoprotein (NP), matrix

protein (M), phosphor protein (P), hemagglutinin-neuraminidase (HN), and fusion protein (F).[3] A diagnostic dilemma considering the F protein cleavage site is a trusted molecular indicator of NDV virulence that sufficiently predicts the virulence potential of NDV isolates and important for characterization and complete genome analysis.[7]

A previous study conducted using serological and molecular tool for NDV in village chickens in selected rift valley of Ethiopia indicated 11.6% overall sero-prevalence and 38.4% detection rate for virulent NDV strains targeting F-gene by qPCR.[8] In 2014 an attempt to characterize the genotype of Newcastle Disease Virus in live poultry markets in Ethiopia by Mulisa et al[9] revealed virulent strains belonging to sub-genotype VI.

Early detection and identification of the NDV strain genotype is essential in controlling the disease in terms of vaccine design.[10] There are live (thermostable) and inactivated vaccines derived from the lentogenic genotype of NDV, which are used in Ethiopia to prevent the outbreak of ND,[11] however, there are complaints with these vaccines as they do not produce active immunity to block NDV. Having knowledge of the NDV strain genotype that circulates in some regions would help in designing effective vaccine to control the disease. In this regard, there is little information on NDV strain in chickens in mid Rift Valley and central part of Ethiopia. Therefore, the purpose of this study was to detect and characterize NDV strain genotype from chickens in mid- Rift Valley and central part of Ethiopia and test whether this NDV strain genotype matches the vaccine strain currently used in the study area.

Methods
Study Area
The study was conducted from February 2020 to October 2020 on village (non-vaccinated) and commercial chickens in Mid-Rift Valley areas (Bishoftu, Batu, Shashemene, Hawassa, and Arba Minch towns) and the central part of Ethiopia (Addis Ababa, Adama, and Bishoftu) (Figure 1). Around 583 species of birds were reported so far from the Rift Valley region of Ethiopia.[12] It has been estimated that around 15 to 16 million chickens are found in this region.[13] Generally, these study areas encompass about 15–40km in width and more than five hundred kilometers in length.
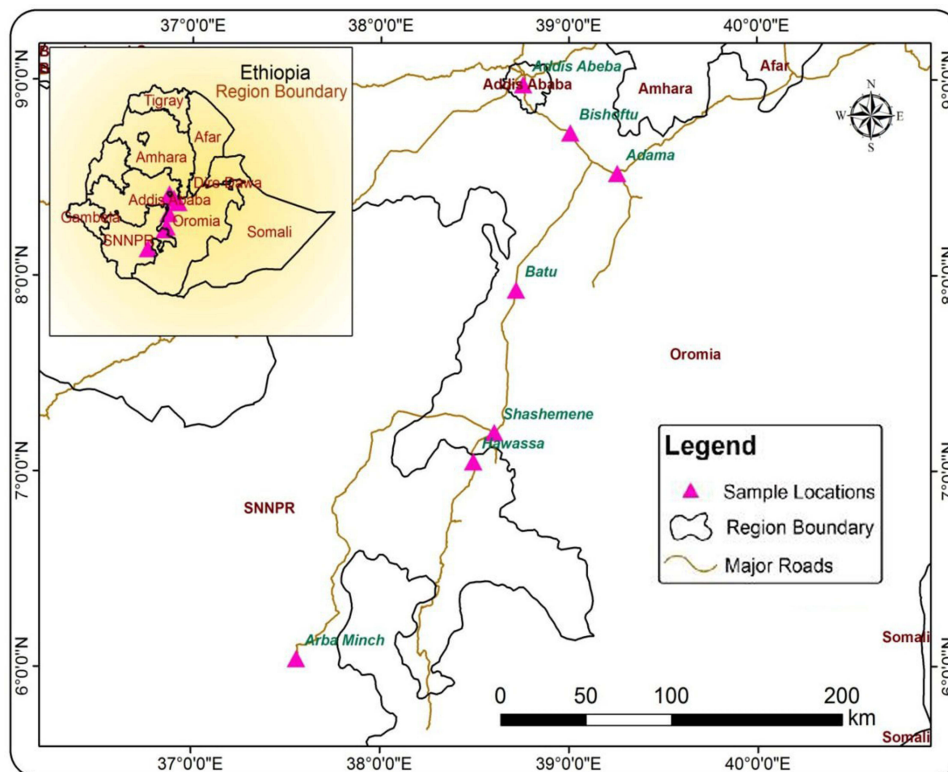

Figure 1 Map showing study areas in the mid-rift valley and central parts of Ethiopia (by ArcGIS 10.4.1).

Sampling, Data Collection, and Virus Detection

A total of 390 live chicken swab samples were collected using a random sampling technique from the village and commercial chickens in the mid-Rift Valley area. A total of 78 tracheal swabs (pool of five) and 78 cloacal (pool of five) were collected in a separate cryovial containing freshly prepared viral transport media (VTM) (2 mL). Twenty tissue samples from sick and freshly dead birds were also collected from the central part of Ethiopia for postmortem examination at Animal Health Institute. Brain, lung, tracheal, and tissue samples (pools of five) from liver, kidney, heart, spleen, and intestine were collected from twenty individual chickens and put in VTM. After sampling, the specimens were transported to the Animal Health Institute molecular biology laboratory; and stored at −80°C until processing. The collection and transport of samples were carried out according to the standard techniques recommended by the OIE.[7] Viral RNA extractions from tracheal and cloacal swabs and postmortem tissue samples were performed using QIAamp Viral RNA Mini Kit as directed by the manufacturer.

Polymerase Chain Reaction Amplification of NDV Strains

Real-time quantitative polymerase chain reactions (qRT-PCR) were performed for 98 samples (78 pooled tracheal and cloacal swabs, and 20 tissue samples) by targeting the matrix (M) gene conserved regions. A forward primer M+4100-5'-AGT GAT GTG CTC GGA CCT TC-3, 'reverse primer M4220-5'CCT GAG GAG AGG CAT TTG CTA-3' and a Probe of M+4100- 5'FAM- TTC TCT AGC AGT GGG ACA GCC-TAMRA-3'[5] were used for M-gene assay. The amplification process was performed by using an Applied Biosystems 7500 fast real-time PCR thermocycler. In addition, conventional PCR was performed using NDV strain positive samples that were identified by qRT-PCR using a second primer set targeting the F gene (270 bp size cleavage site motif region) with NOH-5'-TAC ACC TCA TCC CAG ACA GG-3 'forward primer and NOH-5'- AGT CGG AGG ATG TTG GCA GC −3' reverse primer sequences for sequencing the PCR product to precisely detect the NDV strain for its genotype characterization. Gel electrophoresis was performed by preparing a 2% agarose gel supplied with a power and Gel doc system that was connected for imaging (Gel DocTM XR +, version 5.0 of Molecular Image Lab software) at Animal Health Institute.

Nucleotide Sequencing

Sequencing reaction was performed for the PCR products by using a pair of PCR primers used for amplification of the fusion (F) gene of NDV strain. After completion of the reaction, reaction products were purified using a sodium acetate-ethanol purification method at Armauer Hansen Research Institute (AHRI). The purified products of sequencing reactions were analyzed on an ABI3500 24 capillary genetic analyzer (Sanger Sequencing Machine) at Ethiopian Public Health Institute (EPHI), Addis Ababa, Ethiopia. Finally, the sequences were analyzed and deposited to GenBank with the following accession numbers: ON033821, ON033822, ON033823, ON033824, and ON033825.

Data Management and Analysis

Collected data were encoded on Microsoft Excel 2016 spreadsheet and stored in a computer before it was transferred to SPSS Version 20 for statistical analysis. The outcome was presented using descriptive statistics such as percentage and P-value. For raw sequence data reading and editing, ChromasPor version 2.1.9, and BioEdit version 7.2.5 software were utilized. BLAST was used for comparative studies to collect additional gene sequences for F gene region of NDV strain from GenBank. Multiple sequence alignments were performed for phylogenetic tree construction to align the sequences as nucleotides using the ClustalW algorithm in MEGA 10.2.4.4. The Neighbor-Joining tree construction method was used with Jukes-Cantor and Kimura nucleotide substitution model. A bootstrap value of 1000 replicates was used for phylogenetic tree construction and evaluation.[14]

Results

Molecular Investigation of NDV results

Out of 98 samples tested by qRT-PCR for M gene, 13 samples were found positive for NDV strain. Thus, the overall detection rate for NDV strain in the study areas was 13.26% (13/98) where there were statistically significant differences among the seven districts (P<0.05), although a wide variation was observed (Table 1).

Table 1 Detection Rate of NDV Strain in Village and Commercial Chickens by Geographical Location and Sample Types in the Study Area

Categories	No. examined	No. positive	Detection rate (%)	*P*-value
Geographical location (districts)				
Mid-Rift Valley of Ethiopia				
Arba Minch	30	6	20	<0.05
Shashemene	12	0	0	
Hawassa	12	0	0	
Batu	12	0	0	
Bishoftu	22	3	13.6	
Adama	5	1	20	
Total	93	10	10.75	
Central part of Ethiopia				
Bishoftu	22	3	13.6	
Addis Ababa	5	3	60	
Total	27	6	22.2	
Overall	98	13	13.26	
Sample type				
Swab sample				
Trachea swab	39	4	10.25	<0.05
Cloacal swab	39	2	5.12	
Total	78	6	7.69	
Tissue sample				
Brain	5	2	40	>0.05
Lung & tracheal	5	2	40	
Pool tissue	5	2	40	
Intestinal	5	1	20	
Total	20	7	35	
Overall	98	13	13.26	

Out of the 78 pooled swab samples tested, 4 tracheal swabs and 2 cloacal swab samples were positive for NDV strain with 7.69% detection rate among swab samples. There were statistically significant differences among the swab types (P<0.05). Out of the 20 pooled tissue samples tested, 7(35%) samples were identified as positive for NDV strain. The overall detection rate of NDV strain in the mid-Rift Valley of Ethiopia was 10.75%, where the highest was recorded in Arba Minch (20%) and Adama (20%) followed by Bishoftu (13.6%). There was a statistically significant difference (p< 0.05) among the geographical study locations (Table 1). On the other hand, the detection rate of NDV strain in the central part of Ethiopia was 22.2%, where the highest was recorded in Addis Ababa (60%) followed by Bishoftu (13.6%).

The current molecular findings indicated an overall detection rate of 16% (8/50) NDV strain in village chickens, whereas 10.41% (5/48) in commercial chickens in the study area (Table 2). There was a statistically significant difference (p< 0.05) between the production systems. In village chickens, the highest detection of NDV strain was recorded in Arba Minch (41.6%) followed by Adama (33.3%) whereas in commercial chickens the highest detection was recorded in Addis Ababa (60%).

The present study also revealed 13.72% and 12.76% detection rates of NDV strain in female and male chickens, respectively, even though there was no statistically significant difference (p >0.05) by sex. On the other hand, a higher detection rate of NDV strain was recorded in young chickens compared to the older groups, with a statistically significant difference by age (p < 0.05) (Table 3).

Table 2 Detection Rate of NDV Strain in Chickens Based on the Production Systems (Village and Commercial) in the Study Area

Study Area	No. of Chicken Examined		No. Positive		Detection Rate (%)		p-value
	Village	Commercial	Village	Commercial	Village	Commercial	
Mid-Rift Valley of Ethiopia Swab Sample							<0.05
Arba Minch	12	18	5	1	41.6	5.5	
Shashemene	7	5	0	0	0	0	
Hawassa	9	3	0	0	0	0	
Batu	8	4	0	0	0	0	
Bishoftu	6	6	0	0	0	0	
Sub total	42	36	5	1	11.9	2.7	
Central part of Ethiopia Tissue Sample							
Bishoftu	5	5	2	1	40	20	
Addis Ababa	–	5	–	3	–	60	
Adama	3	2	1	0	33.3	0	
Sub total	8	12	3	4	37.5	33.3	
Grand Total	50	48	8	5	16	10.41	

Table 3 Detection Rate of NDV Strain in Chickens by Sex and Age

Categories	No. Examined	No. positive	Detection rate (%)	P-value
Sex				
Female	51	7	13.72	>0.05
Male	47	6	12.76	
Total	98	13	13.26	
Age				
Old (>6 months)	56	5	8.92	<0.05
Young (3–6 months)	42	8	19.04	
Total	98	13	13.26	

Characterization of NDV Strain Genotypes

Phylogenetic analysis for a partial region of F gene nucleotide sequences of the current isolates from different areas (with accession number ON033822 from Bishoftu, ON033824 from Addis Ababa, ON033823 from Arba Minch, ON033825 from Adama, and ON033821 from NVI, Bishoftu) clustered with genotype VIIg (Figure 2) showing 78% nucleotide similarity to the Ethiopia NDV strain genotypes found in GenBank database (accession number KC851848.1). Similarly, our analysis revealed that NDV isolates of ON033824, ON033823, and ON033821 clustered with the genotype I, II, and III of NDV strain.

The phylogenetic analysis demonstrated that NDV of ON033822 is found in genotype VII (Figure 2). The isolate of the current NDV vaccine strain genotype (ON033821) in use in the study area is distantly associated with NDV strain genotype reference sequences (GenBank accession number AY175710.1) and clustered with genotype II strain.

Discussion

The current study showed an overall molecular detection rate of 13.26% for NDV strain with a statistically significant (P<0.05) difference among the study districts in chickens from selected areas in mid-Rift Valley and central part of Ethiopia. Our study also revealed a 10.75% and 22.2% detection rate of NDV strain in Mid-Rift Valley and central part of Ethiopia,
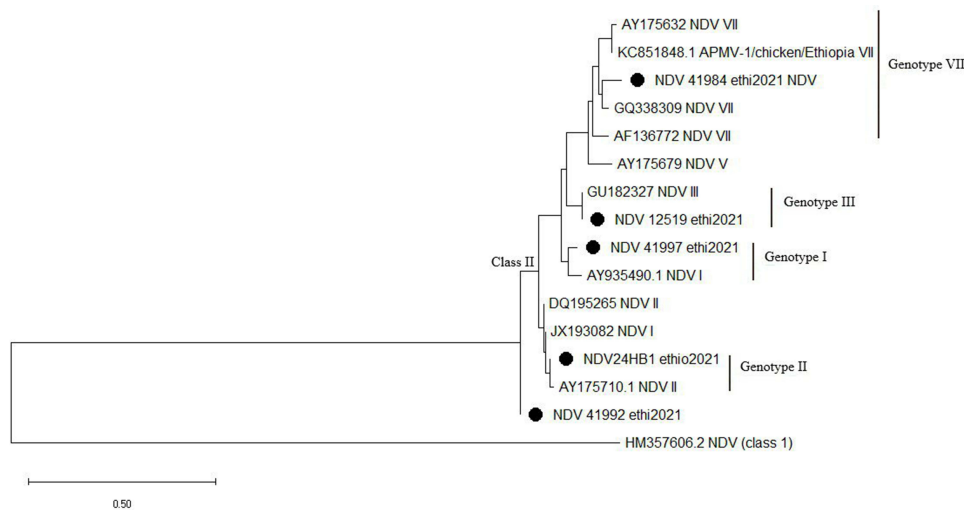
Figure 2 Phylogenetic relationships between the Ethiopian NDV strain genotype (with accession number: ON033821, ON033822, ON033823, ON033824, and ON033825) and reference sequences with accession number: GU182327.1, AY175710.1, AY935490.1, GQ338309.1, JX129807.1, and KC851848.1,[24] the out group accession number: HM357606.2,[18] based on the partial F gene region nucleotide sequence by MEGA 10.2.4.

respectively. The result of the current study in Rift Valley is comparable with previous reports of 12.7% and 11.6% by Gelana[16] and Terefe et al,[8] respectively; however, it is relatively lower (15.47%) than the report of Milkesa et al.[17] The result of the study in the central part of Ethiopia did not agree with the findings of Miressa et al,[18] 26.7% in Adama and Bishoftu; and Gelana,[16] 40%, in Bishoftu. This difference might be due to the difference in the study population.

The detection rate of NDV strain from pooled swab samples was 7.69%, which is different from the previous reports by Milkesa et al[17] and Gelana[16] with 15.47% and 16.7%, respectively, in the Mid-Rift Valley area. There was a statistically significant difference between the different swab types (tracheal swab and cloacal swab samples) in the occurrence of NDV strain ($p<0.05$) where higher detection rate was recorded from tracheal swabs. This is in agreement with the report of Chaka et al.[19] The increased detection rate from tracheal swabs might be due to the presence of velogenic genotype (viscerotropic velogenic) of NDV strain in chickens.[16] On the other hand, in the present study, we found no statistically significant difference among the different tissue sample types for the occurrence of NDV strain ($p>0.05$), which is contrary to the report of Guo et al[20] from China who noted significant variation of viral loads among individual chickens in different tissue types. This variation might be due to the presence of different virulent strains of NDV in the study areas and factors that affect the replication of the virus.

In the current study, a higher detection rate of NDV strain was recorded in village chickens compared to that of the commercial chickens with a statistically significant difference ($p<0.05$) between the different management systems. This is in agreement with the findings of other researchers,[21,22] who reasoned that the high detection rate in the village may be due to poor sanitary conditions, nutritional deficiencies, lack of vaccination, and contact of chickens in one village with those in other villages that can facilitate the spread of NDV strain.

The present study characterized five NDV strain isolates obtained from village and commercial chickens in Mid-Rift Valley and central part of Ethiopia. Accordingly, we found two different cluster NDV strain genotypes (one velogenic and three lentogenic genotypes) with four separate NDV strain genotypes, namely I, II, III, and VII in chickens in the study area. This is in agreement with the recent unified classification system proposed by Dimitrov et al[23] and previous findings from Central part of Ethiopia[9,18] and northwest part of Ethiopia.[24]

Further bioinformatics analysis of NDV strain isolates with ON033822 that were isolated from the freshly dead chicken brain in Bishoftu showed they belong to the velogenic genotype of genotype VII, and revealed 78% similarity to the Ethiopia NDV strain genotype from the database (accession number KC851848.1). However, this isolate showed higher similarity (97.49% to 98.32%) to the reference sequences of the Iranian NDV strain genotype from the database (KP771863.1, JX131357.1, and KU201410.1). The current report of the velogenic genotypes is in agreement with the recent report by Bari et al[19] where they revealed that these genotypes, commonly known as pigeon paramyxovirus type 1

strain, were found in Ethiopia from 1976 to 2007. NDV strain with ON033823 isolate was found to be a lentogenic genotype that belongs to genotype III having more similarity of 88.15%, 88.15%, and 87.68% to USA (JX901307.1), China (DQ217740.1), and Egypt (MN481244.1) NDV strain genotype, respectively.[15,25] NDV strain with ON033824 isolated from the freshly dead chicken intestine in Addis Ababa also belonged to the lentogenic genotype I and was found to have higher similarity (96.43%) to the South Korea NDV strain genotype (JQ966084.1).[26] NDV strain vaccine with ON033824 isolates, isolated from the currently used vaccine for commercial and village chickens in Ethiopia belonged to the lentogenic genotype II and was found to show higher similarity (96.76%) to the HB1 vaccine strain genotype (MK796810.1) and LaSota vaccine strain genotype (MK310259.1) of vaccines in-use in India and Pakistan. In this study, the findings of the phylogenetic analysis are consistent with the report of several researchers,[27–30] showing the continuous evolution and mutation in the partial region of F gene in NDV strain genotype.

Even though this[31] is the first study that attempted to characterize NDV strain genotype from chickens from selected areas in mid-Rift Valley and central parts of Ethiopia, it has some limitations such as not isolating positive samples through egg inoculation and small number of NDV strain isolates' sequencing due to limited access to facilities and sequencing machine, respectively. Despite this challenge, we tried to obtain preliminary data about the NDV strain genotypes that can serve as baseline information for future large scale study in the country.

Conclusion

The present study showed an overall molecular detection rate of 13.26% for NDV strain in chickens, indicating the Newcastle disease is widespread in chickens in the Mid-Rift Valley and the central part of Ethiopia. Furthermore, molecular characterization and phylogenetic analysis of the NDV strain genotype revealed evidence of the presence of velogenic and lentogenic genotypes with at least four distinct genotypes circulating in the study area. Even though large scale characterization using several isolates is required at national level, the current study laid baseline information for the existence of variations between field NDV strain genotype and the currently used vaccine strain genotypes against Newcastle disease in the country.

Data Sharing Statement

All data collected during this study are included in the manuscript.

Ethical Approval and Consent to Participate

Ethics approval was obtained from Adama Science and Technology University Ethical Review Committee with the verification number RECSoANS/BIO/03/2020. Informed written consent statement was also obtained from bird owners for swab and tissue sample collection. In addition, all methods were performed in accordance with the relevant guidelines and regulations of Animal Health Institute for the welfare of animals.

Acknowledgments

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically

reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

Disclosure

The authors declared that they have no competing interests in this work

References

1. Boynukara B, Gülhan T, Çöven F, Kiziroğlu İ, Durmuş A. Determination of Newcastle disease virus among wild bird populations in lake van basin, Turkey. *Turk J Vet Anim Sci*. 2013;37(1):86–93.
2. Alexander DJ. *Ecology and Epidemiology of Newcastle Disease. Avian Influenza and*. Newcastle disease. Springer, Milano, 2009. 19–26
3. Aldous E, Alexander D. Detection and differentiation of Newcastle disease virus (avian paramyxovirus type 1). *Avian Pathol*. 2001;30(2):117–128. doi:10.1080/03079450120044515
4. Melkamu S, Asrat M. Review on economic importance and current diagnostic techniques of Newcastle disease in poultry. *Int J Adv Res Biol Sci*. 2018;5:117–125.
5. Wise MG, Suarez DL, Seal BS, et al. Development of a real-time reverse-transcription PCR for detection of Newcastle disease virus RNA in clinical samples. *J Clin Microbiol*. 2004;42(1):329–338. doi:10.1128/JCM.42.1.329-338.2004
6. Hines NL, Miller CL. Avian paramyxovirus serotype-1: a review of disease distribution, clinical symptoms, and laboratory diagnostics. *Vet Med Int*. 2012;2012:1–17. doi:10.1155/2012/708216
7. OIE WOfAH. Manual of diagnostic tests and vaccines for terrestrial animals. Newcastle disease. 2012; Availble from: https://www.oie.int/doc/ged/D12009.PDF. Accessed april 26, 2024.
8. Terefe D, Belaineh R, Chaka H, et al. Serological and molecular study of Newcastle disease virus in village chickens in selected rift-Valley areas, Ethiopia. *J Vet Sci Technol*. 2015;6(06):1–4. doi:10.4172/2157-7579.1000264
9. Mulisa DD, W/Kiros MK, Alemu RB, et al. Characterization of Newcastle disease virus and poultry-handling practices in live poultry markets, Ethiopia. *SpringerPlus*. 2014;3(459). doi:10.1186/2193-1801-3-459
10. Bello MB, Yusoff K, Ideris A, Hair-Bejo M, Peeters BP, Omar AR. Diagnostic and vaccination approaches for Newcastle disease virus in poultry: the current and emerging perspectives. *Biomed Res Int*. 2018;2018:1–18. doi:10.1155/2018/7278459
11. NVI. Poultry disease and Newcastle disease. *Debere Ethiop*. 2019. Availble from: https://www.nvi.com.et/products/vaccines-against/poultry-diseases/newcastle-thermostable/.
12. Handbook of the birds of the world and birdLife International digital checklist of the birds of the world. 2018. Available from: http://datazone.birdlife.org/userfile/file/Species/Taxonomy/HBW/-Bird Life_check list_v3_Nov18.zip. Accessed 02 May 2024.
13. FAO. Poultry Sector Ethiopia. FAO Animal Production and Health Livestock Country Reviews. FAO, Rome Cited in Guteta Alemayehu (2021) Dynamics of Poultry Production System, Constraints, Opportunities and Marketing in Ethiopia. 2019; Available from: http://www.fao.org/3/ca3716en/ca3716en.pdf). Accessed april 26, 2024.
14. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol Bio Evolu*. 2018;35(6):1547. doi:10.1093/molbev/msy096
15. Shi B, Zhan X-M, Zheng J-X, et al. Identifying key bird species and geographical hotspots of avian influenza A (H7N9) virus in China. *Infect Diseases Poverty*. 2018;7(1):1–11. doi:10.1186/s40249-018-0480-x
16. Gelana H. Conventional and molecular epidemiology of new castle disease in wild and domestic birds in selected rift valley areas. *Ethiopia*. 2017(1):1–10.
17. Milkesa A, Dinka H, Belaineh R, Aliy A. Molecular surveillance of avian paramyxovirus type-1 in nonvaccinated village chickens in central rift valley of Oromia, Ethiopia. *Poultr Sci*. 2021;100(4):101004. doi:10.1016/j.psj.2021.01.027
18. Miressa Y, Cherinet Y, Bsrat A, Tadesse F. Investigation of Newcastle disease virus using reverse transcription polymerase chain reaction in selected Districts of Eastern Shewa, Ethiopia. *J Vet Sci Technol*. 2016;7(384):2. doi:10.4172/2157-7579.1000384
19. Chaka H, Goutard F, Bisschop SP, Thompson PN. Seroprevalence of Newcastle disease and other infectious diseases in backyard chickens at markets in Eastern Shewa zone, Ethiopia. *Poultr Sci*. 2012;91(4):862–869. doi:10.3382/ps.2011-01906
20. Guo H, Liu X, Xu Y, et al. A comparative study of pigeons and chickens experimentally infected with PPMV-1 to determine antigenic relationships between PPMV-1 and NDV strains. *Vet Microbiol*. 2014;168(1):88–97. doi:10.1016/j.vetmic.2013.11.002
21. Kondombo S, Nianogo A, Kwakkel R, Udo H, Slingerland M. Comparative analysis of village chicken production in two farming systems in Burkina Faso. *Trop Anim Health Prod*. 2003;35(6):563–574. doi:10.1023/A:1027336610764
22. Tadesse S, Ashenafi H, Aschalew Z. Seroprevalence study of Newcastle disease in local chickens in central Ethiopia. *Int J Appl Res Vet Med*. 2005;3(1):25–29.
23. Dimitrov KM, Abolnik C, Afonso CL, et al. Updated unified phylogenetic classification system and revised nomenclature for Newcastle disease virus. *Infect Genet Evol*. 2019;74:103917. doi:10.1016/j.meegid.2019.103917
24. Fentie T, Heidari A, Aiello R, et al. Molecular characterization of Newcastle disease viruses isolated from rural chicken in northwest Ethiopia reveals the circulation of three distinct genotypes in the country. *Trop Anim Health Prod*. 2014;46(2):299–304. doi:10.1007/s11250-013-0487-z

25. Liu H, Wang Z, Wu Y, et al. Molecular epidemiological analysis of Newcastle disease virus isolated in China in 2005. *J virolog meth*. 2007;140(1–2):206–211. doi:10.1016/j.jviromet.2006.10.012

26. Kim B-Y, Lee D-H, Kim M-S, et al. Exchange of Newcastle disease viruses in Korea: the relatedness of isolates between wild birds, live bird markets, poultry farms and neighboring countries. *Infect Genet Evol*. 2012;12(2):478–482. doi:10.1016/j.meegid.2011.12.004

27. Aldous E, Mynn J, Banks J, Alexander D. A molecular epidemiological study of avian paramyxovirus type 1 (Newcastle disease virus) isolates by phylogenetic analysis of a partial nucleotide sequence of the fusion protein gene. *Avian Pathol*. 2003;32(3):237–255. doi:10.1080/0307945031000097831

28. Palya V, Kiss I, Tatar-Kis T, Mato T, Felföldi B, Gardin Y. Advancement in vaccination against Newcastle disease: recombinant HVT NDV provides high clinical protection and reduces challenge virus shedding with the absence of vaccine reactions. *Avian Diseases*. 2012;56(2):282–287. doi:10.1637/9935-091511-Reg.1

29. Liu J, Zhu J, Xu H, et al. Effects of the HN antigenic difference between the vaccine strain and the challenge strain of Newcastle disease virus on virus shedding and transmission. *Viruses*. 2017;9(8):225. doi:10.3390/v9080225

30. Alsahami A, Ideris A, Omar A, Ramanoon S, Sadiq M. Isolation, identification and molecular characterization of Newcastle disease viruses in vaccinated chickens from commercial farms in the sultanate of Oman. *Int J Vet Sci Med*. 2018;6(2):248–252. doi:10.1016/j.ijvsm.2018.08.007

31. Esubalew Endale. Molecular Characterization for Strains of Newcastle Disease Virus Circulating in Chickens in Selected Migratory Birds Abundant Areas in the Mid Rift Valley and Central Part of Ethiopia. MSc Thesis, Adama Science and Technology University, Adama, Ethiopia. 2021:1–86.