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Original Article

Seroprevalence of Newcastle Disease among Unvaccinated Village Chickens in Coimbatore District, Tamil Nadu, India

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Abstract *- Newcastle disease causes enormous economic losses to the poultry industry. Despite vaccination efforts, Newcastle Disease (ND) remains endemic in many developing countries, causing major economic losses and impacting food security. A serological survey for antibodies against Newcastle Disease virus (NDV) was conducted in 294 unvaccinated village chickens in Coimbatore District, Tamil Nadu, India. Sampling was conducted between March and August 2025. All chickens were confirmed to be unvaccinated based on interviews with the farmers and veterinary records. This study aimed to assess the seroprevalence of NDV antibodies in village chickens and identify the factors influencing their susceptibility to NDV infection. The haemagglutination inhibition (HI) test was used to detect NDV antibodies in the village chickens. The lowest seroprevalence was detected in Valparai at 32%, and the highest in Cambotore South at 62%. Only the Mettupalayam village chickens had a protective antibody titer above the recommended minimum protective antibody titer of ≥ 3(log2) against NDV. These research findings indicate continued NDV exposure among unvaccinated village chickens, underscoring the urgent need for vaccine deployment and improved poultry health management before the virus reaches large commercial farms. Strengthening active surveillance and laboratory diagnostic capacity will be crucial for sustainable NDV control in Coimbatore District. Although molecular confirmation was beyond the scope of the current study, future work should employ RT-PCR and sequencing of the F gene cleavage site to identify circulating NDV genotypes. A longitudinal seromonitoring study is also planned to assess antibody persistence and seasonal variation.*

Keywords *- Newcastle disease virus, Protective antibody, Seroprevalence, Unvaccinated village chicken, Zoonotic disease.*

1. Introduction

Despite vaccination efforts, Newcastle Disease (ND) remains endemic in many developing countries, causing major economic losses and impacting food security [1]. Newcastle Disease Virus (NDV) spreads quickly among free-range chickens. It is commonly transmitted through respiratory, contaminated feed, water, and faecal-oral routes. NDV is a zoonotic disease that sometimes affects humans, causing conjunctivitis and an influenza-like disease [2, 3, 4].

The NDV, which belongs to the Paramyxoviridae family as per the International Committee for the Taxonomy of Viruses (ICTV, has a non-segmented, enveloped, single-stranded, negative-sense RNA genome. Its genome is between 15,186 and 15,198 nucleotides long. The 3` end has an untranslated leader sequence and the 5` end has a trailer sequence and in between, it has genes that encodes six major structural proteins, such as 3′-nucleocapsid (NP), phosphoprotein (P), matrix (M), fusion (F), hemagglutinin-neuraminidase (HN), and large polymerase (L)-5′ [1] (Figure 1). The NP encapsidates the viral RNA genome to form a helical ribonucleoprotein (RNP) complex, which protects the viral genome from degradation. The phosphoprotein forms a functional polymerase complex of RNA-dependent RNA polymerase (L protein), which catalyses the replication and transcription, bridging the NP and L proteins as a cofactor.

The matrix protein resides beneath the viral envelope. It plays a major role in viral assembly, budding, regulating transcription, and replication. The surface fusion glycoprotein is one of the determinants of Virulence. It mediates the interaction between the viral and host cell membranes during the viral entry into the host cell. The Hemagglutinin-Neuraminidase (HN) is a multifunctional surface

glycoprotein, and one of its main functions is viral attachment to the host cell's sialic acid-containing receptors and final release by receptor cleavage. Both HN and F glycoproteins are major determinants of NDV virulence and infection. The cleavage position of the F glycoprotein determines the Virulence of NDV [5].

Indeed, the basis of NDV virulence classification is dependent on the cleavage of the F glycoprotein; avirulent strains have a mono-basic F protein cleavage site, which restricts it to a few specific sites, while virulent strains have a multi-basic F protein cleavage site, which can lead to severe systemic infection in many sites. Furthermore, NDV produces two structural proteins, V and W, through RNA editing of the P gene. These two proteins are used by the NDV to evade the host's innate immunity by interfering with the Interferon (IFN) pathway.

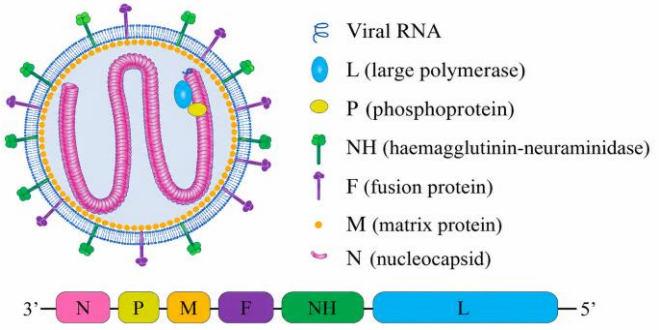

Fig. 1 Schematic diagram of Newcastle Disease Virus. Adapted from Kondakova *et al*., 2025 [43]

NDVs are genetically diverse, even though they all belong to a single serotype. There are two major recognized genotypes of NDV: I and II classes. Class II contains over 21 genotypes (I-XXI), with many other sub-genotypes. The major contributors to NDV genetic diversity are the HN and F genes, which play a major role in Virulence, tissue tropism, and pathogenicity. Changes in these genes create a continuous evolution hurdle in vaccine design and disease control. Newcastle disease spreads easily among free-range chickens because the chickens are in direct contact with the other birds and faecal matter, in which the NDV can reside.

Due to a lack of awareness, cost, and logistical constraints, most village backyard chickens are rarely vaccinated. Because of the free-range systems, these chickens serve as reservoirs for silent viral circulation, posing a serious threat to the poultry industry. High mortalities, ranging from 80 to 100 percent, caused by NDV have been reported in both village/backyard and commercial chicken (6, 7, 8). The village chicken farming plays a very important role in the livelihoods of the rural community as it provides meat, eggs, feathers, and manure as fertilizer for vegetable farming and income through the selling of chickens at the local markets (9, 10, 11). The best viable option to overcome ND in endemic developing countries is through vaccination [12, 13]. Because village chicken farmers are scattered around villages, and the chickens themselves are free range, it sometimes becomes very difficult to sensitize the farmers about the ND and vaccination [14]. It is very important that ND vaccines are heat-stable and do not require cold-chain adherence for easy administration [15, 16].

Recent studies have shown that heat-stable ND I2, I-2, V4-HR, Mukteswar strain (developed at Indian Veterinary Research Institute (IVRI) campus in Mukteswar, India) that can be administered through eye drop (intraocular) or intranasal, drinking water, coarse spray, and injection, is suitable for free-range village chickens [17, 18, 19, 20, 21]. The I_2 vaccine is currently being used in Mozambique and Nigeria [22], Malawi [23], Tanzania [22, 24], and many countries of South-East Asia [25]. The Mukteswar Strain is commonly used in India and Bangladesh [19, 26].

The main objective of this study was to determine the prevalence of NDV in village chickens in 11 villages of Coimbatore, Tamil Nadu, India. There have not been recent NDV serological surveys in Coimbatore District. Therefore, this study fills a key geographical gap by including these areas and comparing villages with distinct ecological and poultry management conditions. Recent studies in some states in India [26, 27, 28, 29, 30] highlight continued NDV circulation in village chickens.

2. Materials and Methods

2.1. Description of Study Sites

The study was conducted in Coimbatore South, Perur, Madukkarai, Sulur, Coimbatore North, Annur, Mettupalayam, Pollachi, Kinathukadavu, Valparai, and Anaimalai villages of Coimbatore, northwestern Tamil Nadu, India (Figure 2). Coimbatore district, which has had a municipality status since 1866, has its headquarters at Coimbatore and has been administered by Coimbatore Municipal Corporation since 1981. The capital city, Coimbatore, was established in 1804. Based on the 2011 population census, Coimbatore district had a population of 3,458,045, of which 1,050,721 resided in Coimbatore city.

The 2021 population census was not conducted due to COVID-19; thus, the current population is estimated at 3,159,000, of which 1,534,000 live in the city. The total area of Coimbatore district is 642.12 square kilometers and lies between Longitudes 11°1′6″N and Latitudes 76°58′21″E. Coimbatore lies 427 m above sea level. It is located on the banks of the Noyyal River in the southern boundary of the city. To the north and west of the city, there are the Western Ghats mountain range and the Nilgiri Biosphere Forest Reserve on the north side. The district has a tropical wet and dry climate, with the northern part rich in evergreen forest, while the eastern side is dry.

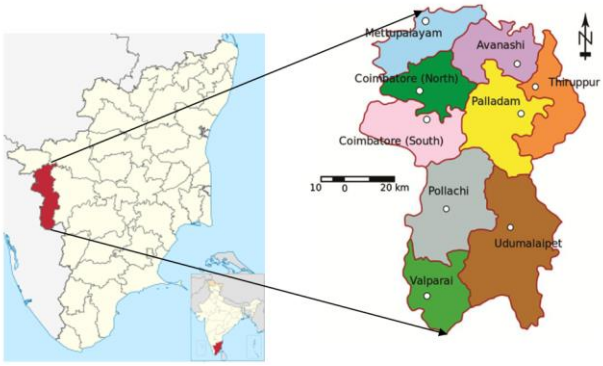

Fig. 2 Map of Coimbatore district of India where the studies were conducted [40]

2.2. Ethical Considerations

A serological survey for antibodies against Newcastle Disease Virus (NDV) involving village chickens was carried out following ethical standards. Participation of all farm owners was entirely voluntary. They were made fully aware of the purpose and procedures of the study before collecting blood samples from their chickens. Informed consent was obtained from each farm owner. The farm owners' identities were protected, all data, including biological samples, were kept confidential, and blood samples were collected humanely to minimize stress on the animals in accordance with Ethical Guidelines for Animal Welfare [31, 32].

2.3. Study Design

This study followed a cross-sectional design to determine the presence of antibodies against Newcastle Disease Virus (NDV) in selected villages of Coimbatore district, as described in the Description of Study Sites section. Villages were selected using stratified random sampling to ensure representation of different geographical zones within the district, poultry density, and accessibility, and sampling was proportional to village flock size. Based on the list of village chicken owners and the number of unvaccinated chickens they had, every fifth chicken on the list was selected so that the selection was completely unbiased and random. For serological analysis, the presence of NDV antibodies was determined from the chicken's blood samples.

2.4. Sample Size Determination

The sample size was determined using Cochran's formula, considering the desired confidence level (Z score for a 95% confidence interval), proportion (p), and margin of error (e) [33].

$$n_0 = \frac{Z^2.p.(1-p)}{E^2}$$

Where:

- n_0 = initial sample size
- Z = z-value (e.g., 1.96 for 95 % confidence),
- p = estimated population proportion,
- E = margin of error.

For this study, the following were assumed:

- Level of significance: 95% (Z = 1.96).
- Margin of error (d) = 0.05 (±5%).
- Estimated population proportion (p) = 0.257 was derived from the study of Ravishankar *et al*. [28], who found 25.7% NDV seroprevalence in Kerala, which borders Coimbatore. Their work showed that NDV seroprevalence varied among broilers, layers, and backyard birds. Their study did not cover Coimbatore villages.

Using the above parameters, a sample size of 294 was established. The village chickens were selected using a systematic random sampling technique.

2.5. Data Collection

Each backyard chicken farmer, using a structured questionnaire, was asked to provide information on bird age, sex, flock size, management system, history of vaccination, arrival of new birds, and contact with wild or neighboring poultry.

2.6. Sample Collection

A total of 294 unvaccinated village chickens aged above 4 weeks were sampled from 11 villages. Sampling was conducted between March and August 2025. Birds showing clinical signs of disease, other than those of NDV, were excluded to avoid confounding results. Blood samples were collected from 294 village chickens randomly selected from different flocks in Coimbatore South, Perur, Madukkarai, Sulur, Coimbatore North, Annur, Mettupalayam, Pollachi, Kinathukadavu, Valparai, and Anaimalai villages of Coimbatore. Using aseptic technique and new disposable needles and syringes, 2 ml of blood samples were withdrawn from the brachial veins of the village chickens. The blood sample was then transferred into a labelled sterile Falcon® 15 mL high-clarity polypropylene (PP) conical centrifuge tube. The samples were immediately stored at 4 °C in a portable fridge and transported to the laboratory. Upon arrival at the laboratory, the samples were removed from the portable fridge, and the tubes were placed in a slanted position against the wall to allow the blood to clot at room temperature for 10 minutes. The samples were then centrifuged at 2,500 revolutions per minute (rpm) for 10 minutes. The serum was then transferred into Eppendorf tubes and stored at −20 °C until further analysis.

2.7. Red Blood Cells for HA and HI Tests

Blood for Hemagglutination (HA) and Hemagglutination Inhibition (HI) tests was aseptically withdrawn from the village chickens and transferred into sterile Light Blue Top tubes, which contained 3.2% (0.109 M) buffered sodium citrate solutions (pH 7) to prevent blood clotting. The

anticoagulant solution was gently mixed with blood in the ratio of 1:9 ratio of sodium citrate solution to blood. Once the samples arrived in the laboratory, they were thoroughly washed 3 times in equal volume Phosphate-Buffered Saline (PBS), pH 7.2. The wash steps involved centrifugation at 2,500 rpm for 15 minutes, discarding the supernatant each time, and resuspending the cells in PBS buffer. After the final wash step, 0.1 ml of RBCs was suspended in 9.9 ml of PBS buffer. A 1% suspension of chicken red blood cells was used as an indicator in the HA and HI tests. The NDV-positive, negative control serum, and NDV-free chicken red blood cells were acquired from the Indian Veterinary Research Institute (IVRI) in Izatnagar, Bareilly, India, a reference laboratory for avian influenza, and used for the detection of antibodies against NDV in the serum samples. The standardization of HA titers of the NDV antigen followed the FAO protocol [34]. Briefly, the HA titer was determined to be 64 (i.e., 1 in 64 dilution). Thus, to obtain 4HA units, the HA titer was divided by 4, which gave a dilution factor of 16. The antigen was diluted in PBS.

2.8. Hemagglutination Inhibition Test

This test was used to detect and quantify antibodies against NDV according to the FAO guidelines [35]. This is achieved by the ability of the antibodies to inhibit the hemagglutination of the red blood cells. When antibodies in the serum neutralize NDV, they inhibit hemoagglutination of the red blood cells; this means the results are positive. For the HI assay, 25 µl of PBS was dispensed in each of the 96-well microtiter plates. Then, 50 µl of each of the serum samples was added to each well of column 1. A two-fold serial dilution of the serum was performed by transferring 25 µl from one well to the next, across the plate, except column 12, which had control wells. This was followed by the addition of 25 µl of the standardized 4 HA NDV antigen to all wells, except the control wells. After gentle agitation, the mixture was incubated for 30 minutes at room temperature. Finally, 50 µl of 1% RBC was added to all the wells. The mixture was again gently mixed and incubated at room temperature for 30 minutes. After the mixture had settled for 30 minutes, the plate was tilted to observe the pattern of the RBCs at the bottom of the wells. All sera were tested in duplicate, and results were accepted only when replicate titers agreed within one dilution. Positive and negative control sera obtained from the Indian Veterinary Research Institute (IVRI) in Izatnagar, Bareilly, were included in each run. The $\geq 3\log_2$ protective threshold was defined following FAO (2023) and OIE (2024) guidelines [36]. Confirmatory diagnosis of ND was made by the HI test based on the procedure of OIE (2024) [36]. The HI antibody titer for each of the 294 serum samples was determined and expressed in $\log_2$.

2.9. Data Analysis

Data was entered into a Microsoft Excel® 2021 spreadsheet to categorize and calculate mean titers. It was later transferred to the SPSS package version 31.00 (IBM Corp., Armonk, NY, USA). The seroprevalence of NDV among village chickens in Coimbatore district villages was determined as the proportion of positive samples among the total tested, with 95% Confidence Intervals (CIs) calculated using the binomial exact method. Univariate analysis was performed to identify potential risk factors associated with NDV seropositivity using the Chi-square test. Variables with $p < 0.20$ were entered into a multivariable logistic regression model to estimate adjusted odds ratios (aORs) and 95% CIs. A p-value ≤ 0.05 was considered statistically significant.

3. Results

3.1. Seroprevalence of Newcastle Disease Virus Antibodies among Unvaccinated Village Chickens

There was a total of 294 serum samples that were analysed for the presence of NDV antibodies in the 11 villages of Coimbatore District. Of these, 138 samples (46.9%) tested positive for NDV-specific antibodies based on the haemagglutination Inhibition (HI) test (≥1:8 titre). Seropositivity at the village level ranged from 32% to 62% (Table 1 and Figure 3). The presence of NDV antibodies in free-range, unvaccinated village chickens in all the villages clearly demonstrated widespread natural exposure to NDV.

Table 1. Seroprevalence of Newcastle Disease Virus Antibodies among Unvaccinated Village Chickens

Name of the Village	No. of Samples	No. of Positive Samples	% Seroprevalence
Coimbatore South	29	18	62
Perur	25	12	48
Madukkarai	30	16	53
Sulur	28	12	43
Coimbatore North	22	11	50
Annur	28	16	57
Mettupalayam	30	12	40
Pollachi	27	9	33
Kinathukadavu	25	15	60
Valparai	25	8	32
Anaimalai	25	9	36

The Chi-square test indicated no statistically significant difference ($p > 0.05$) in seroprevalence among the 11 villages ($\chi^2 = 12.35$, df=10, $p = 0.262$). This meant seroprevalence was relatively similar across the villages. Geometric Mean Titers (GMTs) ranged from 20 to 320, with the highest titers in Mettupalayam, Coimbatore North, and Valparai. Approximate 95% confidence intervals indicated overlapping ranges across most locations (e.g., Coimbatore South, 30–54; Mettupalayam, 240–430), suggesting no statistically significant variation in antibody levels among villages. Overall, all sites exhibited measurable HI antibody responses, indicating widespread seropositivity across the Coimbatore District. Antibody responses at or above 3 $\log_2$ (1:8) were detected in all locations, consistent with broad seropositivity (Table 2).

Table 2. Distribution of geometric mean titer of NDV antibodies among unvaccinated village chickens in Coimbatore District

Name of the Village	Average log_2 HI Titer	Geometric Mean Titer (GMT)	95% CI (approx.)
Coimbatore South	5.32	40	31.1 – 51.5
Perur	5.91	60	45.7 – 78.7
Madukkarai	5.32	40	31.2 – 51.2
Sulur	6.32	80	61.9 – 103.1
Coimbatore North	7.32	160	119.9 – 213.6
Annur	4.32	20	15.5 – 25.9
Mettupalayam	8.32	320	249.5 – 409.9
Pollachi	5.32	40	30.8 – 52.0
Kinathukadavu	6.32	80	61.0 – 104.9
Valparai	7.32	160	121.9 – 209.4
Anaimalai	5.32	40	30.5 – 52.5

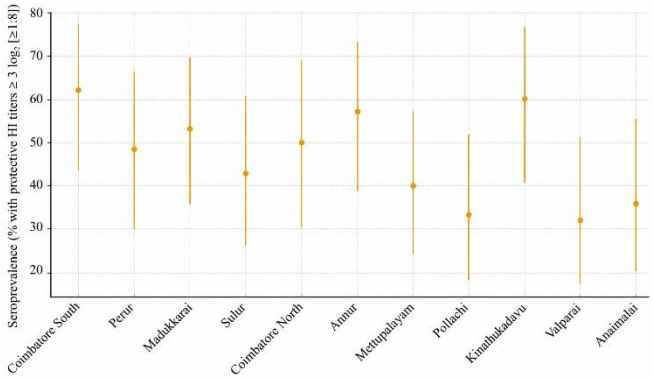

Fig. 3 Hemagglutination inhibition titer seroprevalence by village in Coimbatore District (protective titer threshold set at ≥3log2)

3.2. Management Practices on the Distribution of Newcastle Disease Virus Antibodies

The age of the unvaccinated village chickens had a profound impact on their seropositivity, with seropositivity significantly associated with age, with aOR = 2.35; 95% CI: 1.28–4.31; p = 0.006. Indeed, adult birds (>4 months) showed a higher prevalence (51.7%) compared to growers (34.2%). The gender of the unvaccinated chickens showed no significant association with seropositivity (p = 0.42).

4. Discussion

The backyard chickens tend to be rarely vaccinated based on veterinary records and the owners. This means that the NDV antibodies that were detected were from a virus that was circulating in the villages. The village chickens are highly exposed to the virus because they move freely in villages. Analysis of Table 1 showed that Valparai had a seroprevalence of 32%, which was the lowest, while Coimbatore South had the highest seroprevalence at 62%. In general, all village chickens showed the presence of NDV antibodies in their blood. This showed that the chickens were widely exposed to the virus. Indeed, previous studies by Chitradevi [37] showed that ND was the most prevalent disease (25.45%) among all the diseases that affected backyard chickens in Coimbatore district. The relatively high antibody prevalence among apparently healthy birds supports the hypothesis of endemic virus circulation and frequent subclinical infections, as observed in similar low-input systems elsewhere [38]. In Tirupur district of Tamil Nadu, again ND was the highest cause of mortality among unvaccinated village chickens, with seroprevalence of the virus's antibodies recorded at 27.59% [38]. In Tamil Nadu, the prevalence of ND is seasonally dependent, with the highest cases recorded during Summer (March to June) at 47.22%, followed by Rainy (July to November) at 38.89%, and Winter (December to February) at 13.89% [38]. This compares favourably with this study, which was conducted between March and August 2025, with the seroprevalence range of 32% to 62%. Flocks maintained under free-range scavenging systems tend to exhibit higher seroprevalence compared to semi-intensive systems, likely due to greater environmental exposure and contact with other birds. Similar patterns have been reported in studies from Ghana and Tanzania, where free-range management facilitated horizontal virus transmission [8]. The study conducted by Mramba *et al*. [14] showed that despite 80% of village chicken farmers being aware of ND, only 58% vaccinate the chickens against the disease [14]. This was mainly attributed to a lack of information on the available vaccines, their effectiveness, and also a strong belief in the use of herbal medicines to treat ND among the village chicken farmers [14]. Some farmers in Ghana associated ND with chemical poisoning due to a lack of knowledge of ND [8].

Analysis of Table 2 showed that only Mettupalayam village had a mean HI titer above the recommended minimum protective antibody titer of $\geq 3log_2$ [36], despite high percentages of seroprevalence of NDV antibodies in all the Coimbatore District villages. Annur had the lowest mean titer of 4.32. Some of the factors that have been attributed to low protective antibody titer against ND in free-range village chickens include antigenic mismatch, where the protection is reduced due to the use of a wrong vaccine strain that does not match the circulating village strain [39]. The chick's active immunity may not develop properly due to antibody interference from maternal passive immunity it receives from the mother through the egg. If the chick is vaccinated too early, the passive antibodies can neutralize NDV antigens. The village chickens may not develop their immunity properly due to immunosuppression and co-existing diseases such as infectious bursal disease (Gumboro), infectious anemia, Marek's disease, or mycotoxicosis. The free-range village chickens are prone to various diseases because they move a lot and feed on waste [39]. When chickens are housed in poorly ventilated rooms, overcrowded, exposed to extreme temperatures, rough transportation, and poor nutrition, they tend to be stressed and do not develop their immunity efficiently. Additionally, the immune response may vary with

genetics and age, where very old or very young chickens may have a reduced ability to mount a strong immune response [39].

The higher seroprevalence observed in Coimbatore South may be attributed to its ecological setting along the Noyyal river basin, proximity to the Western Ghats, and a mix of urban, wetland, and dry deciduous/shrub forest ecosystems, which facilitates greater poultry mixing during seasonal migrations and trade fairs. Coimbatore South features an extensive, interconnected network of lakes and canals fed by the Noyyal River basin. Major lakes in the southern part of the city include Valankulam, Ukkadam Periyakulam, and Singanallur Lake. These wetlands are crucial for groundwater recharge and serve as habitats for a variety of resident and migratory birds. In contrast, Valparai flocks are more geographically isolated. The ecology of Valparai is a unique and complex mosaic where high-biodiversity natural ecosystems coexist with human-modified landscapes, primarily extensive tea and coffee plantations. Located in the Anaimalai Hills of the Western Ghats, its ecology is defined by its montane Shola forest-grassland mosaics. High seroprevalence implies that these backyard chickens have become natural NDV reservoirs that can later silently transmit to commercial chickens; at the same time, it can also offer protective immunity against NDV. That is why some chickens appeared healthy despite high levels of circulating NDVs [41, 42].

5. Conclusion

These research findings indicate continued NDV exposure among unvaccinated village chickens, underscoring the urgent need for thermostable vaccine deployment and improved poultry health management. Strengthening active surveillance and laboratory diagnostic capacity will be crucial for sustainable NDV control in Coimbatore District.

Although molecular confirmation was beyond the scope of the current study, future work should employ RT-PCR and sequencing of the F gene cleavage site to identify circulating NDV genotypes. A longitudinal seromonitoring study is also planned to assess antibody persistence and seasonal variation.

Conflicts of Interest

The authors declared no conflict of interest.

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