EasyChair Preprint

№ 8050

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May 23, 2022

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ABSTRACT

Influenza A viruses are amongst the most difficult viruses that compromise both human and creature wellbeing. Constantly evolving and crossing species hindrance, the rise of novel zoonotic pathogens is one of the greatest challenges to global health security. During the last decade, considerable attention has been paid to influenza virus infections in dogs, as two canine H3N8 and H3N2 subtypes caused several outbreaks throughout the United States and Southern Asia, becoming endemic. At this time, we review the most recent knowledge of the influenza A virus epidemiology in dogs, and existing evidence for the abilities of these species to host, sustain intraspecific transmission, and generate novel flu A genealogies through genomic reassortment. We undertook this detailed investigation study conducted on dog cases entered to Baghdad Veterinary Hospital, Baghdad, with canine influenza-like symptoms from 2016, 2017, and October 2018 to September 2019 and to determine a laboratory parameter to identify canine influenza among dogs presenting with influenza-like symptoms while awaiting nasal swab culture and virus isolation reports. They were divided into canine influenza-positive (CI+) and canine influenza negative (CI-) groups, based on their nasal swab culture reports and laboratory data. Neutrophils to lymphocyte (N/L) ratio or neutrophils to monocyte (N/M) was calculated for every dog and the mean N/L ratio or the mean N/M ratio for positive groups. Mean white blood cell count (WBC) was also noted for three groups. N/L < 2 or 3, or N/M > 5 along with a decrease in WBC count can be used as a screening tool in suspected dogs presenting with influenza-like symptoms while awaiting nasal swab culture reports for confirmation. Such enhanced understanding suggests a need to reinforce surveillance of the role played by companion animals-human interface, considering the "One Health" idea and the expected development of novel zoonotic viruses.

Keywords: H3N8 influenza; lymphocytes; monocyte; neutrophils; screening tool; canine influenza.

INTRODUCTION

Influenza type A or type B viruses is an acute infectious respiratory disease in humans. Influenza A viruses (IAV) can be isolated from a wide variety of animal species, while Influenza B circulates only among humans. Wild migratory birds and bats are the main natural reservoirs, from where viruses are used to spill over into other animal hosts like ducks, chickens, horses, pigs, whales, cats, dogs, etc. IAV commonly exhibit a restricted host range, but occasionally transmits from one species to another host (Joseph et al., 2017). Notably, most human pandemics have emerged from avian and swine hosts (Peiris et al., 2007; and Yang et al., 2015). In a world where the number of cat and dog owners is increasing, social behaviour tends to enrol these animal species as family members (Charles et al., 2015; Irvine et al., 2017).

As we know, viral nasal swab culture and reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) are time-consuming procedures and delay significant results in suspected case confirmation. The first detail conducted investigation study of dog cases providing an up-to-date picture of the epidemiology of IAV in the Iraq dog community and their transmission modes that had presented to Baghdad Veterinary Hospital, Baghdad, with symptoms of influenza-like illness during 2016, 2017, 2018, and 2019 (Abdulkareem et al.,

2020). The first aim of this study is to find out a sensitive laboratory parameter that could play a major role in identifying influenza virus infection or any influenza subtype among dogs presenting with influenza-like symptoms while awaiting nasal swab culture or RT-qPCR reports. The second aim of this study is to improve our previous study.

MATERIAL AND METHODS

This detailed investigation study is done for improving our previous conducted study on dog cases that owners brought to Baghdad Veterinary Hospital, Baghdad, from 2016, 2017, and October 2018 to September 2019 and they showed two or more of the following symptoms: sub-normal temperature, rhinorrhoea, and mild fever in some cases. An RT-qPCR tests report for nasal swab were obtained from the Forensic DNA Centre for Research and Training, University of Al-Nahrain, Baghdad, for each suspected dog (Abdulkareem et al., 2020). Fifty-one canines out of 150 canines suspected of canine influenza (CI), who had a positive RT-qPCR test for canine influenza virus were grouped into 3 canine influenza-positive groups (CI+ group (G) 1, 2, and 3) per month which include 51 suspected canine cases (see table 1). Ninety-nine canines out of 150 canines suspected with CI who had a negative RT-qPCR test for canine influenza virus, were labelled as canine influenza negative group (CI- group), which we aren't mentioning in table 1. Some of them were H3N8 negative but influenza A positive, while some were negative for both H3N8 and influenza A.

Haematological parameters and total WBC were measured by using Abacus veterinary Junior an automated hematologic analyser, and all blood samples were analysed at the veterinary hospital in Baghdad. Laboratory data were collected for each suspected dog in two groups that included complete blood count and RT-qPCR from our previous study. Neutrophils to lymphocyte ratio (N/L ratio) or neutrophils to monocyte ratio (N/M ratio) was calculated for each suspected dog. The mean N/L ratio, mean N/M ratio, and mean WBC count for the three groups were also calculated.

The Statistical Analysis System- SAS, (2012) program was used to detect the effect of different factors on study parameters. Least significant difference –LSD test (Analysis of Variation-ANOVA) was used to compare significant differences between means.

RESULTS AND DISCUSSION

WBC count is important in the diagnosis of influenza because differential WBC count is presenting a mirror for body condition with infection and other causes (Dengler et al., 2014). The normal value of WBC count for dogs is 6000-17000/µl as a general, lymphocyte 1000-4800/µl, monocyte 150-1350/µl, and granulocyte 3000-11500/µl according to Rizzi et al., (2010).

The haematological study showed no significant differences in granulocytosis at 5-10 days after infection (data shown in table 1 and figure 1). Also, the analysis showed significant differences in lymphocytosis revealed in the first 3-4 days after infection, and no significant differences in monocytosis which a higher proportion of lowest normal value in CIV infected dogs, especially in G2 and G3 and these results are very close to Dengler et al., 2014 results.

There was a relative decrease in the WBC count of some dogs in the CI+ groups compared to dogs in CI- group, which was consistent with the earlier studies (Cunha et al., 2009; Cao et al., 2009; Saha et al., 2010; and Indavarapu et al., 2011). The WBC count in the CI+ group varied from 2690 to 14260 (mean for each group 14525, 21504, 8418 respectively), while in the CI- group it varied from7790-12270 (mean for all groups 9896). N/L ratio or N/M ratio was more than two for dogs in the CI+ group except for a few cases. It was greater than three for dogs in the CI- group.

Some canine CI+ cases had low lymphocyte counts. While the average lymphocyte count for the CI+ group was 2453 cells/µl while that for the CI- group was 2193 cells/µl. Earlier studies indicated the role of lymphopenia, monocytosis, and lymphocyte to monocyte ratio in detecting influenza (Cunha et al., 2009; Merekoulias 2010; and Indavarapu et al., 2011), but the N/L ratio of less than two and the N/M ratio of more than three observed in this study have not been reported before. So, the cause of lymphopenia was a simultaneous increase in the frequencies of proliferating cells correlated with an increase of leukocytes infiltrating in lungs that similar result to Schwaiger et al., (2019). N/L ratio was found to be sensitive and

specific for canine influenza virus infection within 48 hours from first clinical onset signs and may serve as a rapid screening test or the opposite if sampling takes more than 48 hours. While the N/M ratio as a screening tool was found to be sensitive and specific for CI+ more than 48 hours. It is suggested that dogs presenting with influenza-like symptoms, N/L ratio or + N/M ratio and lymphocytosis together can be used as a screening tool for canine influenza. When all the three parameters i.e., influenza-like symptoms, lymphocytosis and N/L ratio less than two or three and N/M ratio more than 5 are taken into consideration, these are highly indicative of canine influenza infection. RT-qPCR test can also be used alongside the N/L ratio test influenza detection tests to increase the possibility of diagnosis.

CONCLUSIONS

In conclusion, in a dog presenting with two or more influenza-like symptoms (subnormal temperature, rhinorrhoea, and mild fever in some cases) a decrease in WBC count and an N/L ratio less than two, three, and N/M ratio more than 5 indicate the possibility of canine influenza. Nevertheless, if the final diagnosis is possible must be based on an RT-qPCR test, nasal swab culture and virus isolation. N/L ratio of less than two, three and N/M ratio of more than 5 may serve as an excellent screening tool for isolation while awaiting nasal swab culture reports for confirmation of the diagnosis. It is a time-saving and cost-effective procedure. Consequently, to reducing the complications and mortality due to delayed treatment the anti-viral treatment should be started early.

Whether the diagnostic rule of influenza patients described in the previous studies and our current study exclusively applies to dogs with canine influenza needs to be evaluated in larger samples.

Table 1: Comparison among different groups in WBC count and differential.

Groups	**Mean ± SE of Total WBC count**					
	WBC count	Lymphocyte (%)	Monocyte (%)	Neutrophil (%)	N/L ratio	N/M ratio
G1: from October and November 2018	14525.00 ±152.99 ab	15.10 ±0.51 b	10.25 ±1.18 a	74.65 ±1.70 a	4.94	7.28
G2: from February to May 2019	21504.38 ±2596.38 a	26.47 ±1.86 ab	11.74 ±1.36 a	61.78 ±3.04 a	2.33	5.26
G3: from August and September 2019	8418.67 ±937.20 b	29.13 ±5.45 a	9.06 ±0.83 a	61.79 ±5.42 a	2.12	6.82
LSD value	11586.0 **	13.75 *	6.25 NS	17.36 NS	>2	>5
Means having with the different letters in the same column differed significantly *(P≤0.05), ** (P≤0.01).						

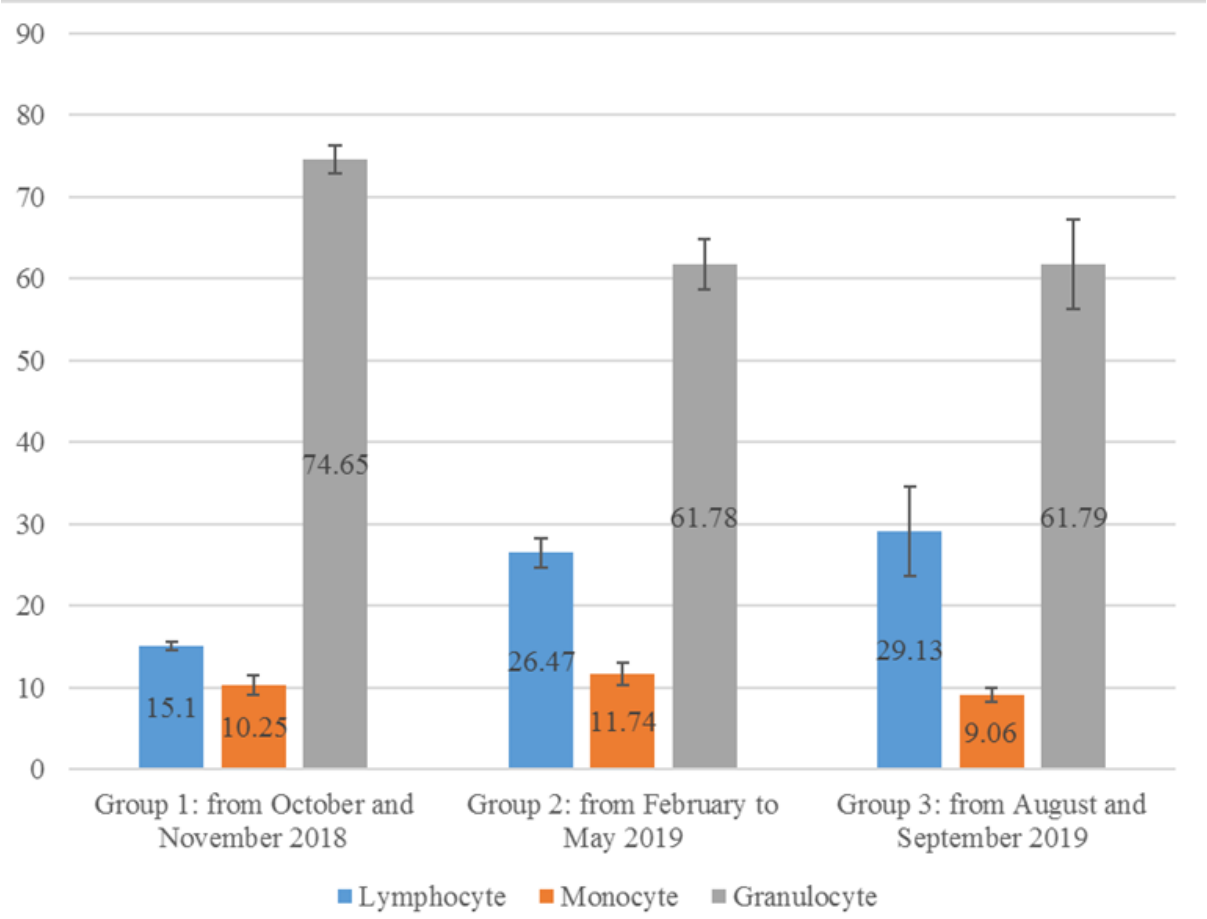

Figure 1: Comparison among different groups in WBC count.

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