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Original Article

Circulation of Infectious Bronchitis Virus Among Guineafowls in Al-Ahsa Province, Saudi Arabia

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ABSTRACT

Infectious Bronchitis Virus (IBV) is a highly contagious pathogen that causes a serious illness with global circulation. While there is extensive data available on the virus's existence and transmission in commercial chickens in Saudi Arabia, there is a lack of such information regarding guineafowls. Therefore, this study aimed to investigate possible IBV infection among guineafowls in the Al-Hassa Governorate of the Eastern Province, Saudi Arabia. Oropharyngeal and cloacal swabs were collected from several unvaccinated flocks of guinea fowls without respiratory clinical symptoms in November and December 2022, totaling 350 samples. Total RNA was extracted from the swab samples, and a conventional reverse transcription-polymerase chain reaction was employed to detect IBV. The results revealed varying amounts of IBV in oropharyngeal and cloacal swabs at different points in time, suggesting that IBV may be widely distributed among guineafowls without exhibiting any symptoms. These findings indicate that guineafowls could act as reservoirs, influencing the ecology and epidemiology of the disease. Notably, this study reports the first occurrence of IBV in the province of Al-Ahsa, highlighting that guineafowls have been naturally exposed to the virus. To support the development of effective vaccination techniques and control measures for the disease in Saudi Arabia, the recommendation for future research endeavors is conducting ongoing surveillance, viral isolation, sequencing, phylogenetic tree analysis, and serotype characterization of IBV in guineafowls.

INTRODUCTION

Infectious Bronchitis Virus (IBV) is a highly contagious pathogen known to cause respiratory, kidney, and reproductive issues in chickens. IBV primarily replicates in the epithelial tissues of the upper respiratory tract, such as the turbinate, before progressing to the lower respiratory tract, including the trachea (Dolz *et al.*, 2012; Jackwood & de Wit, 2013; Al-Rasheed *et al.,* 2022). In the 1930s, Infectious Bronchitis Virus (IBV) was initially identified as the causative agent of "chicken respiratory disease" in the United States. Since then, it has been found to be prevalent in various regions worldwide, including among non-domestic Galliforms (Beach & Schalm, 1936; Cavanagh, 2007; Ramakrishnan & Kappala, 2019; Kariithi *et al.*, 2023). Moreover, it has been documented that IBV can cause damage to the kidneys and negatively impact the egg size, quality, and production. This viral infection continues to be a significant factor contributing to financial losses in the poultry industry, affecting both meat production and table egg-laying hens (Cavanagh, 2007; Bande *et al.*, 2016). IBV is highly contagious and can be transmitted through contaminated food or water, feces, or airborne transmission. Numerous serological and genetic studies have been conducted to determine the prevalence of regional IBV strains (de Wit *et al.,* 2011;

Jackwood, 2012; Jackwood & de Wit, 2013; Awad *et al.,* 2014). IBV exhibits a wide distribution across Africa, Asia, Australia, Europe, and the Americas. According to the International Council on Taxonomy of Viruses (ICTV), Infectious Bronchitis Virus (IBV), Turkey Coronavirus (TCoV), and Guinea Fowl Coronavirus (GfCoV) are classified as members of the avian coronaviruses (ACoVs) species within the subgenus igacovirus, genus Gammacoronavirus, subfamily Orthocoronavirinae, and family Coronaviridae (Cavanagh & Naqi, 2003; Cavanagh, 2007). It should be noted that this genus also includes coronaviruses isolated from wild birds and non-avian hosts (Carstens, 2010; de Wit & Cook, 2020).

The genome of the infectious bronchitis virus (IBV) is comprised of a single-stranded positive-sense RNA that codes for four structural proteins: spike, envelope, membrane, and nucleocapsid (Jackwood & de Wit, 2013). Due to point mutations, genetic recombination processes, and selective pressure in the hyper-variable regions of the genome, IBV exhibits multiple serotypes (IBV). Various serotypes of the virus have been identified in poultry populations worldwide, with some serotypes lacking cross-protection against each other (Lin & Chen, 2017). Given the continuous emergence of novel IBV serotypes, rapid detection is crucial for implementing effective control measures, and understanding the epidemiology and evolution of the virus is imperative (de Wit, 2000). Molecular techniques such as reverse transcription-polymerase chain reaction (RT-PCR) are commonly employed for the rapid detection and identification of IBV. RT-PCR assays offer fast, sensitive, and highly specific detection of IBV RNA from clinical samples or environmental samples containing the virus (Acevedo *et al.*, 2013; Alp Onen & Ozgur, 2017).

The guineafowl (*Numida meleagris*) belongs to the Numididae family within Galliformes order. They are also commonly referred to as speckled hens or distinctive poultry (Dyke *et al.*, 2003). Serum samples from Guinea fowl in the Caribbean and various islands were analyzed for IBV antibodies, yielding positive results that indicate natural exposure of birds to the virus (Kumthekar *et al.*, 2011). In Nigeria, cloacal and tracheal swabs collected from guineafowls were examined using real-time RT-PCR, leading to the detection of IBV RNAs (Bitrus *et al.*, 2020). Additionally, IBV-like viruses have been identified in turkeys, teal (Anas crecca), geese (Anserinae), pigeons (Columbiformes), partridge (Alectoris), ducks (Anseriformes), and guineafowls (Numida meleagris), as well as in pheasants (Phasianus colchicus) and peafowls (Galliformes) (de Wit & Cook, 2020; Jackwood & de Wit, 2020). Currently, there is no documented evidence regarding the presence of Avian Coronaviruses (AvCoVs) in guineafowl from the Al-Ahsa Province or the eastern regions of Saudi Arabia. Hence, the objective of the present study is to investigate IBV infection in guineafowls located in the Al-Ahsa Governorate within the Eastern Province of Saudi Arabia.

MATERIALS AND METHODS

Ethical statement

The experimental animal research conducted in this study adhered to the recommendations of King Faisal University's Ethics Council for Experimental Animal Research (Ref. No. KFU-REC-2021-NOV-EA000196). All institutional and national guidelines for the care and use of animals were strictly followed.

Guinea fowls and swab samples

The research was carried out at the Research and Training Station of King Faisal University in the Al-Ahsa Governorate, Eastern Province of Saudi Arabia (KFU). A total of 350 oropharyngeal (OP) and cloacal (CL) swabs were collected from five flocks of unvaccinated guinea fowls aged between 12 and 18 months. These guinea fowl flocks were kept separately, and at the time of sampling, there were no apparent clinical symptoms of respiratory or other illnesses observed in the sampled birds. Each flock consisted of 100 to 150 birds. Swabs were collected from the five evaluated flocks at various intervals (0, 1, 3, 7, 10, 14, and 21 days) during November and December 2022. Five cloacal and five oropharyngeal swabs were independently obtained from each flock at each point in time. Each swab was placed in a labeled microcentrifuge tube containing 1 ml of virus transport media (VTM). The tubes were promptly placed on ice in a cold box and transported to the Central Biotechnology Laboratory at the King Faisal University College of Veterinary Medicine. Due to the large number of samples (350), five tracheal or cloacal swabs per pool of swabs collected from at the same time point were stored at -80°C in 1.5 ml Eppendorf tubes until further testing (Bitrus *et al.*, 2020).

RNA extraction

Total RNA was extracted from the combined oropharyngeal and cloacal swabs using the QIAamp Viral RNA Mini Kit (QIAGEN, USA), following the manufacturer's instructions. A total 140 µl of the samples were lysed by adding a mixture of 560 µl of AVL buffer, carrier RNA, and 560 µl of 100% ethanol. Successive aliquots of 630 µl were added to a QIAamp spin column and centrifuged at 8,000 rpm for one minute.

The bound RNAs were washed twice with 500 µl of AW1 buffer and 500 µl of AW2 buffer and then centrifuged at 14,000 rpm for three minutes. The RNAs were eluted in 50 µl of AVE buffer and stored at -80°C for future use (Jones *et al.*, 2011).

IBV molecular detection

The presence of IBV in the extracted RNAs was determined using a One-step RT-PCR Kit (QIAGEN, USA). The RT-PCR reaction mixture comprised 5 µl of total RNA, 5 µl of 5x Qiagen one-step RT-PCR buffer, 5 µl of Q buffer, 1 µl of dNTPs mix, 1 µl (50 pmol) of each primer (IBV N+) 5'-GAAGAAAACCAGTCCCAGATGCTTGG-3' and (IBV N-) 5'-GTTGGAATAGTGCGCTTGCAATACCG-3' (Handberg *et al.*, 1999), 1 µl of the enzyme mix (containing RT and PCR reaction enzymes), and 6 µl of RNase-free water. The RT-PCR reaction was performed at 50°C for 30 minutes, followed by 95°C for 15 minutes, and then 40 cycles with denaturation at 95°C for 30 seconds, primer annealing at 50°C for 30 seconds, extension at 72°C for 30 seconds, and a final extension at 72°C for 10 minutes. The amplified PCR products were electrophoresed on a 1.2% agarose gel containing 0.5 µg/ml ethidium bromide and visualized using an ultraviolet gel documentation system (BIORAD).

RESULTS AND DISCUSSION

IBV RNAs were detected in various quantities in pooled oropharyngeal and cloacal swabs collected from different flocks at different time points (Tables 1 & 2). This finding highlights the widespread circulation of IBV and its ability to replicate in guineafowls without causing evident symptoms (Cavanagh, 2005; de Wit & Cook, 2020). Avian coronaviruses (ACoVs) have a broad host range, including chickens, turkeys, and wild birds (Suryaman *et al.*, 2019). ACoVs have also been isolated from domestic and wild peafowls, pheasants, turkeys, partridges, pigeons, jungle fowls, and non-Galliformes birds such as teal (Besar *et al.*, 2023). Coronaviruses isolated from turkeys, and pheasants show genetic similarity to IBV, particularly in a highly conserved region of the 3' UTR with 90% nucleotide identity (Cavanagh *et al.*, 2002). However, molecular and sequence investigations have revealed that these ACoVs are genetically distinct from IBVs (Promkuntod, 2016). The detection of IBV genetic material in healthy guineafowls without a history of IBV vaccination suggests natural exposure to the virus. Consequently, guinea fowls may serve as reservoirs, contributing to the spread of the virus in the environment and significantly influencing the disease's epidemiology (Adebiyi & Fagbohun, 2017). Previous studies have demonstrated that IBV can spread between different avian species without manifesting clinical symptoms (Guy, 2000; Ismail *et al.*, 2003; Bitrus *et al.*, 2020). During the acute stage of infection, IBV can be observed in the trachea, but it later establishes itself in the caecal tonsils, where it can be shed in feces for several weeks (De Wit & Cook, 2014). The detection of IBV infection in guinea fowls through oropharyngeal and cloacal swabs at various time intervals suggests latency and ongoing circulation of the virus (Adebiyi & Fagbohun, 2017). Previous studies have reported IBV infection in ducks, geese, doves, turkeys, pigeons, and quails, as well as in chickens (Cavanagh, 2005). The current study's findings regarding IBV detection in guineafowls align with earlier research (de Wit *et al.*, 2011; Bitrus *et al.*, 2020). Furthermore, contrary to a previous report by Ayim-Akonor *et al.* (2018), which stated that conventional RT-PCR could not detect IBV in guineafowls, this study successfully detected IBV in guinea fowls using conventional RT-PCR. The discrepancy in findings may be attributed to differences in the sensitivity of the molecular methods used. This study also demonstrates that direct sampling from oropharyngeal and cloacal swabs is sufficient for IBV detection, which is consistent with findings from previous investigations that obtained positive results using RT-qPCR directly from viral RNA in VTM (Alp Onen & Ozgur, 2017; Bitrus *et al.*, 2020). In other studies, the virus was cultured in embryonated chicken eggs (ECE) prior to RT-qPCR (Lee & Jackwood 2000), while allantoic fluid harvesting of IBV RNAs was an effective method for identifying IBV serotypes (Meulemans *et al.*, 2001).

Table 1 – RT-PCR detection of Oropharyngeal swabs.

Flocks	Swabs	Days of swabs collection						
		0	1	3	7	10	14	21
Flock 1	Oropharyngeal				+		+	+
Flock 2	Oropharyngeal	+						+
Flock 3	Oropharyngeal				+			
Flock 4	Oropharyngeal	+		+		+		
Flock 5	Oropharyngeal			+			+	

Table 2 – RT-PCR detection of Cloacal swabs.

Flocks	Swabs	Days of swabs collection						
		0	1	3	7	10	14	21
Flock 1	Cloacal	+		+	+			
Flock 2	Cloacal	+	+	+				+
Flock 3	Cloacal	+	+		+			+
Flock 4	Cloacal	+		+	+			
Flock 5	Cloacal						+	

CONCLUSION

Using conventional RT-PCR, our study revealed the extensive circulation of IBV in guineafowls. To develop effective vaccination schedules and implement control strategies for IBV infection in Saudi Arabia, we recommend further research focusing on monitoring, viral isolation, sequencing, phylogenetic tree analysis, and identification of prevalent serotypes.

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AUTHORS' CONTRIBUTIONS

All authors contributed to the sampling, laboratory analysis, and preparation of the manuscript. M. Shawky assisted in drafting the text, data analysis, and laboratory testing. All authors contributed to data analysis and manuscript preparation. M. Al-Rasheed was involved in the conception and design of the experiment, lab management, data analysis, and paper editing. The final manuscript has been reviewed and approved by all authors.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest related to the publication of this paper.

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