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Molecular epidemiological insights into respiratory fowl pox virus circulation in backyard poultry of Tamil Nadu, India

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Abstract

Fowl pox is a contagious viral disease of poultry caused by avipox virus and remains an important constraint in both organized and backyard poultry production systems. The diphtheritic or respiratory form of the disease is associated with respiratory distress, reduced productivity, and occasional mortality. Backyard poultry, typically maintained under free-range conditions with limited biosecurity, may function as reservoirs for Fowl Pox Virus (FPV), enabling virus persistence and transmission. The present investigation evaluated the molecular epidemiology of respiratory fowl pox virus in backyard poultry farms of Namakkal district, Tamil Nadu, during the period from June 2016 to April 2017. A total of 239 samples were collected from 117 backyard poultry farms and subjected to virus isolation in specific pathogen-free embryonated eggs, followed by molecular confirmation using PCR targeting the P4b gene. FPV was detected in 21 samples originating from 10 farms, indicating a farm-level positivity of 62.5% among suspected flocks. A higher occurrence was observed in birds below 24 weeks of age, unvaccinated flocks, and birds maintained under free-range systems. The findings demonstrate active circulation of respiratory FPV in backyard poultry and underscore the necessity for molecular surveillance and strengthened preventive measures.

Keywords: Fowl pox virus, respiratory pox, molecular epidemiology, backyard poultry, PCR, P4b gene

1. Introduction

Backyard poultry farming plays a vital role in rural economies by contributing to household income, nutritional security, and livelihood sustainability. In India, indigenous poultry are commonly reared under scavenging or semi-intensive systems, often without structured vaccination schedules or biosecurity practices. Such production systems increase vulnerability to infectious diseases and facilitate the maintenance of pathogens that may threaten commercial poultry enterprises (Sonaiya, 2007; Biswas *et al.*, 2005) [3, 1].

Fowl pox is a viral disease caused by Fowl Pox Virus (FPV), a member of the genus Avipox virus under the family Poxviridae. The disease manifests primarily in two clinical forms: the cutaneous form and the diphtheritic or respiratory form. The respiratory form is characterized by proliferative lesions involving the oral cavity, pharynx, larynx, and trachea, frequently resulting in respiratory distress and compromised performance (Tripathy & Reed, 2020) [5].

FPV is transmitted through direct contact, contaminated materials, and mechanical vectors such as mosquitoes and other biting insects. Backyard poultry, due to free-range management and increased vector exposure, are particularly susceptible to infection. Despite the endemic nature of the disease, limited molecular epidemiological information is available on respiratory fowl pox virus in backyard poultry populations. Polymerase Chain Reaction (PCR) assays targeting conserved genes such as P4b have emerged as reliable tools for sensitive and specific detection of FPV. This study was therefore undertaken to investigate the molecular epidemiology of respiratory fowl pox virus in backyard poultry of Namakkal district and to identify associated risk factors.

2. Materials and Methods

2.1 Study area and duration

The study was carried out in four taluks of Namakkal district, Tamil Nadu, India, over a period extending from June 2016 to April 2017. The area is characterized by dense poultry activity, including both backyard and commercial poultry farming.

2.2 Study population and sampling

A total of 117 backyard poultry farms rearing desi birds were included. Farms were classified based on flock size as small (5-20 birds), medium (21-50 birds), and large (≥ 51 birds). Epidemiological information pertaining to age, vaccination status, management practices, and proximity to commercial farms was collected using a structured questionnaire.

Altogether, 239 samples were obtained, including tracheal swabs ($n = 110$), faecal swabs ($n = 107$), necropsy tissues ($n = 17$), and pox lesion scabs ($n = 5$), from both clinically affected and apparently healthy birds. Samples were transported to the laboratory in chilled conditions using phosphate-buffered saline.

2.3 Virus isolation

Homogenates prepared from respiratory and lymphoid tissues were inoculated onto the Chorioallantoic Membrane (CAM) of 9-11-day-old specific pathogen-free embryonated chicken eggs. Inoculated eggs were incubated at 37°C and examined daily. CAMs exhibiting typical pock lesions were harvested for molecular analysis.

2.4 DNA extraction and PCR reaction mixture

Viral DNA was extracted from CAM tissues and pox scab samples using the QIAamp® DNA Mini Kit according to the manufacturer's instructions then DNA was prepared for PCR reaction (Table 1 & 1a). PCR amplification was performed using FPV-specific primers targeting the P4b gene (Table 2), yielding an expected product size of approximately 578 bp. The BM strain fowl pox vaccine was used as a positive control.

Table 1: Composition of PCR reaction mixture for DNA viruses

Components	Quantity
DNA template	5µl
Master Mix	10µl
Forward primer	1µl
Reverse primer	1µl
Nuclease free water	7µl
Total volume	25µl

Table 1a: Composition of master mix for DNA viruses

Composition of master Mix	TaqDNA polymerase	dATP	dGTP	dCTP	dTTP	MgCl ₂
Volume	50 units/ml	400µM	400µM	400µM	400µM	3mM

Table 2: Primers for fowl pox virus targeting P4b gene

Disease	Target gene	Sequences		Product Size
FPV	P4b	Forward	5'-CAGCAGGTGCTAAACAACAA-3'	578bp
		Reverse	5'-CGGTAGCTTAACGCCGAATA-3'	

2.5 PCR steps for FPV

Slight alterations were made to the steps were illustrated by

Ebrahimi *et al.* (2012). However, time and temperature was slightly changed for denaturation and annealing.

	Steps	Temperature	Time
Step 1	Initial denaturation	95°C	3 min
Step 2	Denaturation	94°C	45 sec
Step 3	Annealing	52°C	45 sec
Step 4	Extension	72°C	45 sec
	No. of cycles	Step 2 to 4 repeated for 35 cycles	
Step 5	Final extension	72°C	5 min
Step 6	Holding temperature 4°C		

2.6 Submarine gel electrophoresis

The electrophoresis tank was filled with 1X Tris acetate buffer until the gel tray was completely submerged. A volume of 5 µl of RT-PCR product was mixed with gel loading buffer and loaded into the wells along with the PCR-amplified samples, positive control (reference vaccine strain), negative control, and a 100 bp DNA ladder. After loading, the lid of the electrophoresis unit was closed and connected to the electrical leads. DNA migration occurred towards the positive anode (red lead). Electrophoresis was performed at 80 V for 45 minutes.

2.7 Visualization of PCR products

Following electrophoresis, the gel was visualized under a UV transilluminator. The amplified products were identified by comparing the band sizes with the 100 bp DNA ladder. Samples were considered positive only when NDV, IBV, FPV, and ILT amplicons produced bands corresponding to 356, 410, 580, and 588 bp, respectively, running parallel to the expected DNA fragments on the gel, with no amplification observed in the negative control. Gel images were documented using a gel documentation system (Bio-Rad, Germany).

2.5 Statistical analysis

Farm-level incidence was calculated, and associations between FPV occurrence and risk factors such as age, vaccination status, management system, and proximity to commercial farms were evaluated using chi-square analysis. Statistical significance was determined at $p < 0.05$.

3. Results

3.1 Pathological findings and virus isolation

Necropsy of affected birds revealed diphtheritic lesions involving the upper respiratory tract, with accumulation of exudates in the trachea in several cases. Following inoculation into SPF embryonated eggs, suspected samples produced characteristic pock lesions on the Chorio-Allontoic Membrane (CAM), suggestive of fowl pox virus infection. (Figure 1 & 2)



Fig 1: 8 weeks old desi chicken showing nodular lesions on comb and eyelids

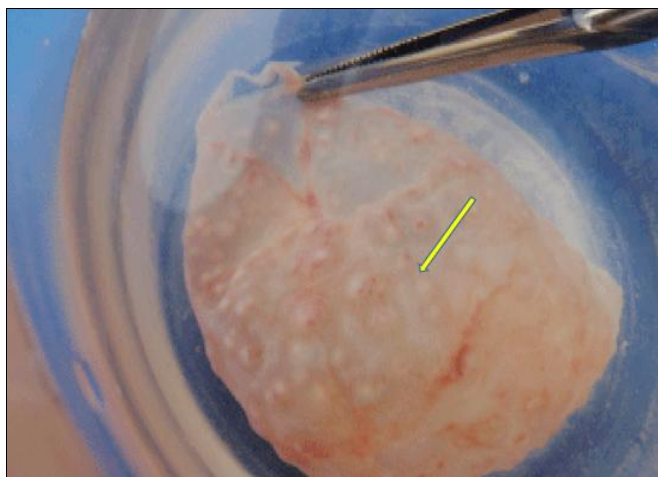


Fig 2: CAM of fowl pox virus infected embryo showing pock lesions, thickening and cloudy appearance

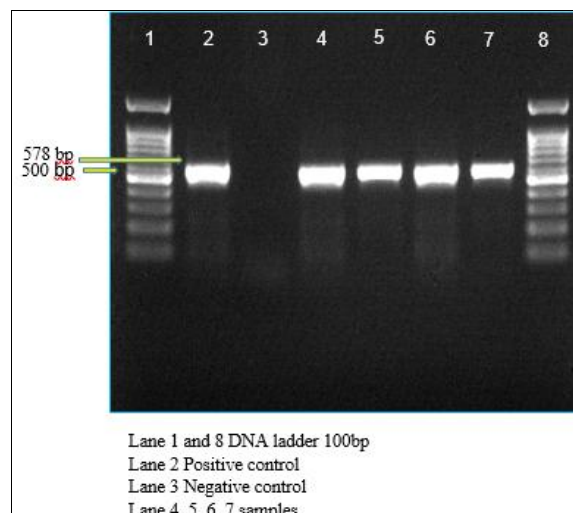


Fig 3: PCR amplified products of fowl pox virus (BM strain) and field samples (from pox cutaneous lesion and CAM of FPV infected embryo) in 1.5 percent agarose gel showing bands at 578 bp

3.2 Molecular detection of fowl pox virus

Among 40 samples collected from 16 clinically suspected farms, 21 samples representing 10 farms were confirmed positive for FPV by PCR targeting the P4b gene at 578bp. All positive samples yielded the expected amplicon size, confirming FPV infection. The vaccine strain used as control also showed specific amplification (Figure 3).

3.3 Incidence of FPV

The overall farm-level positivity for fowl pox among suspected farms was 62.5%. FPV DNA was detected in both clinically affected birds and apparently healthy birds, indicating the presence of subclinical infections within backyard poultry populations (Table 1)

Table 1: Assessing the incidence of fowl pox virus in backyard poultry by conventional method and PCR during study period in Namakkal district

Categories	Number of farms	Conventional method		Polymerase chain reaction		Percentage
		No. of positive	No. of negative	No. of positive	No. of negative	
FPV	16	10	6	10	-	62.5%

3.4 Analysis of risk factors

A higher frequency of FPV detection was recorded in birds below 24 weeks of age compared to older birds. Unvaccinated flocks exhibited greater positivity than vaccinated flocks. Birds maintained under free-range systems showed

significantly higher FPV incidence than those reared under semi-intensive conditions ($p < 0.05$). No statistically significant association was observed between FPV occurrence and proximity to commercial poultry farms (Table 2).

Table 2: Risk factors influencing the fowl pox virus 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
FPV	4	50	6	75	0.608
Age factor	<24 Weeks	Positive percentage	>24 weeks	Positive percentage	
FPV	6	75	4	50	0.362
Vaccine status	Yes	Positive percentage	No	Positive percentage	
FPV	1	25	9	75	0.111
Management system	Free ranging	Positive percentage	Semi-intensive	Positive percentage	
FPV	8	88.88	2	28.57	0.034

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

4. Discussion

The findings of the present study demonstrate the endemic presence of respiratory fowl pox virus in backyard poultry farms of Namakkal district. PCR-based detection targeting the P4b gene proved effective in identifying FPV infections, including subclinical cases that may otherwise go unnoticed. The increased incidence observed in younger birds could be attributed to underdeveloped immune responses and heightened susceptibility to infection. Higher detection rates in unvaccinated flocks highlight the absence of systematic vaccination programs in backyard poultry systems. Free-range birds are more frequently exposed to insect vectors and contaminated environments, which likely explains the

significantly higher incidence recorded under this management system.

The lack of a significant association with proximity to commercial farms suggests that FPV transmission in backyard poultry is predominantly influenced by local ecological factors and vector activity rather than direct transmission from commercial flocks. Comparable observations have been reported in other endemic settings where vector-mediated spread plays a major role (Bolte *et al.*, 1999; Tripathy, 1993)^[2, 4]. Detection of FPV in apparently healthy birds further emphasizes the role of backyard poultry as silent carriers, facilitating viral persistence and sporadic outbreaks.

5. Conclusion

This study provides molecular confirmation of respiratory fowl pox virus circulation in backyard poultry of Tamil Nadu. The higher occurrence among young, unvaccinated birds maintained under free-range conditions underscores the need for enhanced surveillance, vaccination awareness, and vector control measures. Integration of molecular diagnostic tools into routine monitoring programs is essential to minimize the impact of fowl pox and to prevent wider dissemination of the virus.

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