SARS-COV-2 SURVEILLANCE IN IMPORTED MEAT AND ANIMAL PARTS FOR FOOD SAFETY AND SECURITY

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ABSTRACT. The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is the causative agent for the coronavirus disease 2019 (COVID-19) pandemic. COVID-19 is contagious and fatal to humans. In the context of the COVID-19 pandemic, significant concerns on food safety and security are rising due to potential interspecies transmission. As such, surveillance of SARS-CoV-2 on imported meat and animal parts is carried out and reported in this study to safeguard food safety and security. Overall, none of the 225 samples from various livestock (buffaloes, cattle, goat and pig) imported from seven countries were tested positive for SARS-CoV-2 with quantitative reverse transcription polymerase chain reaction (RT-qPCR) from July 2020 to November 2021. This study finding serves as a baseline data for SARS-CoV-2 in imported meat and animal parts. Notably, this study accentuated the importance of active surveillance to prevent zoonosis and to safeguard food safety and security.

Keywords: SARS-CoV-2, COVID-19, surveillance, RT-qPCR, food safety

INTRODUCTION

The coronavirus disease 2019 (COVID-19) is a novel zoonotic viral disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Wu *et al.,* 2020). Similar to the other two *Coronaviridae* members (severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV)), SARS-CoV-2 also causes fatal infection in humans (Dhama *et al.,* 2020). The COVID-19 was first detected in humans in Wuhan, China that spread rapidly through human-to-human transmission, causing global pandemic (Wu *et al.,* 2020). The COVID-19 pandemic has brought and caused tremendous damage globally to human, health societies and economies. From the global perspective, this pandemic has caused over 249 million cumulative cases along with over 5 million cumulative human mortality as of 7 November 2021 (WHO, 2021). Unfortunately, these numbers are still increasing even with vaccination along with the emergence of SARS-CoV-2 variants.

Due to its sudden emergence and rapid global transmission, many aspects of SARS-CoV-2 and COVID-19 are still not understood. SARS-CoV-2 was postulated to be of animal origin with bat suspected as the primary host prior to human population (Wu *et al.,* 2020). Regrettably, the immediate animal ancestor or progenitor virus has yet to be identified and remains a mystery until now. COVID-19 is highly transmissible and contagious. To date, evidence of susceptibility of animals to the SARS-CoV-2 has been demonstrated through either experimental infection or in natural settings when in contact with infected humans (Abdel-Moneim & Abdelwhab, 2020; Haider *et al.,* 2020). Several animal species had been reported to be susceptible to SARS-CoV-2 including

livestock albeit SARS-CoV-2 being reported to be replicated poorly in cattle and pigs (Munir *et al.,* 2020; Goraichuk *et al.,* 2021). Nonetheless, the study of human to animal transmission and *vice versa* of COVID-19 is still lacking. Given the fact that COVID-19 is contagious and its fatal consequences, the potential transmission of COVID-19 via animal-based food products is of concern in terms of food safety and continuous food supply. The potential spread of SARS-CoV-2 along the food chain (production, post-harvest handling, processing, distribution/retail, and finally the dinner plates) is conceivable (Feng *et al.,* 2021). Such transmission could potentially occur through various routes such as direct contact with infected animals during handling and slaughtering, or indirect routes such as handling or consumption of the contaminated, undercooked meat and animal products (Olaimat *et al.,* 2020). Importantly, identifying animal species that are susceptible to SARS-CoV-2 infection not only safeguard food safety but also may elucidate the origin of the virus and mechanisms underlying cross-species transmission to humans, which may have involved an intermediate host. Herein, this study reports the surveillance of SARS-CoV-2 in imported meats and animal parts from July 2020 to November 2021. The finding in this study serves as a baseline data and also emphasizes the importance of better readiness and preparedness for zoonotic risks.

MATERIALS AND METHODS

Samples

Since July 2020, the Department of Veterinary Services has carried out surveillance on imported animal meats and animal parts as a consequence of the COVID-19 emergence and pandemic. A total of 225 samples from various major entering ports imported from seven countries were received for SARS-CoV-2 testing from July 2020 to November 2021 (Table 1). These samples consisted of major livestock species including buffaloes, cattle, goats and pigs.

Ribonucleic Acid (RNA) Extraction

Each sample (10-15 mg) was homogenized with mortar and pestle in the presence of sterile sand prior to centrifugation at 3 000 rpm for 10 minutes. The supernatant was filtered and viral RNA extraction following the manufacturer's instructions was carried out with the IndiSpin Pathogen Kit (Indical Bioscience, Germany). The extracted total RNA was stored at -20 °C until further analysis.

Quantitative Reverse Transcription Polymerase Chain Reaction (RT-qPCR)

The SARS-CoV-2 detection was carried out with RT-qPCR on the RNA-dependent RNA polymerase gene (*RdRP*) following Corman *et al*. (2020) that complies with World Health Organization guidelines (WHO, 2020). The detection was carried out with the RdRP_SARSr-P2 probe that is specific to SARS-CoV-2 only. The 20 uL RT-qPCR mixes contained a final concentration of 0.6 pmol of sense primer, 0.8 pmol antisense primer, 0.2 pmol of probe, 1 X qScript XLT 1 step RT-qPCR Toughmix (Quantabio, USA) and 5 uL of total RNA. The reaction was subjected to 45 °C for 10 minutes for reverse transcription, followed by initial denaturation at 95 °C for 10 minutes, 45 cycles of denaturation at 95 °C for 15 sec, annealing and extension at 58 °C for 45 sec. The amplification was performed on CFX Connect Real-Time PCR System (Bio-Rad, USA). The positive COVID-19 sample was obtained from Ipoh Public Health Laboratory, Perak and the RNA was extracted similar to the sample. The sample was considered and reported as positive for SARS-CoV-2 when the threshold cycle (C_t) value ≤ 35.

Table 1. Details of the sample received and tested for SARS-CoV-2 from July 2020 till November 2021.

Species	Year		Type of sample (2020)[a]	Exporting countries (2020)[a]	Type of sample (2021)[a]	Exporting countries (2021)[a]
	2020	2021				
Buffaloes	67	61	Blade (4) Boneless meat (3) Brisket (1) Cube roll (1) Lung (3) Rump steak (2) Shin shank (2) Slices (35) Tenderloin (12) Trimming (4)	India (67)	Blade (1) Hock tendon (1) Knuckle (2) Rump steak (2) Slices (42) Tenderloin (6) Topside (2) Trimming (5)	India (61)
Cattle	3	0	Striploin (1) Trimming (2)	India (3)		
Goat	8	5	Boneless Leg (2) Trunk (3) Shoulder (3)	Australia (8)	Boneless Leg (1) Meat (1) Shoulder (3)	Australia (2) New Zealand (3)
Pig	15	66	Back fat (2) Belly/sheet belly (10) Stomach (3)	Belgium (7) Germany (2) Spain (6)	Back fat (1) Belly/sheet belly (57) Humerus bone (4) Stomach (4)	Belgium (52) Denmark (10) Spain (4)
Grand Total	93	132				

[a]The number in the brackets indicates the total sample

RESULTS

A total of 93 and 132 samples were received and tested for SARS-CoV-2 in 2020 (July to December) and 2021 (January to November), respectively (Table 1). These samples were derived from numerous animal origins comprising various cuts of meat and animal parts. The most abundant samples received and tested were from buffaloes (n=128) followed by pig (n=81) samples. In contrast, only 13 and three samples from goat and cattle were received and tested during that period, respectively. The major exporting countries for these samples are Australia, Belgium, Denmark, Germany, India, New Zealand and Spain, with India monopolizing our importation of buffaloes and cattle's meat and parts. Of the 225 samples received and tested, none were detected positive for SARS-CoV-2 with the RT-qPCR that was specific to SARS-CoV-2.

DISCUSSION

Food safety is a vital element to secure that every food given to the consumers is safe to avoid potential foodborne infections and illnesses. It was estimated that zoonotic transmission from animals to humans has contributed to 75 % of new emerging infectious diseases in the past three decades (WHO, 2014). As such, the ongoing COVID-19 pandemic presents uncertainties and challenges to authorities responsible for national food safety control systems. To prevent disease transmission and to protect both human and animal health, there is a need for surveillance on animal-based products and meat for SARS-CoV-2 for risk of anthropozoonotic spill-over infections and potential foodborne transmission. From July 2020 to November 2021, a total of 225 various animal origin samples imported from seven countries were tested for SARS-CoV-2 by RT-qPCR. However, none of the tested samples were found to be positive for SARS-CoV-2. Nonetheless, this result is important in mitigating food safety and security risks from farm to fork and serves as timely baseline information for potential anthropozoonotic spill-over infections of COVID-19.

Surveillance and rapid disease detection play an essential role in informing disease prevalence and is of utmost importance especially during a pandemic. As a whole, this study reported the absence of SARS-CoV-2 in imported meat and animal parts. Clearly, the study result is not unexpected and corroborated with the non-existent risk of reverse zoonotic transmission of COVID-19 in farmed animals apart from farmed mink (CDC, 2021). Similarly, the result is also in agreement with a few experimental infection studies, whereby several domestic livestock including pig and cattle have been reported to be not susceptible or low susceptible to SARS-CoV-2 infection in experimental infection setting (Schlottau *et al.,* 2020; Shi *et al.,* 2020; Ulrich *et al.,* 2020). Moreover, the current COVID-19 pandemic is driven and sustained through human-to-human transmission. Nonetheless, the close resemblance of the angiotensin-converting enzyme 2 (ACE2) proteins, a key protein related to SARS-CoV-2 infection and entry to the cell, in several animals including pigs, cats, and ferrets to the human ACE2 receptor cannot entirely exclude the possibility of SARS-CoV-2 anthropozoonotic in farm animals (Zhao *et al.,* 2020), especially when in proximity to humans as evidenced in mink farm (Oude Munnink *et al.,* 2021). Furthermore, with the emergence of many pathogenic and contagious SARS-CoV-2 variants, the potential anthropozoonotic risk cannot be ruled out for now and warrant further study.

The existing state of the information on SARS-CoV-2 transmission through food is lacking, however, that does not dismiss that SARS-CoV-2 will not be transmittable along the food supply chain. To date, SARS-CoV-2 has been detected along the food supply chain involving imported frozen food (frozen shrimp and chicken meats) and COVID-19 outbreaks involving workers in slaughterhouses and meat packing factories (Feng *et al.,* 2021). Moreover, SARS-CoV-2 was

reported to be stable at low temperature (4 °C & -20 °C) (Feng *et al.,* 2021) and at different pH (4 to 11) at room temperature (Chan *et al.,* 2020). As such, SARS-CoV-2 is highly possible to be spread in the food supply chain especially through aerosol and fomite transmission (van Doremalen *et al.,* 2020; Zhang *et al.,* 2021). The risks should not be ignored and require further investigation.

As the COVID-19 is unlikely to disappear soon, proactive global and local monitoring of SARS-CoV-2 along the food chain is still required not only to safeguard food safety and security but also to protect lives. The findings from this study are crucial to the decision and policymakers at both national and global levels to inform effective strategies and policy responses to present and future outbreaks. This will enable timely interventions on disease preparedness and foresight, facilitating better countermeasures to mitigate the minimal impact on humans and animals.

The emergence and rapid transmission of COVID-19 have brought devastating consequences in many aspects. In addition, the exact origin of SARS-CoV-2 remains a mystery, hampering disease control and prevention. Generally, zoonoses and human-to-animal cross-species transmission are driven by human actions and activities in areas high in mammal biodiversity and experiencing anthropogenic land use changes (Allen *et al.,* 2017). Therefore, it is important to engage the relevant authorities in strengthening and expanding the One Health initiative to facilitate cross-sectoral collaborative actions. This will be paramount not only in preventing the next pandemic spill-over but also contributes to the better health of people, animals and the environment.

CONCLUSION

The various imported meat and animal parts (n=225) tested were negative for SARS-CoV-2. This study finding serves as a baseline data for SARS-CoV-2 in imported meat and animal parts. Explicitly, this study accentuated the importance of active surveillance to prevent zoonosis and to safeguard food safety and security.

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