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Molecular insights into infectious laryngotracheitis virus in backyard poultry

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Abstract

Infectious Laryngotracheitis (ILT) is an economically important respiratory disease of chickens caused by Infectious Laryngotracheitis Virus (ILTV), a member of the subfamily *Alphaherpesvirinae*. Backyard poultry production systems, which are commonly characterized by free-range management, inadequate biosecurity, and absence of systematic vaccination, may contribute to the maintenance and dissemination of ILTV. These birds can harbor the virus subclinically and serve as epidemiological links between village flocks and commercial poultry operations. The present study aimed to investigate the molecular epidemiology of ILTV in backyard poultry through PCR-based detection and genetic characterization. Clinical and subclinical backyard chickens were screened for ILTV using molecular techniques targeting conserved viral genes. Detection of ILTV DNA in backyard poultry highlights their role in viral persistence and underlines the importance of molecular surveillance, enhanced biosecurity, and strategic disease control measures to mitigate transmission risks to commercial poultry.

Keywords: Infectious laryngotracheitis, backyard poultry, molecular detection, PCR, herpesvirus

Introduction

Backyard poultry farming is an integral component of rural livelihoods, contributing to household income, food security, and nutritional well-being, particularly in low- and middle-income countries (Alexander *et al.*, 1998; Bagust, & Johnson, 1995) ^[1, 2]. Despite these benefits, backyard poultry systems often operate with minimal disease prevention strategies, limited veterinary support, and poor biosecurity, rendering them susceptible to infectious diseases and facilitating pathogen transmission across poultry sectors (Sonaiya, 2007; Hamilton-West *et al.*, 2012) ^[13, 8].

Infectious Laryngotracheitis (ILT) is a contagious viral disease of chickens that primarily affects the upper respiratory tract and is associated with considerable economic losses (Biswas *et al.*, 2005) ^[4]. Clinical manifestations include respiratory distress, gasping, conjunctivitis, coughing, and expectoration of bloody mucus, with disease severity varying depending on host immunity and viral virulence (Bagust *et al.*, 2000) ^[3]. While ILT has been extensively studied in commercial poultry, information on its occurrence and epidemiological significance in backyard poultry remains limited (Callison *et al.*, 2007) ^[5]. The increasing application of molecular tools has improved the detection of ILTV and facilitated differentiation between field strains and vaccine-related variants, thereby enhancing understanding of viral transmission dynamics (Coppo *et al.*, 2013; Kirkpatrick *et al.*, 2006) ^[6, 11].

2. Materials and Methods

2.1 Study design and sampling strategy

A molecular epidemiological investigation was conducted to detect infectious laryngotracheitis virus in backyard poultry populations. Birds reared under free-range and semi-intensive systems were included, particularly from areas located near commercial poultry farms or with a reported history of respiratory illness.

2.2 Sample collection

Samples including tracheal swabs, conjunctival swabs, and respiratory tissues (trachea and larynx) were collected aseptically from both clinically affected and apparently healthy backyard chickens. Collected samples were transported to the laboratory in virus transport medium under refrigerated conditions and stored at -80°C until further processing.

2.3 Extraction of viral DNA

Positive control of ILT virus was obtained from PDDSL, Namakkal and Viral DNA was isolated from clinical samples using a commercial DNA extraction kit (QIAmp® DNA Mini Kit obtained from Qiagen, Germany) in accordance with the manufacturer's guidelines (Tab. 1 & 2). DNA quality and concentration were assessed spectrophotometrically (nano drop method), and extracted DNA was preserved at -20°C for molecular analysis.

Table 1: Composition of PCR reaction mixture for DNA viruses

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

Table 2: Composition of master mix for DNA viruses

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

2.4 Polymerase chain reaction

PCR assays were performed using forward and reverse primers targeting conserved ILTV genes such as p32 (Tab. 3). Amplification reactions were carried out in a thermal cycler under optimized cycling conditions (Tab. 4).

Table 3: Oligonucleotide primers used for PCR

Disease	Target gene	Sequences		Product size
ILT	P32	Forward	5'- CTACGTGCTGGGCTCTAATC- 3'	588bp
		Reverse	5'- AAACCTCTCGGGTGGCTACTG-3'	

Table 4: For extraction of ILT virus following steps were followed as per Jorge and Antonio (2009)

	Steps	Temperature	Time
Step 1	Initial denaturation	95 $^{\circ}\text{C}$	3 min
Step 2	Denaturation	94 $^{\circ}\text{C}$	1 min
Step 3	Annealing	61 $^{\circ}\text{C}$	1 min
Step 4	Extension	72 $^{\circ}\text{C}$	1 min
	No. of cycles	Step 2 to 4 repeated for 35 cycles	
Step 5	Final extension	72 $^{\circ}\text{C}$	10 min
Step 6	Holding temperature 4 $^{\circ}\text{C}$		

2.5 Submarine gel electrophoresis

Tris acetate buffer (1X) was added in the electrophoresis tank to submerge the gel tray. A volume of 5 μl PCR product are added after mixing with gel loading buffer, PCR amplified product, negative control and positive control (reference vaccine strain) and 100 bp DNA ladder was also loaded into

the wells. The lid of the electrophoresis tank was closed and attached to the electrical leads. DNA would migrate towards the positive anode (red lead). The electrophoresis was carried out at 80 volts for 45 min.

2.6 Visualization of PCR products

The gel was visualized under UV transilluminator and bands of appropriate size were identified by comparison with the 100 bp ladder. The sample was scored as positive only if ILT runs parallel to 588 bp DNA fragment of expected size was identified on the gel and no signal was obtained with negative control. The images were documented using gel documentation system (Bio-Rad, Germany).

3. Results

Molecular screening of backyard poultry samples using PCR assays targeting conserved ILTV genes p32 yielded no detectable ILTV DNA in the analyzed specimens (Fig. 1). All tested tracheal swabs, conjunctival swabs, and respiratory tissue samples from both clinically suspected and apparently healthy birds were negative for ILTV amplification.

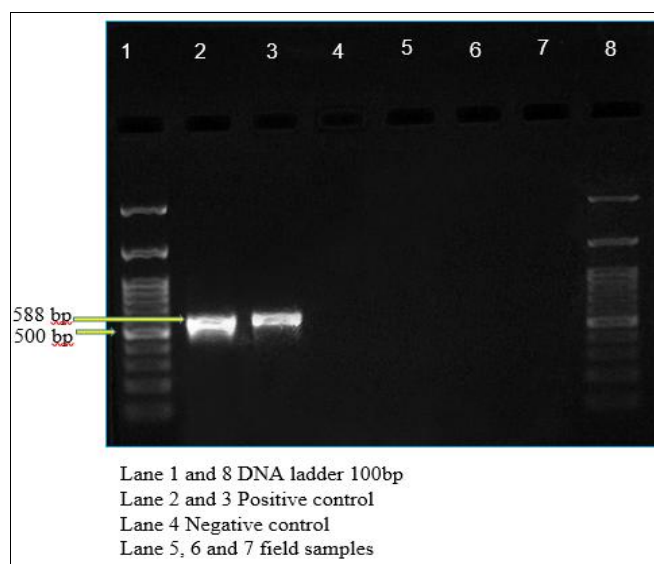


Fig 1: PCR amplified products of infectious laryngotracheitis virus (positive sample obtained from PDDSL) in 1.5 percent agarose gel showing bands at 588 bp

Internal controls confirmed the integrity of DNA extraction and PCR reactions, indicating that the negative results were not due to technical failure or poor sample quality. These findings suggest the absence of active or detectable latent ILTV infection in the sampled backyard poultry populations at the time of investigation.

4. Discussion

The absence of ILTV DNA in backyard poultry samples indicates that infectious laryngotracheitis virus was not circulating at a detectable level within the investigated flocks during the study period. This finding contrasts with reports from other regions where backyard poultry have been identified as reservoirs or carriers of ILTV, including subclinical infections (Hughes *et al.*, 1991; Bagust & Johnson, 1995) [9, 2]. The discrepancy may be attributed to differences in geographical location, poultry density, proximity to commercial farms, and disease control practices. Several factors may explain the negative molecular findings. First, ILTV distribution is often sporadic and outbreak-driven, with viral detection closely associated with active disease

events. Sampling conducted outside outbreak periods may therefore yield negative results, particularly given the intermittent nature of viral shedding during latent infections (Davison *et al.*, 2009) [7]. Second, limited interaction between backyard flocks and commercial poultry in the study area may have reduced the likelihood of viral introduction.

Additionally, environmental conditions flock size, and bird movement patterns may influence ILTV transmission dynamics. Backyard systems with lower stocking densities and reduced bird-to-bird contact compared to intensive commercial operations may inherently limit sustained viral circulation. Similar observations have been reported in backyard poultry surveys where major viral respiratory pathogens were absent despite minimal biosecurity (Hamilton-West *et al.*, 2012) [8].

While PCR is a highly sensitive diagnostic tool, its ability to detect ILTV depends on viral load and tissue tropism at the time of sampling. Latently infected birds may harbor the virus in neural tissues with minimal viral replication, rendering detection in respiratory samples challenging. Therefore, the negative findings should not be interpreted as definitive evidence of ILTV absence but rather as an indication of low prevalence or absence of active infection during the study period.

Importantly, the present results provide baseline molecular data that can support future surveillance efforts. Continuous monitoring remains essential, as changes in poultry management, introduction of vaccinated birds, or increased interaction with commercial flocks could alter the epidemiological landscape and facilitate ILTV emergence.

5. Conclusion

This study attempted to demonstrate the molecular presence of infectious laryngotracheitis virus in backyard poultry and emphasizes their epidemiological significance in maintaining and disseminating the virus. Molecular epidemiological approaches provide critical insights into ILTV circulation and genetic relationships among strains. Strengthening molecular surveillance, promoting biosecurity awareness among backyard poultry owners, and implementing appropriate preventive strategies are essential to limit viral spread and protect commercial poultry enterprises.

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