Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN:2410-8863
E-ISSN:2958-6178
Vol. 1, No. 4, December 2023

Molecular detection of IBV from backyard chickens in Diyala province

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Abstract

Globally, chickens are infected with the economically significant viral disease known as infectious bronchitis virus (IBV). Finding endemic IBV strains helps reduce production losses and improves control of these illnesses. Nine backyard flocks in Diyala were used to collect samples from 90 birds using Oropharyngeal and cloacal swabs. Using part-S1 gene primers for IBV, RT-PCR was performed on swabs from each sample. Nine samples of chicken were positive for IBV, with an incidence rate of 9.9% overall and 1.1% in each investigated location. These positive samples were genotyped, and they shared similarities with M41 and 1494/06, similar to IBV. Identified viruses, especially mutant IBV strains, may still be a threat to backyard and commercial chickens even though no disease was evident at the time of sampling in poultry in Diyala province.

Keywords: IBV, Swabs, Diyala, PCR, Backyard chickens.

Introduction:

Infectious bronchitis IB, an acute viral disease that is highly contagious. Infectious bronchitis virus (IBV), RNA coronavirus, is the cause. The virus typically causes respiratory illnesses, but it has also been demonstrated to infect non-respiratory organs such the kidney and genitalia (1; 2). Although the virus may be isolated from feces, there is evidence that it replicates in the colon and other digestive system organs (3; 4).

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New genotypes may appear as a result of genetic point mutation processes (such as deletion, insertion, and recombination) (5;6). Although the first 400 bp of the S1 gene has been shown to be highly changeable, most recombination events take place in a separate 600–700 bp region of the S1 gene (7). IBV must be continuously monitored in order to identify novel varieties, especially in endemic locations. The spike S1 glycoprotein is important for virulence because it is involved in adhesion to host cell receptors and has the necessary epitopes for antibody neutralization. Moreover, it may occasionally be involved in the fusion of the viral envelope with the host cell and cell-to-cell combination (8; 9). The three hyper variable regions (HVR) that make up IBV S1 are HVR 1, HVR 2, and HVR 3(5; 10). The locations of the three HVRs are amino acids 38–67, 91–141, and 274–387, in that order. These explanations explain why S1 changes and IBV antigenic evolution are correlated (11). There is no denying that low vaccine cross-protection resulting from serotype specificity and antigenic changes across IBV serotypes make vaccination a potentially problematic means of controlling IBV (12). Therefore, current epidemiology data are required in order to identify any novel pathogenic variants that might be in circulation(13). It has previously been shown that genetic evolution can be identified and the epidemiological assessment of IBV strains can be accomplished using nucleotide sequencing and phylogenetic analysis of whole or partial S1 gene sequences (13; 14).

Recent research has identified and genotyped IBV strains based on the S1 subunit in Iraq and the Middle East

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(15). Findings show that 4/91 is in circulation, along with Sul/01/09 and three other closely similar variations of IBV isolates in Israel (IS/720/99 and IS/885) and Egypt (Egypt/Beni-seuf/01) (16; 17). It is commonly recognized that renal and respiratory infections in Middle Eastern flocks are primarily caused by IS/885/00 and IS/1494/06 (18; 19; 20). Following sample collection, with FTA cards, which were previously negative utilizing cloaca and trachea swabs, recent study from Saudi Arabia has identified distinct strains. IBV/CHICKEN/KSA/101/2010 and IBV/CHICKENS/KSA/102/2010 were the isolates' genotypes, and 21.95% of the samples tested positive when using Flinders Technology Associates (FTA) cards (21).

IBV has previously been identified using RT-PCR analysis of serum samples collected from Duhok in northern Iraq (22). Additionally, the prevalence of IBV and other respiratory illnesses such AIV and Mycoplasma gallisepticum (MG) was examined in a recent study. Using quantitative polymerase chain reactions, the results showed that the prevalence of IBV alone was 20% (qPCR) (23). We will apply partial-S1 detection and sequencing to identify the circulating IBV genotypes within backyard chickens in Diyala province.

Martials and methods

Sampling methodology:

Samples were taken from backyard hens in the Diyala province (Kanaan, Kanaan 2, Buhriz, Baladroz, Budaa, Joba, Alshik temim, Shumer, Faris) of Iraq. All information entered will remain anonymous. Nevertheless, this might be prolonged based on the overall quantity of positive samples. Swabs from the oropharynx and cloaca of 90 backyard

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flocks in all of Diyala's governorates and regions were obtained. Throughout the whole investigation, sterile cotton wool swabs from Medical Wire and Equipment Co. Ltd. were utilized. Each bird's sample was taken aseptically, and it was then put in marked bijou tubes. The laboratory received these samples in a safe manner, and the samples were processed right away. Each set of swabs (OP or CL) was dipped in 1.5 ml of Tracheal organ culture (TOC) media and pooled as a single sample. The QIAamp viral RNA small kit was used to extract RNA from swab samples in accordance with the manufacturer's instructions.

RT-PCR and primers:

The incomplete S1 gene of IBV strains will be found with RT-PCR (24). Sequence with an IBV positive as previously reported (25) After purifying the PCR-positive isolates with 0.99 µl shrimp alkaline phosphatase (SAP) and 0.15 µl Exonuclease 1 (EXO) at 37 °C for 30 minutes, and then 10 minutes at 80 °C, they will be submitted for commercial bi-directional sequencing using the forward and reverse primers (SX3+ and SX4-, respectively).

Table 1. Oligonucleotide primers used in the detection of IBV as previously described (21).

Primer Name	Sequence(5′-3`)	Product

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		Size (bp)
SX1F SX2R	5'-TCCACCTCTATAAACACCYTTAC - 3′ 5'-TCCACCTCTATAAACACCYTTAC - 3′	393bp
SX3F SX4R	5'-TAATACTGGYAATTTTTCAGATGG - 3′ 5'-AATACAGATTGCTTACAACCACC - 3′	393bp

Partial sequencing and phylogenetic analysis:

To guarantee good read data, ChromasPRO (Technelysium Pty Ltd, Helens vale, Australia) will be used to initially assess the incomplete S1 sequences. Next, a BLAST search against the NCBI database (GenBank) will be used to establish the identities of the sequences.

Every IBV positive sample will have its alignments to reference genomes performed in MEGA6 (26), and phylogenetic analysis will be inferred using the neighbor-joining approach with 1000 bootstrap repetitions. In order to verify high-quality read data, sequences were first analyzed using ChromasPRO v1.7.3 (http://technelysium.com.au/). Using Clustal W, alignments were performed in MEGA6 (Tamura et al., 2013). Following alignment, BLAST searches were conducted to confirm isolate identification. Obtained IBV sequences were compared against reference strains for S1 retrieved from GenBank (National Centre of Biotechnology Information). Reference strains used throughout this study Maximum likelihood analysis was utilised to infer phylogeny of both isolate and reference sequences, with default settings and 1000 bootstrap re-sampling.

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The results

Incidence rate in each region

The results are illustrated in Table 1, which show that in each sampled region there was a positive result when tested by PCR. The incidence rate was 1.1 % for each region.

Region	Total samples	Positive samples	Positive % ratio by PCR
Kanaan	10	1	10%
Kanaan 2	10	1	10%
Buhriz	10	1	10%
Baladroz	10	1	10%
Budaa	10	1	10%
Joba	10	1	10%
Alshik temim	10	1	10%
Shumer	10	1	10%
Faris	10	1	10%

Table 1.Show the incidence rate for each tested region from nine sampled backyard flocks

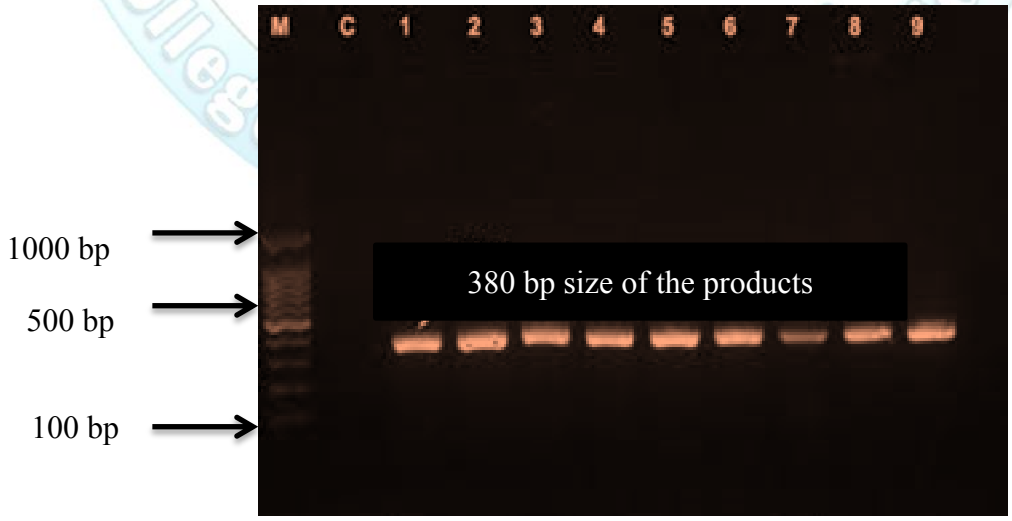

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Figure 1. Amplicons of the RT-PCR method using certain primer pairs to identify the infectious bronchitis virus. Using two primers (SX1 and SX2, SX3 and SX4), the S1 region of IBV is identified, yielding a 393-bp PCR product. 1.5% agarose gel was used for the electrophoresis process. M: A 100 bp DNA molecular weight marker; Lane 1 (NTC): Non-template control; Lane 2: Positive control; Lanes 1–9: Positive samples.

Genotyping and detection of IBV

9.9% IBV positive (Figure 1). Most of the samples had the highest resemblance to genotype 1494/06, whereas the isolates that remained had a strong relationship to M41.This study included each of the nine sequenced samples.

Phylogenetic examination of isolates of IBV Based on the genotypes of previously described strains, the maximum likelihood analysis results showed that the isolates grouped into five different clusters (Figure 2). Between 56% and 97% of the nine isolates that clustered with 1497/06 shared nucleotide similarity. The nucleotide homology of the remaining nine isolates, which clustered with M41, ranged from 97%

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To 99%.

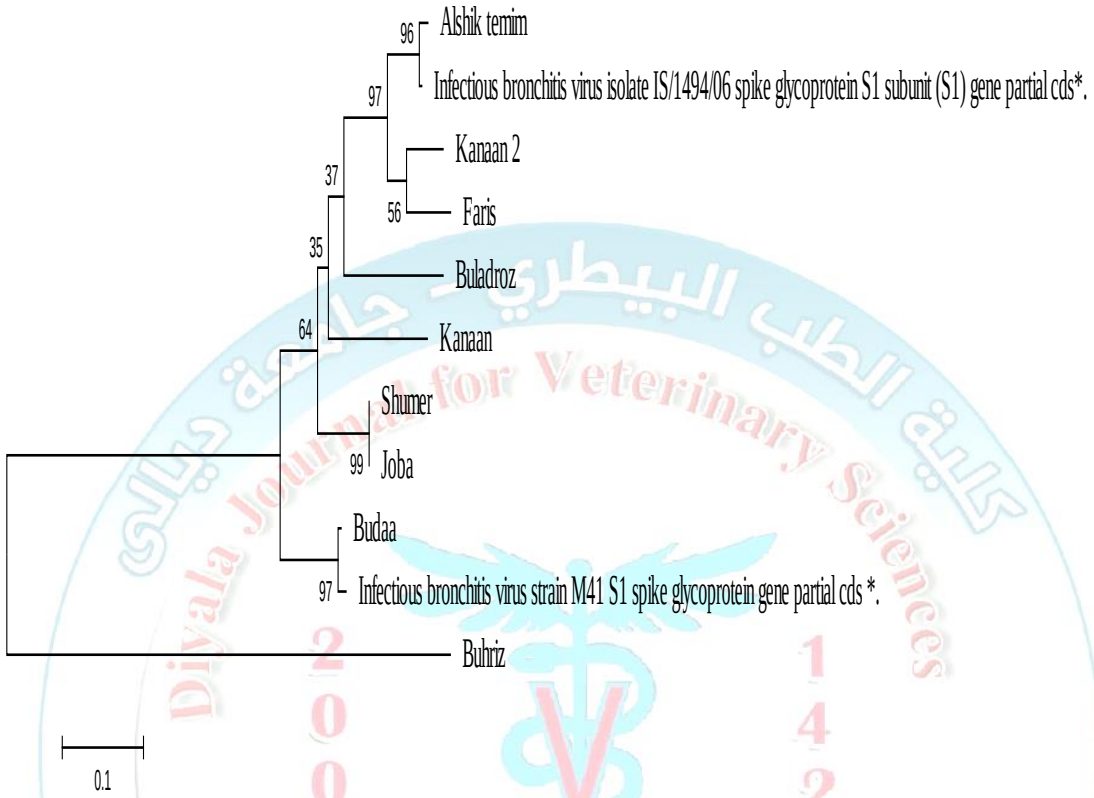

Figure 2. phylogenetic tree based on the partial S1 nucleotide sequence between the reference strain (Astra dote) and the IBV strains employed in this investigation Maximum likelihood analysis using Tamura 3-parameter and 1000 bootstrap repetitions was used to infer the analysis.

Discussion

Presenting and identifying the circulating IBV genotypes in backyard flocks in Diyala is the main goal of the current investigation. Swabs were used to transfer a number of epidemiologically representative samples from various Diyala locations to a private laboratory for this

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investigation. Once returned in the lab, the RNA extraction was helpful in analyzing field isolates that were extracted far from the sample location. We determined the frequency of the viruses in backyard flocks in response to location and chicken species using RT-PCR and direct amplicon sequencing. There were nine flocks who tested positive for IBV, yielding a 9.9% prevalence overall. This result is less than what has been previously documented on producing farms in nearby nations, such as 58.8% and 42.8% in Jordan and Iran, respectively(27; 28; 29).In contrast to private flocks, both studies sampled hens in environments with larger densities, which would have contributed to the increased incidence rate. The predominant IBV genotype in this study's backyard flocks infected with M41 among IBV-positive flocks was 1494/06. It has already been documented that other Middle Eastern nations including Iran, Jordan, and Israel have variable populations in their commercial flocks (30;31) The tight phylogenetic cluster established by the isolates found in the backyard flocks suggests that the same 793/B strain may be spreading throughout various backyard flocks and areas in Diyala. We found flocks of chickens that resembled M41 in the current investigation. Although at a far greater incidence than seen during this investigation, both genotypes have been previously documented in commercial flocks in the Middle East (32; 29) Originally isolated in Israel, IBV genotypes IS/1494/06 and IS/885/00 are presently circulating in several Middle Eastern nations (33; 34). In addition to backyard flocks, the commercial poultry business in Diyala and probably other parts of the region

are also at risk from these diseases. IBV has previously been found in Diyala flocks using RTPCR. It seems like this is the first time IBV backyard flocks have been found in Diyala flocks. It seems that this is Diyala's first report of IBV backyard flocks. Given its association with respiratory and reproductive diseases (35;36), backyard and commercial poultry in Diyala may be at risk from this pathogen's spread. Our knowledge of the epidemiology of IBV strains must grow in order to better control these infections, given the growing importance of backyard poultry in Diyala and the commercial chicken sector.

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