Detection and Molecular Characterization of Canine Influenza Genotype H3N8

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Abstract- Canine Influenza (CI) remains a major public health problem and an endemic disease in Iraq, therefore this study was conducted to identify the canine influenza virus (CIV) subtype existed, and to find out the best method for diagnosis. One hundred and fifty samples suspected with CI appeared in canine training and qualification kennels and Baghdad veterinary hospital from Baghdad, Al-Najaf, Karbala, and Babel cities during the period extended from October 2018 to September 2019. The demographical study dealt with the number of infection, reverse real-time quantitative polymerase chain reaction (RT-qPCR), and sequencing results were showed that the majority of positive infected dogs with CIV at Baghdad province (78.38%) higher than other provinces (21.62%). The Molecular detection by RT-qPCR technique was concluded that 51/150 (34%) of suspected patients carried Influenza A infection according to the cycle threshold (Ct) value less than 35 that considered as positive result 51 (34%). The next-generation sequence was indicated that Influenza type A belongs to the genotype H3N8, and very close to the USA strains according to Hemagglutinin and Neuraminidase genes phylogenetic analysis. This indicates that H3N8 CIV may be a common pathogen for kennelled dog populations in Iraq at present.

Keywords: Canine Influenza, RT-qPCR, H3N8, Molecular detection, Iraq.

I. INTRODUCTION

Influenza A virus can infect a varied range of hosts, from birds to mammals, and exhibits varying degrees of host alteration [1]. Given international communication among humans, birds, pigs, and further mammalian types [2]. Epidemiological studies of CIVs have revealed several outbreaks of inter-species transmission, such as the equine-origin H3N8 [1] and the avian-origin H3N2 influenza virus that crossed the dog host barrier [3]. Sialic acid (SA) receptors in dogs have a distribution similar to that of the avian SA receptor [4], and the equine SA receptor [5], which enables influenza viruses to enter the respiratory epithelial cells. Infected dogs by influenza viruses showed respiratory clinical signs which are susceptible to highly pathogenic influenza [6]. Avian and equine influenza viruses crossed the host barrier to give rise to the canine influenza virus (CIV), which indicates that dogs co-infected with various influenza viruses may act as intermediate hosts for avian and equine influenza viruses' re-assortment.

As well as avian-to-canine and equine-to-canine transmission, evidence has been reported for transmission of seasonal human H3N2 and pandemic H1N1 (pH1N1) virus to canines. Dogs had experienced seasonal H3N2 infection since 2008, and pH1N1 infection alone or in blending with H3N2 CIV after 2009 were revealed by Serum samples gathered from canines. Pathological variations in the lungs caused by the infectivity of pH1N1 and migrant H3N2 viruses in canines proved by artificial injection of viruses with lively viral shedding [7]. The possibility of re-assortment between the two viruses in canines was suggested by Studies on seroprevalence and artificial infection; subsequently, M segment-swapped CIV between pH1N1 and wild-type H3N2 CIV and H3N1 were isolated [8, 9].

The dog showed similar symptoms of sneezing, copious nasal discharge, and subnormal to low fever 36 °C-39.5 °C when the dogs entered the clinics. Until now, no such genetically study about H3N8 CIV infections

were carried out in kennelling dogs in Iraq. This study will provide an important insight into pathogenesis, transmission, and evolution of CIVs, which emerged recently in Iraq, and help determine future countermeasures.

II. MATERIALS and METHODS

To display the prevalence of the equine-derivation H3N8 CIV in the kennelled dog population, four large-scale dog kennels were selected from four different cities of Iraq Provinces. Regular inspections were conducted to assess the prevalence of CIV in dog kennels from October 2018 to September 2019. A total of 150 samples, nasal swabs were collected from dogs at a different sex and age from four dog kennels. Four cities included Baghdad, Babel, Karbala, and Najaf. These samples were transferred in VTM after collection and stored at -70 °C before tested.

The isolation of viruses from canine nasal swabs was carried out according to report protocols for the World Health Organization, [10]. Viral RNA was extracted using the Kit Ribo Virus (Sacace, Italy). The subtyping experiments primers used for detection influenza A types by RT-qPCR in this study according to Terrier et al., [11]. And reverse transcription RNA to synthesizes complementary DNA (cDNA) performed by using the Go Script™ Reverse Transcription (Promega, USA). cDNA delivered to generate through widely used next-generation sequence (NGS) technologies (Illumina) for whole-genome sequence (WGS).

Reading quality analysis and improvement. This unit is the first stage in almost WGS bioinformatics analyses and refers to the quality control and improvement of the raw sequencing data. Raw data accepted single-end and paired-end reads (fastq.gz format) to generate NGS technologies (Illumina). Reads' quality control in this study performed by using FastQC software [12], while Trimmomatic achieved quality improvement [13].

Variant uncovering and accord generation. This stage of the pipeline contains mapping the quality processed reads against user reference sequences, followed up by SNP/indel calling and annotation, and generation of consensus nucleotide sequences.

The reference database of canine influenza includes reference sequences of equine-origin Influenza A virus (MK690099.2 Influenza A (H3N8) (HA) gene USA 2020). Reference sequences are publicly available at the National Centre for Biotechnology Information (NCBI). The reference file, both in ".fasta" and ".gbk" (GenBank) format annotation accomplished by using (Prokka) [14], was prepared to fit amplicon-based schemas capturing the whole coding sequences (CDS) of the eight genes of influenza virus. ".Fasta" files uploaded and explained by using Prokka. In this unit, by Snippy advantage, an extremely elastic multi-program tool for fast readability mapping (by using Burrows-Wheeler Aligner – BWA) [15], SNP/indel calling (by using samtools and freebayes) [16, 17], variant annotation (by using SnpEff) [18] and consensus generation (by using vcftools) [19].

III. RESULTS

The recent genetic screening revealed that from 150 nasal swabs 51 (34.0%) was found positive by M gene-specific RT-qPCR in kennelled dogs (Figure 1). Kennelled dogs were examined by M gene-specific RT-qPCR for equine-origin H3N8 CIV from four cities in Iraq. Kennelled dogs from Baghdad were detected with the highest prevalence when tested by M gene-specific RT-qPCR (78.38%), followed by Babel, Najaf (8.1%) and Karbala (5.4%) (Figure 2). Phylogenetic tree was mainly divided into subgroups that correlative with equine lineages. As shown in the four constructed phylogenetic trees, the CIV grouped with the newly isolated Equine influenza viruses H3N8 from France, Malaysia, Chile, and the USA. Hemagglutinin (HA), and Neuraminidase (NA) genes of CIV H3N8 closely relate and cluster in the same clade with the equine H3N8 viruses from the USA, while CIV from other countries isolated in different clades. Furthermore, the HA gene of the canine H3N8 strain from kennelled dogs seemed to be derived from the (A/equine/Oregon/78356/2012(H3N8)) strain (Figure 3). The internal genes of A/canine/Iraq/2020(H3N8) most closely connected to the (A/equine/Oregon/78356 /2012(H3N8)). Similarly, we compared the gene sequence of the gene NA from the CIV isolate against the equine isolate from the USA and the most similar influenza viruses strain, (A/Equus caballus/USA/154390 /2018(H3N8)) strain (Figure 4). All these analyses revealed that few changes happened in CIVs after years of spreading.

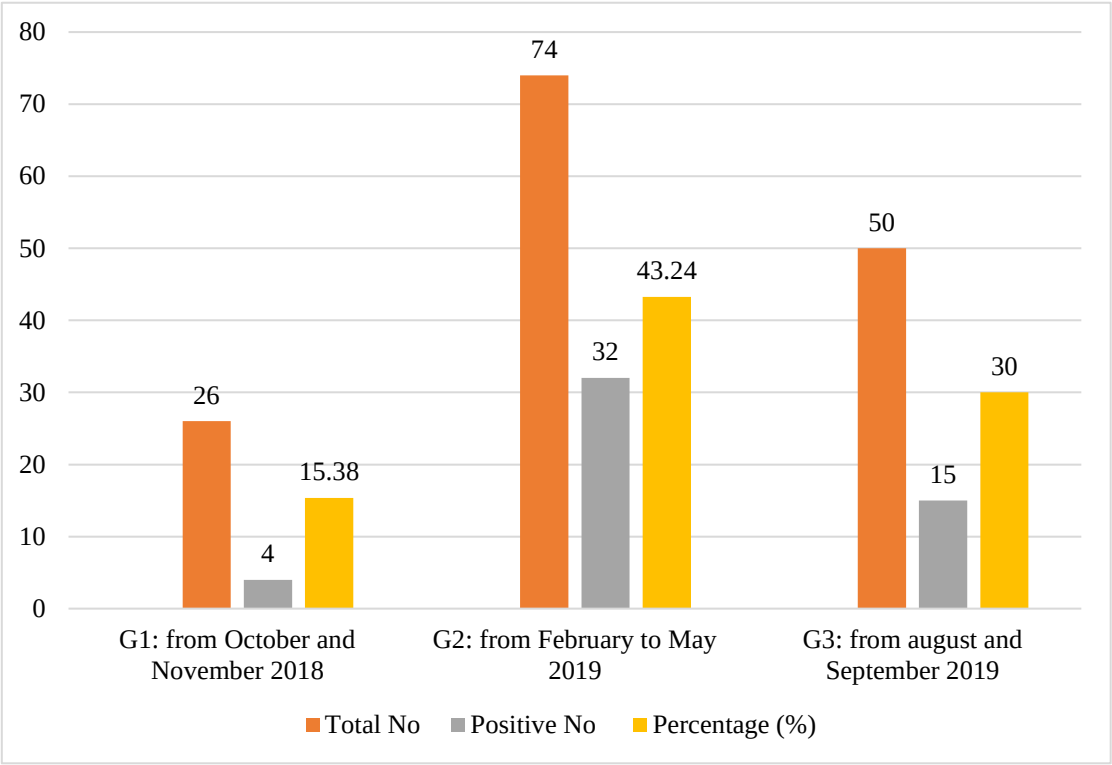

Figure 1. Distribution of samples according to results of RT-qPCR.

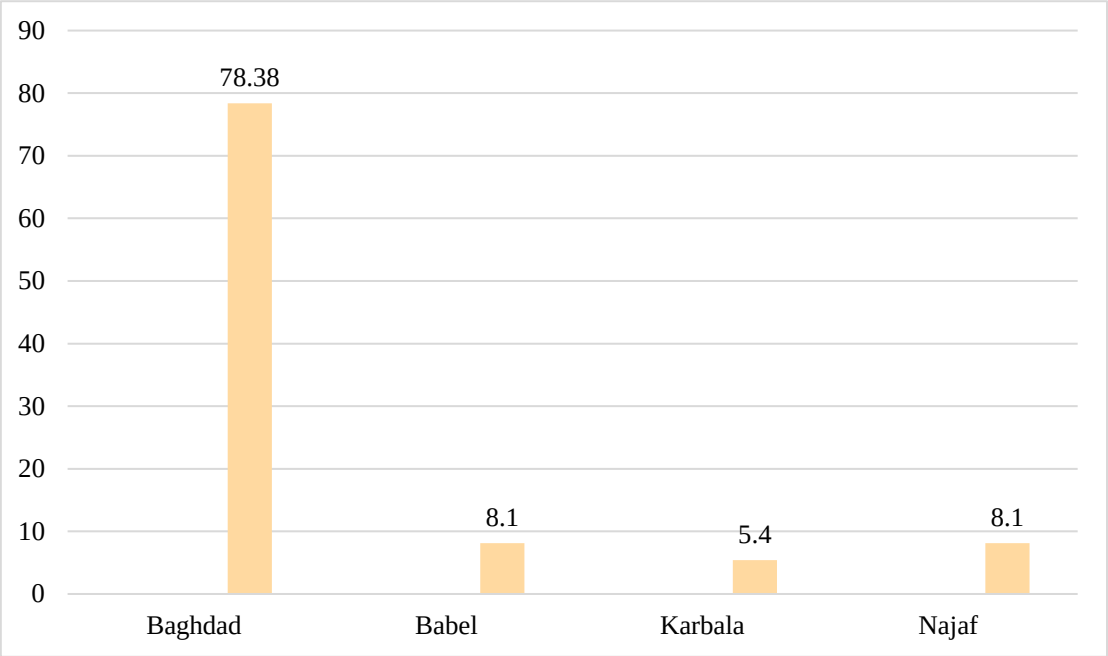

Figure 2. Distribution of collected samples study according to Provinces.

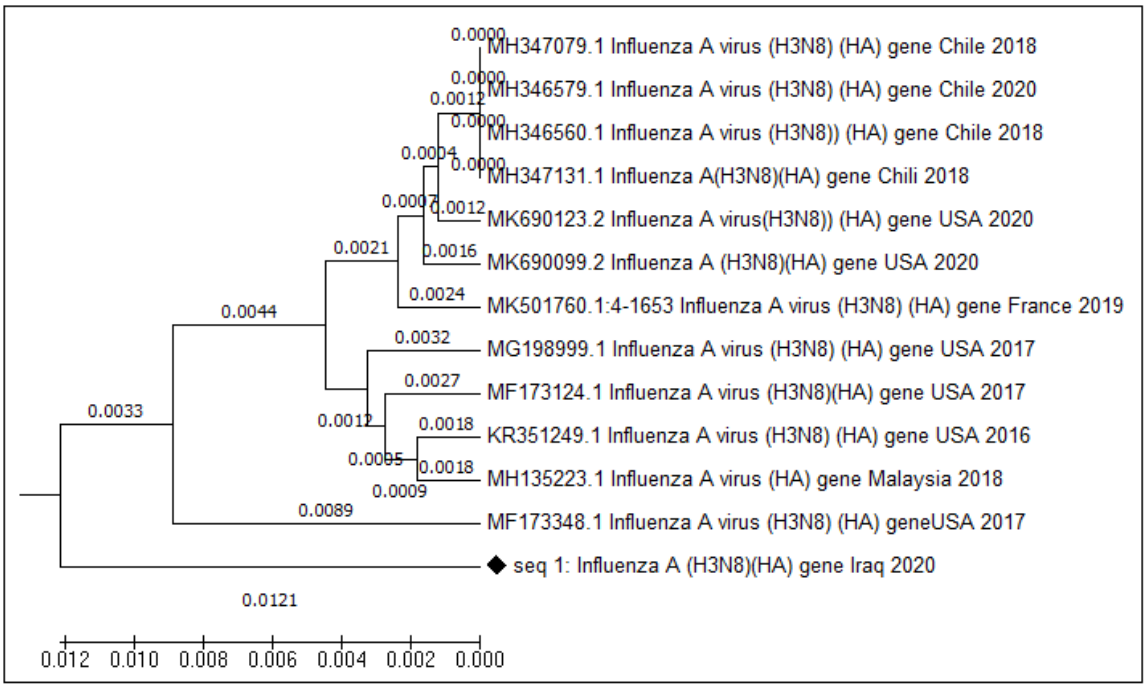

Figure 3. Phylogenetic trees for the A/canine/Iraq/2020(H3N8) HA gene and those of other influenza A viruses. The analysis was based on the nucleotide sequences in the open reading frames of the HA gene. The trees were generated with the MEGA program (version 6.0) by using neighbour-joining analysis.

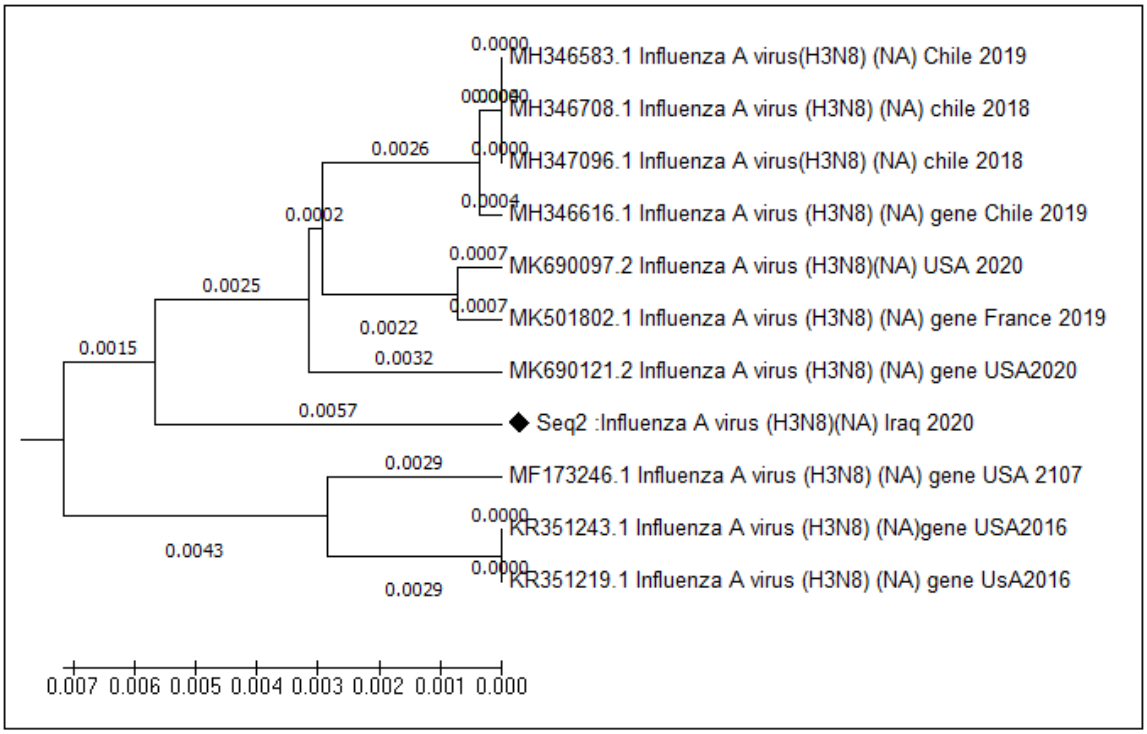

Figure 4. Phylogenetic trees for the A/canine/Iraq/2020(H3N8) NA gene and those of other influenza A viruses. The analysis was based on the nucleotide sequences in the open reading frames of the NA gene. The trees were generated with the MEGA program (version 6.0) by using neighbour-joining analysis.

IV. DISCUSSION

This is the first epidemiology survey to assess the risk of H3N8 CIV transmission among different dog populations in Iraq. One strain was isolated from kennelled pet dog in the veterinary hospital of Baghdad. This indicates that H3N8 CIV may currently be a common pathogen for kennelled dog populations in Iraq. Similarly, our study has shown that the infection rate of this equine-origin canine influenza in kennelled dogs were (34.0%), as determined by M gene-specific RT-qPCR test. The close contact is most probably the route of spread occurred between infected canines with H3N8 CIV and uninfected canines from different dog populations as data showed. The most possible way of infection is dog-to-dog spread. Our findings strengthened the previous data by showing that after long-term adaptation in dogs, the equine-origin H3N8 CIV has already circulated in various dog inhabitants in Iraq [20, 3]. As it is known that dogs accompany humans, so direct contact with humans is possible, and around this information, no direct transmission of CIV H3N8 from dogs to humans has been documented. Because of the similarity of the cellular receptors of the virus between dogs and humans, according to Song et al., [4]; and Daly et al., [5]; this is likely to cause CIV a new pandemic outbreak. Which may pose a great threat to our human life, these findings highlight the importance of monitoring dogs in pet hospitals and on dog kennels.

V. CONCLUSION

As CIV H3N8 outbreaks among dogs continue in Iraq provinces, areas where are densely populated and with frequent animal trade, there are a non-stop dangers for pets CIV H3N8 infections, and for mutations, or genetic re-assortment foremost to first-hand CIV strains with greater than before transmissibility between dogs. Furthermore, vaccine development for CIV H3N8 is very urgent. Further study is required as the CIV H3N8 confirmed in various dog inhabitants and pose a potential danger to community health. The kennelled dogs' population may serve as a more sensitive sentinel for monitoring emerging CIV H3N8 in the future.

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AUTHOR CONTRIBUTIONS

Yahya A. AbdulKareem, Oday K. Luaibi, and Nadira S. Mohamed conceived and designed the study; Yahya A. AbdulKareem and AbdulRaheem Wali collected samples; Yahya A. AbdulKareem, and Nadira S. Mohamed performed the experiments and sequencing analysis. Yahya A. AbdulKareem, Oday K. Luaibi, and Nadira S. Mohamed contributed to the writing and revision of the manuscript and approved the final one.

ETHICAL APPROVAL

This study does not cover any trainings with human/ animal members implemented by authors.

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