NIGERIAN VETERINARY JOURNAL
ISSN 0331-3026
Nig. Vet. J., September 2024 Vol 45(3): 77-95.
https://dx.doi.org/10.4314/nvj.v45i3.6 ORIGINAL ARTICLE

# Pathogenicity of Newcastle Disease Virus Isolated from Ethiopia

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## SUMMARY

Newcastle disease represents an enormous problem for African breeders, and country-specific fears and economic exchanges complicate its elimination and control. Slow laboratory diagnosis creates additional delays during initial epidemic discovery. To address these concerns, researchers undertook a study in Ethiopia to examine the impact of the circulating Newcastle disease virus on chicken farms.

The aim of the study is to identify the virus from likely outbreak cases, confirm its pathogenicity, and evaluate its effects on vaccinated and uninfected chicks Forty chicks were separated into 02 groups and exposed to 02 virus samples at the National Veterinary Institute in Bishoftu, Ethiopia. The isolated viruses were classified as velogenic, with the BOG strains scoring 1.67 and the HAR strains rating 1.55. Immunized chicks demonstrated high herd immunity, while single immunization failure was observed. In the challenged group, the death rate was 55%, with all 20 unvaccinated chicks dying from the sickness, whereas just 2 out of 20 vaccinated chicks were afflicted. Statistical analysis showed that the virus produced general inflammation, which was more common in the BOG group (87.50%) than in the HAR group (12.50%). Vaccination significantly reduced clinical signs and gross lesions, such as irritation in multiple organs.

The study showed the persistence of velogenic Newcastle disease virus strains in Ethiopia and the importance of vaccination. The findings have improved our understanding of the disease's physiopathology and provided vital information for responsible authorities to implement early intervention techniques.

**Key words:** Newcastle disease, Velogenic Strains, Pathogenicity, Vaccination, Ethiopia.

## INTRODUCTION

Newcastle disease is one of the most prevalent viral diseases affecting domestic poultry and wild birds worldwide. According to Ferreira et al. (2019) and Getabalew et al. (2019), the Newcastle disease virus is also known as an avian paramyxovirus serotype 1 (APMV-1). It is a member of the *Paramyxoviridae* family, subfamily *Avulavirinae*, genus *Orthoavulavirus*, and species Avian orthoavulavirus. The genome encodes six structural proteins: nucleocapsid protein, phosphoprotein, matrix protein, fusion protein, haemagglutination-neuraminidase protein, and RNA polymerase protein (Brown & Bevins, 2017).

According to the Terrestrial Animal Health Code, this disease is a minor zoonosis requiring to be reported to the World Organization for Animal Health (WOAH) as it is highly contagious, extends rapidly across a large area, may have high mortality rates, and can result in significant economic loss (Shittu et al., 2016). Five continents worldwide are host to the disease (Absalón et al., 2019). Even though this disease is now under control in Western European nations and the North American continent, it was identified in southern California sixteen years before the most recent epidemic in May 2018. (Figueroa and coworkers, 2022). Additionally, many chicken farms in Asia, South America, and Africa continue to be affected by it (Amoia et al., 2021). It is estimated at 8.1% of Indians farms still contaminated by the circulationg NDV, with a 95% confidence interval of 4.8 to 13.4% (Sahoo et al., 2022). The incidence averaged 39.1% in countries in Southern America, with variations by area in Brazil of 6.5 to 58.4% (Orsi et al., 2010). Lybia, which has a prevalence of 45%, is noted to have a high prevalence in Africa (Sahoo et al., 2022).

In Ethiopia, the occurrence of the disease is very high. Four types of farming are present in the country: large-scale commercial, medium-scale commercial, small-scale commercial production and backyard farming (Tirfie & Tirfie, 2023). However, backyard chicken production supplies more than 90% of the national demand for meat and eggs (Sime, 2022). A low level of biosecurity, low input investment, and low productivity characterize backyard chicken production. This situation increases the risk of the introduction of Avian disease and its spread (Tafesse et al., 2008), especially Newcastle disease, with a seroprevalence between 22.22% and 34.94% in three districts of the Oromia regional state (Mamo & Yimer, 2021) and 19.7% to 25.5% in the Shewa zone (Chaka et al., 2013). In 2014, three distinct motif genotypes of the virus were isolated: one velogenic strain and two lentogenic strains (Fentie et al., 2014).

Even though the lack of a good biosecurity program is a relevant issue to prevent the disease, farmers must consider the vaccination of their flocks to avoid an outbreak, especially in commercial chickens during the introduction of new chickens or bands. Good practice and a specific vaccination schedule are essential to protecting the entire flock. Herd immunity is only achieved if 85% of the flock is effectively immunized (Rehmani et al., 2015; Wajid et al., 2017). The common vaccines used in Ethiopia are

Hitchener's B1 or H1, IOENDV, Thermo-stable I-2, and La Sota vaccines (Robi, 2020). In Bishoftu, the spread of disease continues to cause serious problem for the exportation of chicken's product, such as eggs and meat. In fact, this area is supplying the essential chicken's production for the country.

However, researchers in the country actively focused mainly on backyard studies, which are predominant and in which prophylaxis is not effective (Mamo & Yimer, 2021).

The physiopathology of the virus in birds is complex and differs based on a multitude of factors, including virus strains, species, age of the birds, and immune status (Creelan et al., 2002; Fentie, Dadi, et al., 2014; Ferreira et al., 2019). Both pathogenicity and tropism are employed to categorize the virus. According to strain type, researchers divide the virus into five pathotypes: viscerotropic and neurotropic velogenic, mesogenic, lentogenic, and asymptomatic enteric (Amoia et al., 2021).

Clinical signs depend on the strain of the virus. Severe strains like viscerotropic velogenic strains affect the respiratory tract and cause lethargy, anorexia, fluffy feathers, conjunctivitis, dyspnea, edema of the head, and diarrhea. With the neurotropic velogenic strains, the chickens may develop tremors, torticollis, wing or leg paralysis, and tonic or clonic spasms (Dimitrov et al., 2019). In the two cases, a drop in egg production, misshapen eggs, and thin shell eggs also occur in commercial-layer chickens. Also, mortality occurs in 2–6 days, with a 50% rate for non-vaccinated flocks. Sometimes, no clinical signs are observed because of the quick evolution of the disease. However, mortality depends not only on the strains but also on the immune system, vaccination status, and the birds' environment (Getabalew et al., 2019). Then, the mesogenic strains caused respiratory or neurologic signs but a low mortality rate (Rashi et al., 2021). However, co-infection with bacteria will always complicate the disease and its management. No clinical signs are associated with the asymptomatic or lentogenic strains. Nonetheless, mild clinical signs could appear in the chicks affected by the lentogenic strain (Bello et al., 2018).

Necropsy is very helpful in deciding the management of the farm before the laboratory's isolation and characterization of the virus. Some lesions are characteristic of the virus, such as hemorrhage in the gizzard. Even hemorrhage affects all of the intestines. In another part, mucosal secretion can also be seen in the trachea.

The similarity of respiratory diseases in avian species can confuse the diagnosis of Newcastle disease. The similarity of the symptoms does not provide an accurate diagnosis according to the clinical signs and lesions. Many diagnosis methods are available for virus identification, such as virus isolation, serological tests, molecular-based assays, microarray hybridization techniques, biosensor diagnostics, next-generation sequencing and random priming technologies (Bello et al., 2018). Furthermore, according to the international agreement, a definitive assessment of virus virulence is based on the intracerebral pathogenicity test (ICPI) (Rashi et al., 2021). The virulence of the disease is determined by the intracerebral pathogenicity

index (0.7–2.0), according to the WOAH. Virulent viruses' indices will approach the maximum score of 2.0, and lentogenic or asymptomatic enteric strains will give values close to 0.0. Even a score of ≥ 0.7 is considered virulent (Dimitrov et al., 2019). In this way, understanding the pathogenicity and effect of the virus in breeder farms is essential to know the main health issue of the virus in poultry farms, and it is helpful to differentiate the type of disease rapidly and to conduct the best method to protect the farm in the region. On the one hand, identifying the circulating virus and understanding its effect on poultry is critical. Therefore, the aim of this study was to identify the virus from outbreak cases, confirm its pathogenicity, and evaluate its effects on vaccinated and uninfected chicks.

## MATERIALS AND METHODS

### Area of Study

Ethiopia is an eastern African country located in the Horn of Africa. The capital is Addis Ababa, situated at the center of the land. According to the World Bank, the Ethiopian population was estimated to be 120 million in 2021. The economy is based on agriculture. More than 70% of Ethiopians are employed in agriculture. Agriculture activities comprise about 0.52 of the gross domestic product or GDP. The estimation of the Ethiopian poultry population is around 56.06 million. In Ethiopia, farmers actively raise three types of chickens, with indigenous chickens dominating the poultry population. Chicken farming is an essential element for Ethiopian households. However, more than eggs and meat are needed to meet the high demand from the vast population in the country. Also, poor input has the worst repercussions on productivity and increases the risk of disease (Sime, 2022). The importance of this activity was the reason for the creation of the National Veterinary Institute, or NVI, in 1964 to support the development of the poultry industry in the country. Realising research at the National Veterinary Institute, or NVI, Bishoftu, in Ethiopia was a vast opportunity to improve the disease control of the Newcastle disease, which is a real challenge for undeveloped countries, mainly in Africa. The study improves the knowledge of the disease, and the presence of the institute disease, like the NVI, makes it essential to study and manage the animal disease and produce vaccines that control its spreading. However, a mitigation of the vaccination method and an underestimation of the vaccination from the stakeholders are the main challenges that reduce the control program in veterinary medicine (Betela et al., 2023).

### Study Design

#### Design

The study was conducted at the National Veterinary Institute from April 2023 to June 2023. The duration of the study is three months. The study was an experimental study with a double-blind sampling process. Ninety-five eggs, obtained from the NVI poultry farm, were incubated for all of the experiments. 45/95 eggs were used for the isolation of the virus sample (n = 10/45), the ICPI test (n = 30/95). The 50 remaining eggs were hatched for the challenging purposes (n = 40/50), and necropsy control (n =

10/50). The day-old chicks (n = 50) had been raised inside one penhouse with standard management. Then, they were separated on day seven and distributed into five penhouses containing 10 chicks each.

At weeks 3 and 5, the HI test proceeded to appreciate the response of the vaccination of the chicks on days 7 and 21. The commercial vaccine produced and available in the city was used for this study. Then, the flock will be challenged with the previous two isolated strains of the NDV. Every day, the post-effect of the disease, or PED, will be noted during 14 days (PED 1–PED 14) (Appendix 7, Appendix 8). The affection of the general being, clinical signs, the incidence per day, the mortality, and the gross lesions on the necropsy were recorded (Roy et al., 1999; Sachan et al., 2015; Vogel, 2000).

**Isolation of the Virus**

Two suspected NDV samples solution (PBS + Swab'sample) from two different areas were collected from the cloacal and oropharyngeal of the sick bird. The sample solutions were inoculated in a 10-day-old embryonated chicken egg, or ECE, intra-allantoically to isolate the virus. In the absence of SPF eggs, NDV antibody-negative eggs were used. Five embryonated eggs were inoculated intra-allantoic with 0.2 ml of suspected NDV supernatant, and five control eggs were inoculated with 0.2 ml of 1% PBS. After inoculation, the ECE is incubated at 60% humidity and the viability of the ECE is checked twice every five days. Allantoic' fluid of dead embroyenated eggs were harvested, sent to the serology laboratory and the biomolecular laboratory.

**Virus Identification**

First, a spot hemagglutination test was performed to indicate the presence of hemagglutinin protein. Then, a Hemagglutination test was performed to determine the HA titre of the virus in the sample. For confirmation, a sample from the AAF was sent to NVI's molecular laboratory to identify the virus. This step is performed to confirm the presence of the NDV by RT-PCR. Finally, the ICPI test was performed to determine the pathogenicity of the two NDV samples.

**Statistical Analysis**

The statistical analysis used during the study was the non-parametric statistical methods: the Fisher test, Mann-Whitney test, and survival analysis. First, the Fisher test (comparison of two proportions) was used to see the relation between clinical signs and the pathogenicity of the virus, as well as the effect of the vaccination on the clinical signs and the gross lesions. Then, the Mann-Whitney test (comparison of two means) was used to appreciate the difference between the mortality, the morbidity from each virus sample, and the immune status of the chicks. Finally, the survival analysis with the rank log-rank of the Kaplan-Meir estimate was performed to determine the proportion of surviving chicks.

## Ethical Consideration

According the National veterinary protocol, animal behavior and welfare were cared for during all of the experimentation. Ad libitum feed and water, lighting was provided to the chicks to ensure their growth during all stages of the experimentation. Their health was checked daily; the environment was considered good, and the chicks could show typical behavior. An experimentation was advisable for a virus with a comprehensive strain like the Newcastle disease virus because of the replicability of the methods, which allows the verification and implementation of the experimentation for the different strains (Cardona, 2003). The experimentation permitted control of the environmental conditions of all subjects (temperature, humidity, diet, vaccination, lighting, etc.). The main challenge was the consideration of ethics when conducting an experimental study. It involves experimentation on animals, which includes minimizing pain, ensuring their welfare, and protecting the area of study against rodents and others (Hanlon et al., 2007).

## RESULTS

### Identification of the Virus

AAF of two virus samples was collected from dead ECE. The spot hemagglutination test was positive. RT-PCR test was performed and confirmed the Newcastle disease virus. The RNA of the sample was extracted, prepared, and amplified. Then, gel electrophoresis was used to separate the amplified cDNA. This involved loading the PCR products onto an agarose gel and applying an electric current to separate the DNA fragments according to size.

The titer of the virus was 28 for the Haramaya group (HAR) virus strain and 29 for the Bishoftu original group (BOG) virus strains.

### Pathogenicity

The two strains were classified as velogenic strains (Index > 1.4) according to the ICPI result, which shows a score of 1.67 for BOG strains and 1.55 for HAR strains.

### Immunity Status

The Hemagglutination assay of bulk HB1 was used as an antigen to quantify the Newcastle virus antibodies produced. The result showed that 11/20 chicks that received the first dose of the vaccine (thermostable vaccine) developed a protective titer against the disease (>1:16), and one case of none responding to the vaccine was recorded (HI = 0). After the booster doses, 19/20 developed a protective antibody titer among the NDV (>1:16), and one case of failed vaccination was confirmed (HI = 0).

### Morality, General Affection, and Clinical Signs

The mortality was 55% (n = 22/40) for all flocks, which appeared at the mean of 4.09 (IC 95% = ±0.48) dpi. For both groups, 11 chicks (n = 20) were counted as dead after the observation period of 15 days (Table 1). The general affection was recorded in 33% (n = 13/40) of the flock (Table 2), which appeared at a mean of 3.26 (IC 95% = ±0.51) Dpi. After the survey, 25% (n = 40) of the

flock presented a clinical sign (Table 3), which appeared at 3.33 (IC 95% = ±0.62) Dpi.

According to the type of virus, the respective group had a mean day of death of 4.36 ±0.80 (BOG) and 3.80 ±0.51 (HAR), with a non-significant difference (p = 0.36). Further, the mean day of apparition of the body condition affection was not different (p = 0.68). The clinical sign apparition was also not significantly different (p = 0.56) (Table 4).

The mean day of death (p = 0.76) for the vaccinated and unvaccinated groups was 5.50 (IC 95% = 4.90) and 3.95 (IC 95% = 0.33). The unvaccinated group showed early deterioration in body condition, which was at 2.94 (0.31) Dpi, as opposed to the vaccinated group, which was only noticeable at 6.00 (0.00) Dpi, and the difference in Dpi was statistically significant, p = 0.01. The same situation was observed for the clinical sign, which appeared at 2.92 (±0.34) and 6.00 (±0.00) Dpi, (p = 0.02) (Table 5).

**Censored Data**

A daily survey of the flock allowed us to appreciate the evolution of the importance of the flock for two weeks. The mortality of the chicks started at day three and reached about 50% of the flock at day 5.

**Necropsy**

**Body scoring**

The flock's mean score was 2.38 out of 5 (IC 95% = 0.01). According to the isolated virus, the HAR group was heavier than the BOG group: 2.40 (IC 95% = 0.02), p = 0.64. However, the immunological status of the chicks has no influence of the gain weight of the chicks p = 0.11.

**Inflammation**

20% (n = 8/40) of the flock presented external skin lesions. According to the group of viruses, the generalized inflammation was associated with the BOG group, p = 0.04, with a proportion of 87.50% (n = 7/8) and 12.50% (n = 1/8) for the HAR group. Moreover, none of the chicks that presented with this lesion were vaccinated during the experimentation (p = 0.003).

During the necropsy, 48% (n = 19/40) of the flock presented internal lesions. According to the group of viruses, no difference was found between the two groups of study (p = 0.527), with a proportion of 57.90% (n = 11/19) for the BOG group and 42.10% (n = 08/19) for the HAR group. However, the internal lesion was predominant in the unvaccinated group (89.47%, n = 17/19) rather than in the vaccinated group (10.53%, n = 02/19), p < 0.003.

**Head Affection**

23% (n = 09/40) of the flock was affected on the head. According to the group of viruses, no difference was found between the two groups of study, p = 1.000, with a proportion of 55.55% (n = 05/09) for the BOG group and 44.45% (n = 04/09) for the HAR group. However, the internal lesion was predominant in the unvaccinated group (88.88%, n = 08/09) rather than in the vaccinated group (11.12%, n = 01/09), p = 0.019.

**Respiratory Tract**

50% (n = 20/40) of the flock had a lesion on the respiratory tract. There is no difference between the two groups of study, p = 1.000, with a proportion of 50.00% (n = 10/20) for the BOG group and 50.00% (n = 10/20) for the HAR group. However, the internal lesion was predominant in the unvaccinated group (90.00%, n = 18/20) rather than in the vaccinated group (10.00%, n = 02/20), p < 0.003.

The mean number of respiratory tract lesions identified was 0.87 per chick (IC 95% = ±0.04). According to the isolated virus used during the experimentation, the mean score difference between the two groups was not significant even if the BOG group (0.90 (IC 95% = ±0.10) had more lesions than the HAR group (0.85 (IC 95% = ±0.09), p = 0.94.

Nevertheless, unvaccinated chicks developed more lesions, 1.60 (IC 95% = ±0.08), than the vaccinated chicks, 0.15 (IC 95% = ±0.04), and the difference is significant (p < 0.003).

**Heart Affection**

The flock had a cardiac lesion of 25% (n = 10/40). Even the most affectionate chicks belong to the HAR group, with 70% (n = 07/10) and 30% (n = 03/10) belonging to the BOG group. There was no association between the studied parameters, which were the group of viruses, the immune status of the chicks, and the heart's affection (p = 0.270). During the necropsy, a mean of 0.40 (IC 95%= ±0.03). Lesion per chick was discovered in the group. According to the isolated virus used during the experimentation, the mean difference between the two groups was not significant even if the BOG group; 0.20 (IC 95% = ±0.05) had more lesions than the HAR group (0.60 (IC 95% = ±0.08), *p* = 0.12.

Also, the same observation was concluded to be associated with immune status. The presence of the lesion on the heart was not associated, even if the unvaccinated group presented more lesions with a mean of 0.60 (IC 95% = ±0.08) rather than the vaccination of the flocks, where a mean of 0.20 (IC 95% = ±0.05) was established, *p* = 0.12.

**Gastrointestinal Tract**

More than half of the chicks in the flock, 67.50% (n = 27/40), had at least one lesion on the gastrointestinal tract of the flock. On the one hand, the virus inoculated into the chicks did not influence the occurrence of lesions in their gastrointestinal tracts (p = 0.170).

On the other hand, the vaccination gave strong protection against gastrointestinal symptoms in the flock, which represented 25.92% (n = 7/27) of the carcasses with gross gastrointestinal lesions. Also, all chicks without lesions were vaccinated, p < 0.003.

A mean of 1.40 (IC 95% = ±0.06) lesions per chick was discovered during the experimentation on the gastrointestinal tract. According to the isolated virus used during the experimentation, the BOG virus was more virulent with a mean of 1.75 (IC 95% = ±0.14) lesion than HAR group 1.05 (IC 95% = ±0.11). Nevertheless, this difference was not significant (p = 0.11).

Furthermore, the number of lesions on the gastrointestinal tract was associated with the immune status of the chick. The vaccination protected or reduced the apparition of the lesion on it, with a mean of 0.20 (IC 95% = ±0.05) for the vaccinated group and 0.60 (IC 95% = ±0.08), $p < 0.003$.

**Others Organ**

Half of the chicks in the flock, 52.50% (n = 21/40), had at least one lesion in an internal organ. On the one hand, the virus inoculated into the chicks did not influence the occurrence of lesions on the gastrointestinal tract of the chicks, p = 0.520, which were 12/21 cases for the BOG group and 09/21 cases for the HAR group.

On the other hand, the vaccination protected the internal organs against the lesions and reduced the cases by nearly 10%, $p < 0.003$.

A mean of 0.40 (IC 95% = ±0.03) lesion per chick was discovered during the experimentation without the natural association of the isolated virus or the status immunity: the BOG group was 0.20 (IC 95% = ±0.05), the HAR group was 0.60 (IC 95% = ±0.08), p = 0.12, and the unvaccinated group was 0.60 (IC 95% = ±0.08), 0.20 (IC 95% = ±0.05) was founded, p = 0.12. A mean of 0.82 (IC 95% = ±0.04) lesions per chick were discovered during the experimentation on the internal organ of the bird. According to the isolated virus used during the experimentation, the BOG virus was more virulent with a mean of 1.00 (IC 95% = ±0.09) lesion than the HAR group 10.65 (IC 95% = ±0.07). Nevertheless, this difference was not significant (p = 0.25). Furthermore, the number of lesions on the gastrointestinal tract was associated with the immune status of the chick. The vaccination protected or reduced the apparition of the lesion on it, with a mean of 0.15 (IC 95% = ±0.04) for the vaccinated group and 1.50 (IC 95% = ±0.06), $p < 0.003$.

A mean of 0.17 (IC 95% = ±0.01) lesions per chick was discovered during the experimentation on the other organs of the bird. According to the isolated virus used during the experimentation, the BOG virus was more virulent with a mean of 0.25 (IC 95% = ±0.04) lesion than the HAR group 0.10 (IC 95% = ±0.03). Nevertheless, this difference was not significant (p = 0.22).

Furthermore, the number of lesions on the other organs was associated with the immune status of the chick. The vaccination protected or reduced the apparition of the lesion on it, with a mean of 0.05 (IC 95% = ±0.02) for the vaccinated group and 0.30 (IC 95% = ±0.0), p = 0.04.

**DISCUSSION**

Our study was experimental with a double-blind sampling process. It allowed the daily observation and control of the viruses 'effects on the embryo, day-old chicks, and chicks. The experimental study accurately assessed the causal relationships between the isolated virus and its pathogenicity, immune status, and pathogenicity. Cost and equipment constraints could also affect the well-being of the study and limit the results obtained. Also, the control of internal parameters during the experimentation limited the capacity to generalize

the absence of the capacity for sequencing tests (Goraichuk et al., 2020; Xiao et al., 2012).

The population of the study was composed of the embryo for the culture of the virus, the day-old chicks to run the ICPI test, and finally, the chick's eggs. First, eggs were collected from the institute's farm and incubated for 21 days for hatching. The institute has been producing regular eggs instead of SPF eggs in the absence of equipment. These SPF eggs are highly recommended in the case of virology research to prevent contamination of the pathogenic germ into the egg's microflora (Burova & Trubitsyn, 2021). Nevertheless, periodic monitoring of the parent's stock is regularly done every three months to check the immunity status of the flock. After titration and virus detection, any suspected infection in the flock results in the removal, application of quarantine, disinfection, and renewal of the parent stock.

The virus strains used for this study were obtained from NVI, Virology Department of Research and Development Directorate, isolated from two different areas of the country. One came from a village farm around Bishoftu; the other was a sample from Haromaya. The sample was collected from the cloaca of chickens presenting clinical signs. This site presented the advantages of being the primary site of NDV shedding, non-invasive methods, low cost, and easy to perform on live birds (Spackman et al., 2013; Zeynalova et al., 2015). However, this method increases the risk of cross-contamination of the sample, and it also has a low viral load compared to samples from internal organs like the liver, brain, and spleen (WOAH, 2022).

Spot hemagglutination was conducted to determine the suspicion of Newcastle disease. This method confirms the presence of the hemagglutinating agent in the fluid sample (WOAH, 2022). However, this method is limited by its inability to differentiate the virus's origin, which could be the Newcastle disease (NDV) or the Avian Influenza virus (AIV). The subjectivity of the result interpretation could also interfere with the result because the test is based on a visual interpretation of the agglutination. The virus titer was 28 HA units in 25 mL for the Haramaya group (HAR) virus strain and 29 HA units in 25 mL for the Bishoftu original group (BOG) virus strains. According to the WHO, if the titer of the hemagglutinin collected is higher, the accuracy of the result is good.

The two strains were classified as velogenic strains (index > 1.4) according to the ICPI result, which shows a score of 1.67 for BOG strains and 1.55 for HAR strains. Nevertheless, this value was less than the mean ICPI value in Ethiopia (1.76) obtained for all strains collected from 1976 until 2008 (Bari et al., 2021). The study showed a higher ICPI value than the study in India, where the highest value of velogenic strains was 1.53 for the eight studied strains (Rashi et al., 2021). However, higher in-vivo pathogenicity with an ICPI of 1.9 was found in Malagasy-isolated viruses (MG-1992 and MG-725/08) (Maminiaina et al., 2010).

Based on the vaccination schedule recommended by the NVI (thermostable I-2 strain vaccine at day 7 and the lentogenic strain Lasota at day 21). 95% of the chicks developed a protective antibody against the NDV (HI> 1:16, max = 1/128, min =

0) (Appendix 7). However, one case of vaccination failure was recorded. In Iran, a study showed a mean of HI = 1:4 and HI = 1:8 after the boost with the thermostable vaccine (Asl Najjari et al., 2017). The RT-PCR test confirmed that the two samples were Newcastle disease viruses. The electrophoresis showed a similar size of amino acid in the two samples. This similarity could mean that the two samples were classified as one similar strain. However, sample sequencing is the only way to confirm the strain groups of the virus. Sequencing is performed to determine the genetic sequence of the amplified viral RNA. This sequencing data can be compared to known reference sequences of different NDV strains to identify the specific strain in the sample (Goraichuk et al., 2020).

Results from the experimental flocks indicates that the mortality was 55% (n = 22/40) with a mean of 4.09 (IC 95% = ±0.48) dpi. According to a report study in Egypt, a rate of 75% of mortality was recorded with an outbreak of Newcastle disease virus velogenic strain (Abdel-Glil et al., 2014). This result confirmed the affirmation of the WHOA, which said that the mortality approach has a rate of 100% on unvaccinated chicken (WOAH, 2022). For the vaccinated group, a similar result was found in Cameroun, which led to a reduction of 90% in mortality with multipotent vaccines, including Newcastle disease (Awa et al., 2008). The vaccination failure could explain the cause of mortality in one vaccinated chick. The chicks did not develop a protective antibody before the experimental outbreak (Appendix 7), an approach that explained their sudden death (WOAH, 2022). However, a study released in 2017 by Asl Najjari showed that the thermostable I-2 vaccine offered good protection for broilers (Asl Najjari et al., 2017). 33% (n = 13/40) of the chicks showed a general affection which appeared at a mean of 3.26 (IC 95% = ±0.51) Dpi (Table 4). A study in 2010 showed a similar result, with the earliest apparition at 2 Dpi and a mean of 3 Dpi (Kattenbelt et al., 2010). The general modification of the behavior touched mainly the unvaccinated group (n =11/20) rather than the vaccinated group (n = 02/20), $p < 0.003$. The general affection was characterized by a modification of the behavior (anorexia) or the general appearance (fluffy feather), depression, and lethargy. Other signs could appear with the velogenic stain, such as edema and conjunctiva (Betela et al., 2023; Getabalew et al., 2019).

In this survey, 25% (n = 10/40) of the flock presented a clinical sign during the survey, which appeared at 3.33 (IC 95% = ±0.62) Dpi (Table 4). The low rate of mortality could be due to the quick evolution of the disease which conduct to the death. Indeed, 07 corpses were identified very early at three dpi and a sudden death (n = 5/7) represented the critical part of it: n = 04/05 were not vaccinated, n= 02/05 were infected with BOG, and the remaining were infected with HAR (n = 03/05). According to Mebrate Getabalew et al., the susceptibility of the chicks is higher at a young age (Mebrate Getabalew et al., 2019). However, respiratory and nervous signs were observed with the velogenic Newcastle disease infection (Mebrate Getabalew et al., 2019). Charlie Franck Arthur N'Guessan Amoia added that nervousness and respiratory affection were associated with the neurotropic strains, and diarrhea and a decrease in

egg production were associated with the viscerotropic strains (Amoia et al., 2021).

Pathogenicity of the virus for each group of studies (BOG and HAR), neither MDT, general affection appearance nor the mortality and the proportion rate of the general sign were significantly different. According the virus inoculated, the mortality rate was equal with 11 chicks each, ($p$ = 1), counted as dead after the observation period of 15 days.

The respective groups had a mean death time, or MDT, of 4.36 ±0.80 (BOG) and 3.80 ±0.51 (HAR), with a non-significant difference ($p$ = 0.36). An experimental study 2019 showed a general MDT of 3.2 for infected birds with high doses of velogenic strains (Ferreira et al., 2019). Further, the mean day of apparition of the body condition affection and clinical signs were generally at 3 Dpi and were not significantly different ($p$ = 0.68). Earlier cases were recorded from the same study by Ferreira et al. in 2019, which affirmed that mild general affection appeared at two dpi. Another study found between 4 and 8 Dpi the first morbidity and clinical signs (Fentie, Dadi, et al., 2014).

Nonetheless, there were more chicks showing clinical in the flock infected with BOG group (n = 08/10, $p$ = 0.02). This case could suppose that the BOG sample were more infectious than HAR even if they were classified at the same class. The immune status of the chicks showed that the mortality rate reached 100% for the unvaccinated group (n = 20/40) and only 10% for the vaccinated group (n = 2/20), p < 0.003. However, MDT were not significantly different between the two groups ($p$ = 0.76). Then, the unvaccinated group showed a significant early degradation of the body condition, which was at 2.94 (±0.31) Dpi rather than the vaccinated group, which was notable only at 6.00 (±0.00) Dpi, $p$ = 0.01. The same situation was observed for the clinical sign, which appeared at 2.92 (±0.34) and 6.00 (±0.00) Dpi, and the difference was very significant ($p$ = 0.02) (Table 5). According to Fentie et al., morbidity and mortality depended on the type of vaccine used. Nevertheless, it appeared at least one-week post-inoculation (Fentie, Dadi, et al., 2014).

The Kaplan-Meier dpi curve showed that mortality started at three dpi. The survey median showed that the mortality of the chicks reached about 50% of the flock on day 5 (Figure 10). A similar result was found in an experimental study using high doses of velogenic for one virus strain (Dudley,1 et al., 2016). However, 50% of mortality was reached on the same day and increased to 80% at six dpi (Ferreira et al., 2019).The results of the necropsy and Body scoring indicated that 95% =The mean of the body score of the entire flock was 2.38 over 5 (IC 95% = ±0.01), which means the chicks were not cachexic or overweight. Also, the mean score difference between the two groups (higher unvaccinated group, p = 0.11). However, assigning scores for some birds was difficult due to the asymmetry of the muscle chest, which could induce an error in scoring. Also, the chick's age did not consider the fat on the score parameter, which could impact the body scoring too (Gregory & Robins, 1998). The Gross lesion observed in 20% (n = 8/40) of the chicks presented generalized inflammation, which was associated with the

BOG group (p = 0.04) with a proportion of 87.50% (n = 7/8). According to the immune status of the chicks, vaccination protected the chicks against this lesion (*p* = 0.003) (Figure 11). According to Juncheng Cai, NDV induces autophagy, associated with inflammation (Cai et al., 2023). NDV action activates pro-inflammatory cytokines and type I interferon (IFN) (Fournier et al., 2012). During the necropsy, 48% (n = 19/40) of the flock presented internal inflammation. First of all, the inflammation was not associated with a specific virus sample group (p = 0.527), with a proportion of 57.90% (n = 11/19) for the BOG group and 42.10% (n = 08/19) for the HAR group. However, the internal lesion was predominant in the unvaccinated group (89.47%, n = 17/19) rather than in the vaccinated group (10.53%, n = 02/19), p < 0.003 (Figure 12). The most gross lesions were observed in the experimentation that studied different commercial vaccines on the broiler in 2022 (Sultan et al., 2021).

The necropsy showed a proportion of 23% (n = 09/40), which was presented lesion on the head. No difference was found between the two groups of study, *p* = 1.000, with a proportion of 55.55% (n = 05/09) for the BOG group and 44.45% (n = 04/09) for the HAR group. However, the internal lesion was predominant in the unvaccinated group (88.88%, n = 08/09) rather than vaccinated group (11.12%, n = 01/09), *p* = 0.019. While, half of the chicks presented a lesion on the respiratory tract (n = 20/40). This problem was not associated with the isolated virus sample (p = 1.000), with a proportion of 50.00% (n = 10/20) for the BOG group and 50.00% (n = 10/20) for the HAR group. However, the internal lesion was predominant in the unvaccinated group (90.00%, n = 18/20) rather than in the vaccinated group (10.00%, n = 02/20), p < 0.003. The mean number of respiratory tract lesions identified was 0.87 per chick (IC 95% = ±0.04), and no difference was observed regarding the isolated virus during the study (p = 0.94). Nevertheless, unvaccinated chicks developed more lesions, 1.60 (IC 95% = ±0.08), than the vaccinated chicks, 0.15 (IC 95% = ±0.04), and the difference is significant (p < 0.003). The same study conducted by Sultant et al. showed that vaccinated chicks with rNDV-inactivated G VII showed eye lesions such as lacrimation and slight occlusion in 5.7% (2/35) of the cases and at least one lesion was present, and the different chick lesions could be classified as mild, moderate, or severe catarrhal tracheitis or lung congestion. Also, nearly 29% (n = 10/35) presented respiratory clinical signs (Sultan et al., 2021).

The flock had a cardiac lesion of 25% (n = 10/40). The lesions observed were hypertrophy, a whitish heart, or (n = hemorrhage. Even the most affectionate chicks belong to the HAR group, with 70% (n = 07/10) and 30% (n = 03/10) in the BOG group. There was no association between the studied parameters, which were the group of viruses, the immune status of the chicks, and the heart's affection (p = 0.270). Accordingly, the heart was not the common organ where the virus grew (Rehman et al., 2020). However, according to the same study, uncommon organs could develop lesions, such as on the testicular organs.

A mean of 0.40 (IC 95% = ±0.03) lesion per chick was discovered during the experimentation without the natural association of the isolated

virus or the status immunity: the BOG group was 0.20 (IC 95% = ±0.05), the HAR group was 0.60 (IC 95% = ±0.08), p = 0.12, and the unvaccinated group was 0.60 (IC 95% = ±0.08), 0.20 (IC 95% = ±0.05) was founded, p = 0.12. Gastrointestinal hemorrhage was mainly identified during the necropsy, and according to Mebrate Getabalew, lesions on the gastrointestinal tract indicated that the virus could be a viscerotropic strain (Mebrate Getabalew et al., 2019). Also, nervous lesions were not observed during the study, which supports this hypothesis. However, sequencing the virus is the only way to confirm the strain's characteristics (Maminiaina et al., 2010). Another study in Ethiopia showed the following gross lesions: enlarged spleen, degeneration, and multifocal necrosis in the liver (Worku et al., 2022). were caused by the NDV.

## CONCLUSION

In conclusion, the Newcastle disease virus experimentation conducted at NVI Ethiopia has provided valuable insights into the characteristics and management of the disease. The assessment of virulence using the ICPI revealed the presence of velogenic strains, indicating the potential for severe outbreaks and high mortality rates in poultry populations.

However, the study also demonstrated vaccination's effectiveness in protecting against Newcastle disease. The vaccinated groups significantly reduced disease severity and mortality compared to the non-vaccinated groups. This finding underscores the importance of implementing robust vaccination programs to mitigate the impact of Newcastle disease and safeguard poultry health.

The mean death rate observed on day 5 highlighted the rapid progression and severity of the disease, emphasizing the urgency for timely intervention and preventive measures. Early detection, prompt vaccination, and strict biosecurity protocols are crucial in minimizing the spread of velogenic Newcastle disease strains and preventing devastating losses in poultry production.

The findings from the Newcastle disease virus experimentation at NVI Ethiopia contribute to the growing body of knowledge on the epidemiology and control of this viral disease. Further research and surveillance efforts are warranted to continuously monitor the prevalence and evolution of Newcastle disease strains, evaluate the long-term efficacy of vaccination strategies, and explore potential improvements in vaccine formulations.

Ultimately, the successful management of Newcastle disease relies on a comprehensive approach integrating vaccination, surveillance, biosecurity, and collaboration between veterinary authorities, researchers, and poultry industry stakeholders. By implementing evidence-based practices informed by studies such as this, we can work towards minimizing the impact of Newcastle disease on poultry populations and ensuring the sustainability of the poultry industry.

## ACKNOWLEDGEMENT

The authors wish to appreciate the assistance of National Veterinary Institute during the work. Our

heartfelt gratitude goes the Research and development staff and others units who contributed to the study.

Funding for this research was provided by the African Union Commission through the Pan African University Life and Earth Sciences Institute (including Health and Agriculture), Ibadan, Nigeria.

## REFERENCES

Absalón, A. E., Cortés-Espinosa, D. V., Lucio, E., Miller, P. J., & Afonso, C. L. (2019). Epidemiology, control, and prevention of Newcastle disease in endemic regions: Latin America. *Tropical Animal Health and Production*, *51*(5), 1033–1048. https://doi.org/10.1007/s11250-019-01843-z

Dudley,1, W. N., Wickham,2, R., & COOMBS,3, N. (2016). An Introduction to Survival Statistics: Kaplan-Meier Analysis. *Journal of the Advanced Practitioner in Oncology*, *7*(1), 91–100.

Tirfie, F. & Tirfie. (2023). *Review on Poultry Production Status and Economic Contribution of the Sector in Ethiopia*. *109*, 1–9. https://doi.org/10.7176/FSQM/109-01

BDEL-GLIL, M. Y., MOR, S. K., SHARAFELDIN, T. A., PORTER, R. E., & GOYAL, S. M. (2014). Detection and characterization of Newcastle disease virus in formalin-fixed, paraffin-embedded tissues from commercial broilers in Egypt. Avian Diseases, 58(1), 118–123. https://doi.org/10.1637/10616-071813-Reg.1

AMOIA, C. F. A. N., NNADI, P. A., EZEMA, C., & COUACY-HYMANN, E. (2021). Epidemiology of Newcastle disease in Africa with emphasis on Côte d'Ivoire: A review. Veterinary World, 14(7), 1727–1740. https://doi.org/10.14202/vetworld.2021.1727-1740

ASL NAJJARI, A. H., NILI, H., ASASI, K., MOSLEH, N., ROHOLLAHZADEH, H., & MOKHAYERI, S. (2017). Efficacy of Thermostable I-2 Newcastle Disease Vaccine Compared to B1 Commercial Vaccine in Broiler Chicken. Iranian Journal of Veterinary Research, 18(2), 103–107.

BARI, F. D., GELAYE, E., TEKOLA, B. G., HARDER, T., BEER, M., & GRUND, C. (2021). Antigenic and Molecular Characterization of Virulent Newcastle Disease Viruses Circulating in Ethiopia Between 1976 and 2008. Veterinary Medicine: Research and Reports, 12, 129–140. https://doi.org/10.2147/VMRR.S297281

BELLO, M. B., YUSOFF, K., IDERIS, A., HAIR-BEJO, M., PEETERS, B. P. H., & OMAR, A. R. (2018). Diagnostic and Vaccination Approaches for Newcastle Disease Virus in Poultry: The Current and Emerging Perspectives. BioMed Research International, 2018, e7278459. https://doi.org/10.1155/2018/7278459

BETELA, Y., HAILEGEBREAL, G., & SHIFERAW, D. (2023). Epidemiology of newcastle disease in village chicken in melokoza district, gofa zone, southwest Ethiopia. Heliyon, 9(3), e14384.

BROWN, V. R., & BEVINS, S. N. (2017). A review of virulent Newcastle disease viruses in the United States and the role of wild birds in viral persistence and spread. Veterinary Research, 48(1), 68. https://doi.org/10.1186/s13567-017-0475-9

CAI, J., WANG, S., DU, H., FAN, L., YUAN, W., XU, Q., REN, J., LIN, Q., XIANG, B., DING, C., REN, T., & CHEN, L. (2023). NDV-induced autophagy enhances inflammation through NLRP3/Caspase-1 inflammasomes and the p38/MAPK pathway. Veterinary Research, 54(1), 43. https://doi.org/10.1186/s13567-023-01174-w

CHAKA, H., GOUTARD, F., ROGER, F., BISSCHOP, S. P. R., & THOMPSON, P. N. (2013). Household-level risk factors for Newcastle disease seropositivity and incidence of Newcastle disease virus exposure in backyard chicken flocks in Eastern Shewa zone, Ethiopia. Preventive Veterinary Medicine, 109(3–4), 312–320. https://doi.org/10.1016/j.prevetmed.2012.10.003

CREELAN, J. L., GRAHAM, D. A., & MCCULLOUGH, S. J. (2002). Detection and differentiation of pathogenicity of avian paramyxovirus serotype 1 from field cases using one-step reverse transcriptase-polymerase chain reaction. Avian Pathology: Journal of the W.V.P.A, 31(5), 493–499. https://doi.org/10.1080/0307945021000005860

DIMITROV, K. M., FERREIRA, H. L., PANTIN-JACKWOOD, M. J., TAYLOR, T. L., GORAICHUK, I. V., CROSSLEY, B. M., KILLIAN, M. L., BERGESON, N. H., TORCHETTI, M. K., AFONSO, C. L., & SUAREZ, D. L. (2019). Pathogenicity and transmission of virulent Newcastle disease virus from the 2018-2019 California outbreak and related viruses in young and adult chickens. Virology, 531, 203–218. https://doi.org/10.1016/j.virol.2019.03.010

FENTIE, T., DADI, K., KASSA, T., SAHLE, M., & CATTOLI, G. (2014). <Effect of vaccination on transmission characteristics of highly virulent Newcastle disease virus in experimentally infected chickens. Avian Pathology: Journal of the W.V.P.A, 43(5), 420–426. https://doi.org/10.1080/03079457.2014.951832

FERREIRA, H. L., TAYLOR, T. L., DIMITROV, K. M., SABRA, M., AFONSO, C. L., & SUAREZ, D. L. (2019). Virulent Newcastle disease viruses from chicken origin are more pathogenic and transmissible to chickens than viruses normally maintained in wild birds. Veterinary Microbiology, 235, 25–34. https://doi.org/10.1016/j.vetmic.2019.06.004

FOURNIER, P., ARNOLD, A., WILDEN, H., & SCHIRRMACHER, V. (2012). Newcastle disease virus induces pro-inflammatory conditions and type I interferon for counter-acting Treg activity. International Journal of Oncology, 40(3), 840–850. https://doi.org/10.3892/ijo.2011.1265

GETABALEW, M., ALEMNEH, T., AKEBEREGN, D., GETAHUN, D., & ZEWDIE, D. (2019). Epidemiology, Diagnosis & Prevention of Newcastle Disease in Poultry. American Journal of Biomedical Science & Research, 3(1), 50–59.

GORAICHUK, I. V., WILLIAMS-COPLIN, D., WIBOWO, M. H., DURR, P. A., ASMARA, W., ARTANTO, S., DIMITROV, K. M., AFONSO, C. L., & SUAREZ, D. L. (2020). Complete Genome Sequences of 11 Newcastle Disease Virus Isolates of Subgenotype VII.2 from Indonesia. Microbiology Resource Announcements, 9(5), e01519-19. https://doi.org/10.1128/MRA.01519-19

GREGORY, N. G., & ROBINS, J. K. (1998). A body condition scoring system for layer hens. New Zealand Journal of Agricultural Research, 41(4), 555–559. https://doi.org/10.1080/00288233.1998.9513338

KATTENBELT, J. A., STEVENS, M. P., SELLECK, P. W., & GOULD, A. R. (2010). Analysis of Newcastle disease virus quasispecies and factors affecting the emergence of virulent virus. Archives of Virology, 155(10), 1607–1615. https://doi.org/10.1007/s00705-010-0739-4

LIU, M., SHEN, X., LI, J., YU, Y., FAN, J., JIA, X., & DAI, Y. (2023). Efficacy of Newcastle disease LaSota vaccine-induced hemagglutination inhibition antibodies against challenges with heterologous virulent strains of genotypes VII and IX. Veterinary Immunology and Immunopathology, 259, 110591. https://doi.org/10.1016/j.vetimm.2023.110591

MAMINIAINA, O. F., GIL, P., BRIAND, F.-X., ALBINA, E., KEITA, D., ANDRIAMANIVO, H. R., CHEVALIER, V., LANCELOT, R., MARTINEZ, D., RAKOTONDRAVAO, R., RAJAONARISON, J.-J., KOKO, M., ANDRIANTSIMAHAVANDY, A. A., JESTIN, V., & ALMEIDA, R. S. DE. (2010). <Newcastle Disease Virus in Madagascar: Identification of an Original Genotype Possibly Deriving from a Died-Out Ancestor of Genotype IV. PLOS ONE, 5(11), e13987. https://doi.org/10.1371/journal.pone.0013987

MAMO, T., & YIMER, L. (2021). Serological Investigation of Newcastle Disease in Selected Districts of Buno Bedelle Zone, Ethiopia. Veterinary Medicine: Research and Reports, 12, 253–259. https://doi.org/10.2147/VMRR.S327892

MEBRATE GETABALEW, ALEMNEH, T., AKEBEREGN, D., GETAHUN, D., & ZEWDIE, D. (2019). <Epidemiology, Diagnosis & Prevention of Newcastle Disease in Poultry. American Journal of Biomedical Science & Research, 3(1), 50–59.

ORSI, M. A., DORETTO JR, L., CAMILLO, S. C. A., REISCHAK, D., RIBEIRO, S. A. M., RAMAZZOTI, A., MENDONÇA, A. O., SPILKI, F. R., BUZINARO, M. G., FERREIRA, H. L., & ARNS, C. W. (2010). Prevalence of newcastle disease virus in broiler chickens (Gallus gallus) in Brazil. Brazilian Journal of Microbiology, 41, 349–357. https://doi.org/10.1590/S1517-83822010000200014

RASHI, U., MINI, M., PRIYA, P. M., SANKAR, S., & VIJAYAKUMAR, K. (2021). <Isolation and pathotyping of Newcastle disease virus isolated from birds in Kerala. Journal of Veterinary and Animal Sciences, 52(3), 245–249. https://doi.org/10.51966/jvas.2021.52.3.245-249

REHMAN, Z. U., REN, S., YANG, B., YANG, X., BUTT, S. L., AFZAL, A., MALIK, M. I., SUN, Y., YU, S., MENG, C., & DING, C. (2020). Newcastle disease virus induces testicular damage and disrupts steroidogenesis in specific pathogen free roosters. Veterinary Research, 51. https://doi.org/10.1186/s13567-020-00801-0

REHMANI, S. F., WAJID, A., BIBI, T., NAZIR, B., MUKHTAR, N., HUSSAIN, A., LONE, N. A., YAQUB, T., & AFONSO, C. L. (2015). Presence of Virulent Newcastle Disease Virus in Vaccinated Chickens in Farms in Pakistan. Journal of Clinical Microbiology, 53(5), 1715–1718. https://doi.org/10.1128/jcm.02818-14

ROBI, D. (2020). Newcastle disease and its different applicable control options in poultry in Ethiopia. International Journal of Agricultural Extension, 8. https://doi.org/10.33687/ijae.008.01.3010

SAHOO, N., BHUYAN, K., PANDA, B., BEHURA, N. C., BISWAL, S., SAMAL, L., CHAUDHARY, D., BANSAL, N., SINGH, R., JOSHI, V. G., JINDAL, N., MAHAJAN, N. K., MAAN, S., RAVISHANKAR, C., RAJASEKHAR, R., RADZIO-BASU, J., HERZOG, C. M., KAPUR, V., MOR, S. K., & GOYAL, S. M. (2022). Prevalence of Newcastle disease and associated risk factors in domestic chickens in the Indian state of Odisha. PLOS ONE, 17(2), e0264028. https://doi.org/10.1371/journal.pone.0264028

SHITTU, I., JOANNIS, T. M., ODAIBO, G. N., & OLALEYE, O. D. (2016). Newcastle disease in Nigeria: Epizootiology and current knowledge of circulating genotypes. VirusDisease, 27(4), 329–339. https://doi.org/10.1007/s13337-016-0344-6

SIME, A. (2022). Review on poultry production, processing, and Utilization in Ethiopia. International Journal of Agricultural Science and Food Technology, 8, 147–152. https://doi.org/10.17352/2455-815X.000156

SULTAN, H. A., ELFEIL, W. K., NOUR, A. A., TANTAWY, L., KAMEL, E. G., EED, E. M., EL ASKARY, A., & TALAAT, S. (2021). Efficacy of the Newcastle Disease Virus Genotype VII.1.1-Matched Vaccines in Commercial Broilers. Vaccines, 10(1), 29. https://doi.org/10.3390/vaccines10010029

TAFESSE, M., YIGEZU, Z., AYANA, I., & KUMSA, T. (2008). Livestock Resource Potentials: Constraints and Opportunities for Intervention by the Private Sector. http://localhost:8080/xmlui/handle/123456789/3015

WAJID, A., DIMITROV, K. M., WASIM, M., REHMANI, S. F., BASHARAT, A., BIBI, T., ARIF, S., YAQUB, T., TAYYAB, M., ABABNEH, M., SHARMA, P., MILLER, P. J., & AFONSO, C. L. (2017). Repeated isolation of virulent Newcastle disease viruses in poultry and captive non-poultry avian species in Pakistan from 2011 to 2016. Preventive Veterinary Medicine, 142, 1–6. https://doi.org/10.1016/j.prevetmed.2017.04.010

WANG, M., DU, D., SUN, Z., GENG, X., LIU, W., ZHANG, S., WANG, Y., PANG, W., & TIAN, K. (2024). Evaluation of the Immune Effect of a Triple Vaccine Composed of Fowl Adenovirus Serotype 4 Fibre-2 Recombinant Subunit, Inactivated Avian Influenza (H9N2) Vaccine and Newcastle Disease Vaccine Against Respective Pathogenic Virus Challenge in Chickens. Journal of Applied Poultry Research, 100410. https://doi.org/10.1016/j.japr.2024.100410

WOAH. (2022). Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2022. WOAH - World Organisation for Animal Health. https://www.woah.org/en/what-we-do/standards/codes-and-manuals/terrestrial-manual-online-access/

WORKU, T., DANDECHA, M., SHEGU, D., ALIY, A., & NEGESSU, D. (2022). Isolation and Molecular Detection of Newcastle Disease Virus from Field Outbreaks in Chickens in Central Ethiopia. Veterinary Medicine: Research and Reports, 13, 65–73. https://doi.org/10.2147/VMRR.S352727

XIAO, S., PALDURAI, A., NAYAK, B., SAMUEL, A., BHAROTO, E. E., PRAJITNO, T. Y., COLLINS, P. L., & SAMAL, S. K. (2012). Complete Genome Sequences of Newcastle Disease Virus Strains Circulating in Chicken Populations of Indonesia. Journal of Virology, 86(10), 5969–5970. https://doi.org/10.1128/JVI.00546-12