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Investigation of spillover evidence of SARS-CoV-2 virus in dogs and cats in Egypt

A thesis presented by

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(2022)

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Specification: Virology
Thesis Title: Investigation of spillover evidence of SARS-CoV-2 virus in dogs and cats in some Egyptian governorates

Abstract

SARS-CoV-2 zoonotic and reverse zoonotic transmission could be resulted from routine activities and interactions between humans and their companion animals. A combination of SARS-CoV-2 high mutation rate and homology of cellular ACE2 receptors enable SARS-CoV-2 to transcend species barriers and facilitate the viral transmission between humans and animals. The aim of this study to investigate spillover of SARS-CoV-2 between humans and companion animals with studying mutations of the detected SARS-CoV-2 spike glycoprotein and the effect of these mutations on the viral structure and function.

Oropharyngeal/Nasopharyngeal swabs, serum and blood samples were collected form 66 companion animals (33 cats and 33 dogs) which were close contact to SARS-CoV-2 positive owners from December 2020 to March 2021. Swabs were screened by rRT-PCR and some positive cases were confirmed by partial spike sequencing. Clinical pathology and pathological studies were also performed. Spillover of SARS-CoV-2 between humans and their companion animals were reported in Egypt with a rate of 30.3% of cats (10/33) and 24% of dogs (8/33) by using rRT-PCR. Partial spike gene sequencing of 6 positive samples collected in December 2020 were identical to SARS-CoV-2 that was detected in humans in Egypt in that time frame. Furthermore, the infected companion animals have suffered from lymphocytopenia, thrombocytopenia with elevation of ferritin, LDH, C-reactive protein and D-dimers levels. The latter infected animals have showed a wide range of clinical signs including

asymptomatic, mild and severe respiratory signs with some deaths in the infected cats. The dead cats exhibited multiple systematic pathological lesions in lung, heart, liver intestine and kidney. Thus, spillover of SARS-CoV-2 may be occurred between humans and pet animals.

Full spike sequencing for some detected SARS-CoV-2 in cats that were collected in December 2020, March 2021 and July 2021 has displayed 7 amino acid substitutions. Structural modelling has revealed that 4 of these mutations could affect the interaction with the neutralizing antibodies and others could influence S1/S2 cleavage, facilitate viral binding to the ACE2 host receptors and enhance viral infectivity. Bioinformatics analysis of ACE2 receptors in different animal hosts has provided in-depth investigation for RBD/ACE2 complex binding affinity and their relationship to SARS-CoV-2 infection susceptibility. Therefore, this thesis paves the way for studying SARS-CoV-2 host susceptibility in different animal species.

Keywords: SARS-CoV-2, companion animals, zoonoses, reverse zoonoses, spike, mutations, ACE2, structural modeling.

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List of Abbreviations:

ACE2	Angiotensin-converting enzyme 2
BaTG13	Bat coronavirus
CH	Central helix
CDR	Complementarity-determining region of antibody
CMs	Convoluted membranes
COVID-19	Coronavirus Disease 2019
CRP	C-reactive protein
CTD1	C-terminal domain-1
CTD2	C-terminal domain-2
DMSs	Double-membrane spherules
DMVs	Double-membrane vesicles
E	Envelope protein
ERGIC	Endoplasmic Reticulum–Golgi Intermediate Compartment
FMV	Formerly monitored variants
FP	Fusion peptide
FPPR	Fusion peptide proximal region
HR1	Heptad repeat 1
HR2	Heptad repeat 2
IC	Intracellular
LDH	Lactate dehydrogenase
M	Membrane protein
MP789	Pangolin coronavirus
N	Nucleocapsid protein
NTD	N-terminal domain
NSP	Non-structural protein
NTD	N-terminal domain
OIE	Office International des Épizooties
ORF	Open reading frame
+ssRNA	Positive sense single stranded ribonucleic acid
RdRp	RNA dependent RNA polymerase
RBD	Receptor binding domain
RBM	Receptor binding motif
ROS	Reactive Oxygen Species
RT-PCR	Reverse transcriptase Polymerase Chain Reaction

rRT-PCR	Real Time Reverse Transcriptase Polymerase Chain Reaction
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
S	Spike glycoprotein
SP	Structural protein
TM	Transmembrane
UTR	Untranslated region
UK	The United Kingdome
USA	The United States of America
VOI	Variants of interest
VOC	Variants of concern
VUM	Variants under monitoring
WHO	World Health Organization
WOAH	World Organization for Animal Health