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Molecular epidemiology of respiratory virus - Newcastle disease in backyard poultry

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Abstract

Newcastle Disease (ND) remains one of the most devastating viral infections affecting poultry worldwide, with backyard poultry serving as an important reservoir for virus maintenance and dissemination. The present study investigated the molecular epidemiology of respiratory Newcastle disease virus (NDV) infections in backyard poultry reared under different management systems in Namakkal district, Tamil Nadu, India, during June 2016 to April 2017. A total of 239 samples comprising tracheal swabs, faecal swabs, necropsy specimens, and pox lesions were collected from 117 backyard poultry farms. Samples were subjected to virus isolation in specific pathogen-free embryonated chicken eggs (SPF-ECE), followed by molecular detection using reverse transcription polymerase chain reaction (RT-PCR) targeting the fusion (*F*) gene of NDV. Fourteen field isolates of NDV were confirmed, yielding a positivity rate of 25.9% among investigated farms. Molecular detection revealed a specific 356 bp amplicon corresponding to the *F* gene, identical in size to the LaSota vaccine strain, confirming the circulation of NDV in backyard poultry populations. Epidemiological analysis indicated higher incidence in birds below 24 weeks of age and in unvaccinated, free-ranging flocks. The study highlights the critical role of backyard poultry in the epidemiology of NDV and emphasizes the need for structured vaccination and biosecurity measures to prevent spillover between backyard and commercial poultry systems.

Keywords: Newcastle disease virus, backyard poultry, molecular epidemiology, RT-PCR, fusion gene

1. Introduction

Backyard poultry production plays a vital role in sustaining rural livelihoods in India by providing nutritional security and supplementary income to smallholder farmers (Sonaiya, 2007; FAO, 2010) ^{[1], [6]}. However, the unorganized nature of backyard poultry rearing, coupled with minimal biosecurity and irregular vaccination practices, predisposes birds to various infectious diseases. Among these, Newcastle Disease (ND) is considered one of the most economically significant viral diseases affecting poultry, capable of causing high morbidity and mortality (Alexander, 2000; Saif *et al.*, 2008) ^{[2], [10]}.

Newcastle Disease Virus (NDV), also known as avian orthoavulavirus-1, belongs to the genus *Orthoavulavirus* within the family *Paramyxoviridae* (Alexander & Senne, 2008) ^[4]. NDV strains vary widely in virulence and are classified as lentogenic, mesogenic, or velogenic based on clinical severity and molecular characteristics. Respiratory involvement is a hallmark of ND, particularly in lentogenic and mesogenic strains, and is frequently observed in backyard poultry due to continuous exposure to environmental stressors and infected birds.

Backyard poultry often acts as a silent reservoir of NDV, facilitating virus persistence and transmission to commercial flocks (Biswas *et al.*, 2005; Hamilton-West *et al.*, 2012) ^{[5], [7]}. Conventional diagnostic methods based on clinical signs and postmortem lesions are insufficient for precise characterization of circulating NDV strains. Molecular techniques such as RT-PCR offer sensitive and specific detection and enable epidemiological tracking of NDV in field conditions. The present study was therefore undertaken to investigate the molecular epidemiology of respiratory NDV infections in backyard poultry in Namakkal district, Tamil Nadu.

2. Materials and Methods

2.1 Study area and period

The study was conducted in four taluks of Namakkal district, Tamil Nadu Tiruchengode, Rasipuram, Paramathivelur, and Namakkal between June 2016 and April 2017. The region is characterized by a high density of both commercial and backyard poultry populations.

2.2 Sampling strategy

A total of 117 backyard poultry farms rearing between 5 and 100 birds were surveyed. Farms were categorized into small (5-20 birds), medium (21-50 birds), and large (>50 birds) holdings. Epidemiological data including age, vaccination status, management practices, and proximity to commercial farms were recorded using a structured questionnaire.

2.3 Sample collection

A total of 239 samples were collected, comprising 110 tracheal swabs, 107 faecal swabs, 17 necropsy specimens, and 5 pox lesions from clinically affected and apparently healthy birds. Samples were transported to the laboratory in phosphate-buffered saline under cold chain conditions.

2.4 Virus isolation in SPF embryonated eggs

Processed samples were inoculated into the allantoic cavity of 9-11-day-old specific pathogen-free embryonated chicken eggs. Eggs were incubated at 37°C and candled daily for five

days. Embryos exhibiting characteristic ND lesions such as haemorrhages were harvested, and allantoic fluid was collected for further analysis.

2.5 RNA extraction and RT-PCR

Viral RNA was extracted from allantoic fluid using the TRIzol LS method. RT-PCR was performed using NDV-specific primers targeting the fusion (*F*) protein gene, yielding a 356 bp amplicon, a widely used molecular marker for NDV detection and characterization (Aldous & Alexander, 2001; Wise *et al.*, 2004) [3, 12]. The LaSota vaccine strain served as a positive control.

2.6 Statistical analysis

Incidence rates were calculated, and associations between NDV occurrence and epidemiological risk factors were assessed using chi-square analysis. A p-value <0.05 was considered statistically significant.

3. Results

3.1 Virus isolation and gross pathology

Out of the total samples processed, fourteen NDV field isolates were successfully propagated in SPF embryonated eggs. Embryos showed characteristic occipital and generalized haemorrhages consistent with NDV infection (Figure 1).

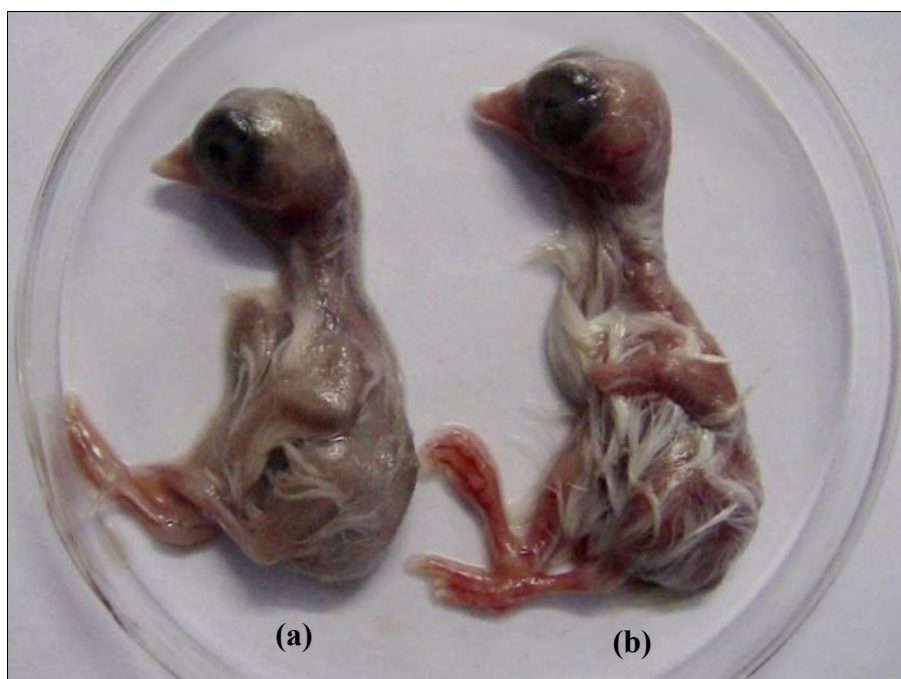


Fig 1: Chicken embryo showing gross pathological lesion of occipital haemorrhage for Newcastle disease. (a) Left- Normal chick of Non-infected embryo; (b) Right - Occipital haemorrhages of NDV infected embryo

3.2 Molecular detection of NDV

RT-PCR analysis confirmed NDV in all fourteen isolates, producing the expected 356 bp amplicon corresponding to the *F* gene. No amplification was observed in negative controls,

indicating high assay specificity (Figure 2). The molecular profile of field isolates was comparable in size to the LaSota vaccine strain.

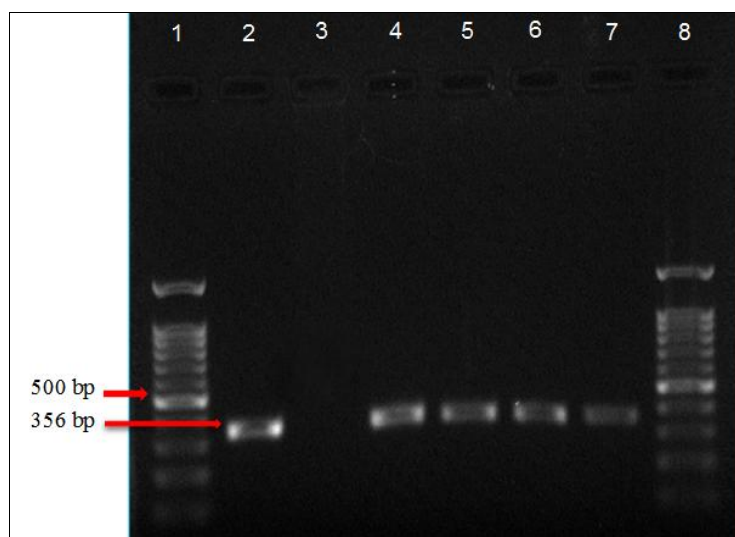


Fig 2: RT-PCR amplified products of Newcastle virus (LaSota strain) and field samples (allantoic fluid from NDV infected embryo) in 1.5 percent agarose gel showing bands at 356bp. Lane 1 and 8 DNA ladder 100bp; Lane 2 Positive control; Lane 3 Negative control; Lane 4, 5, 6 and 7 field samples

3.3 Incidence of NDV in backyard poultry

NDV was detected in 14 out of 54 farms investigated for ND (Table 1), with an overall incidence of 25.9%, which is comparable with earlier reports from backyard poultry systems in Asia and India (Biswas *et al.*, 2005; Roy *et al.*, 2005) [5, 9]. Higher incidence was observed in birds below 24

weeks of age (40%) compared to older birds (12.9%), showing a statistically significant association ($p < 0.05$), which corroborates previous findings that younger birds are more susceptible to NDV infection (McFerran & McCracken, 1988; Saif *et al.*, 2008) [8, 10].

Table 1: Assessing the incidence of respiratory viral pathogens in backyard poultry by conventional method and PCR during study period in Namakkal district

Virus	Number of farms	Conventional method		Polymerase chain reaction		Percentage
		No. positive	No. negative	No. positive	No. negative	
NDV	54	14	40	14	-	25.9%

3.4 Risk factor analysis

NDV incidence was higher in unvaccinated flocks and in free-ranging management systems. Farms located within 1 km of

commercial poultry units showed a slightly higher prevalence, although the difference was not statistically significant (Table 2).

Table 2: Risk factors influencing the respiratory viral pathogens in backyard poultry in Namakkal district between June, 2016 and April, 2017

Location of nearby farms	<1 Km	Positive percentage	>1 Km	Positive percentage	p- value
NDV	4	30.76	10	24.39	0.721
Age factor	<24 Weeks	Positive percentage	>24 Weeks	Positive percentage	
NDV	10	40	4	12.9	0.025
Vaccine status	Yes	Positive percentage	No	Positive percentage	
NDV	5	21.73	9	29.03	0.754
Management system	Free ranging	Positive percentage	Semi-intensive	Positive percentage	
NDV	9	24.32	5	29.41	0.747

*Non-significant as per the calculated P value ($p > 0.05$)

4. Discussion

The present study provides molecular evidence of NDV circulation in backyard poultry in Namakkal district. The detection of NDV in apparently healthy birds underscores the role of backyard poultry as reservoirs facilitating virus persistence. Higher susceptibility in younger birds may be attributed to immature immune responses and lack of vaccination.

Molecular detection targeting the F gene remains a reliable approach for NDV identification, as demonstrated by consistent amplification of the 356 bp product, as also reported by Aldous and Alexander (2001) [3] and Wise *et al.* (2004) [12]. The similarity in amplicon size between field isolates and vaccine strains highlights the need for further

sequencing studies to determine virulence and genotype distribution.

The absence of a structured vaccination schedule and low biosecurity practices in backyard systems significantly contribute to NDV endemicity. Regular vaccination, farmer awareness, and integration of backyard poultry into regional disease surveillance programs are essential to mitigate ND outbreaks.

5. Conclusion

This study confirms the endemic presence of respiratory Newcastle disease virus in backyard poultry through molecular epidemiological investigation. Backyard poultry populations serve as important reservoirs for NDV, posing a

continuous risk to commercial poultry. Implementation of regular vaccination programs, improved management practices, and molecular surveillance is imperative for effective control of ND in mixed poultry production systems (Alexander, 2001; OIE, 2009) [3, 13].

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