



Presence of Neutralizing Antibodies to SARS-CoV-2 in Cats and Dogs Transported to Indonesia

YP ARIOS^{1,2*}, J PAMUNGKAS³, IWT WIBAWAN³, D ISKANDRIATI⁴, CS TAN⁵, SPH RAHMAN⁵

¹Animal Biomedical Science Study Program, School of Veterinary Medicine and Biomedical Sciences (SVMBS), IPB University, Bogor, West Java, Indonesia; ²Balai Besar Karantina Hewan, Ikan, dan Tumbuhan Papua, Indonesian Quarantine Agency, Jayapura, Papua, Indonesia; ³Division of Medical Microbiology, School of Veterinary Medicine and Biomedical Sciences (SVMBS), IPB University, Bogor, West Java, Indonesia; ⁴Primate Research Center, IPB University, Bogor, West Java, Indonesia; ⁵Faculty of Medicine and Health Sciences, Universiti Malaysia Sarawak, Kota Samarahan Sarawak, Malaysia.

Abstract | A substantial number of studies have documented the presence of SARS-CoV-2 in dogs and cats through RNA and/or antibody detection, following the initial reported case of companion animal infection in March 2020. The transmission of SARS-CoV-2 from and to companion animals is crucial because dogs and cats frequently share close proximity with their owners and often engage with people and other animals outside of their household. Earlier research has suggested that pets can create an immune response to SARS-CoV-2; yet, it remains unclear whether the antibodies produced can effectively shield these animals against the virus. Data and studies regarding the presence of COVID-19 in dogs and cats in Indonesia and those being trafficked are useful to provide initial information on the presence of this disease, as no research has been conducted at all in Indonesia. This study was conducted to determine the existence of a specific immune response against COVID-19 in dogs and cats. Samples were taken from two exporting countries and clinics in Indonesia. Samples were tested using the species-independent Surrogate Virus Neutralization Test (SVNT). A total of 12 sera (8 cat serum and 4 dog serum), based on the results of the Surrogate Virus Neutralization Test analysis, formed antibody titers against SARS-CoV-2 in as many as four individuals (33.33%). The existence of protected antibody titers in dogs and cats can provide initial information for further studies.

Keywords | Antibody titer, COVID-19, Dogs and cats, SVNT

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***Correspondence** | YP Arios, Animal Biomedical Science Study Program, School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, Indonesia; **Email:** putrianakbaik126@gmail.com

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INTRODUCTION

Corona Virus Disease 2019 (COVID-19) is a disease caused by Severe Acute Respiratory Syndrome Corona

Virus 2 infection. This virus considered the seventh coronavirus that infects human. The disease most likely emerged from animal sources and became a pandemic through widespread human-to-human transmission. Around 390

million confirmed human cases have been reported worldwide, with more than 5.7 million human deaths as of January 31, 2022. The emergence of animal infections caused by close contact between humans and animals is influenced by several factors, including the spread of novel zoonotic viruses, their effects on various animal species, and the vulnerability of these animals to infection. On the other hand, research indicates that certain animal species can pose a risk of infecting humans through close interaction with infected animals. This report provides a monthly overview of the global situation of SARS-CoV-2 in animals, with a specific emphasis on the recent submissions to the (WOAH, 2022) (Figure 1).

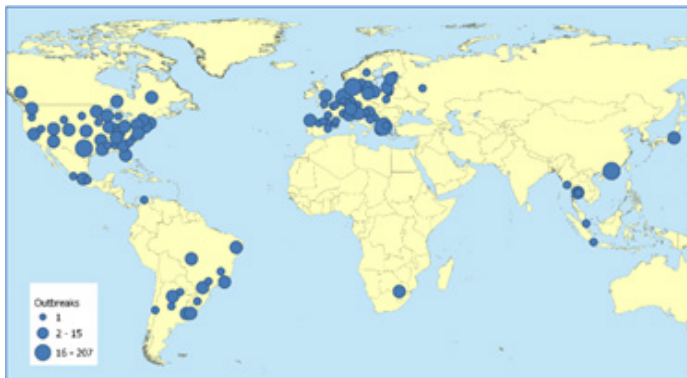


Figure 1: Global Situation distribution of SARS-CoV-2 outbreaks in fifteen animal species reported to the WOA (as of 31 January 2022). Note that dot size on the map is proportional to the number of outbreaks reported.

Indonesia is an archipelagic country with a high population density. Most residents have pets, such as dogs and cats. According to a survey conducted by the Rakuten Insight Global research institute on 97,000 respondents from China, the Philippines, Hong Kong, India, Indonesia, Japan, South Korea, Malaysia, Taiwan, Thailand, and Vietnam, it is revealed that in Indonesia, 59% of the population own cats, while only 10% own dogs. These pets are at a higher risk of contracting COVID-19 due to their close physical interaction with humans. The greater prevalence of dog and cat ownership drives the demand for a wide array of distinctive breeds of these pets, one of which is importing these animals from abroad into Indonesia. This can be seen from the trend of dog and cat importation, which continues to increase from year to year. However, during the COVID-19 pandemic in 2019-2020 the importation of dogs and cats decreased. Until now, there has been no statement that the transmission of COVID-19 in humans does not come from animals (dogs and cats). During the pandemic, many dog and cat owners were frightened and threw away or abandoned beloved pets.

Data on the import of dogs and cats from abroad to Indonesia via Soekarno Hatta Airport were recorded at 1,800 in 2020, 764 in 2021, 3,025 in 2022, and 2,380 in 2023 (Badan

Karantina Indonesia, 2023). The countries that are sources of imports include Argentina, Australia, Austria, Belarusia, Belgium, Brazil, Bulgaria, China, Colombia, Croatia, Denmark, France, Georgia, Germany, Hungary, Hong Kong, India, Iran, Ireland, Italy, Japan, Jordan, Malaysia, Portugal, Taiwan, Thailand, and many other countries (Table 1). Imports, which are carried out with quite a high frequency, are accompanied by an analysis of the risk of carrying animal diseases from the country of origin into Indonesia. The classification types of quarantine animal diseases are regulated in Minister of Agriculture Regulation Number 3238/kpts/pd.630/9/2009 of 2009 concerning Classification of Types of Quarantine Animal Pests, Classification and Classification of Carrier Media. The types of diseases being monitored are divided into quarantine animal pests, class one, and class two.

The Indonesian Quarantine Agency issued rules related to the movement of dogs and cats or animals vulnerable to COVID-19. This regulation is a reference in the implementation of monitoring of SARS-CoV-2 in animal media carriers, as stated in the Decree of the Head of the Agricultural Quarantine Agency number 1041/KPTS/HK.140/K/2/2022. Recently, research conducted by Arios et al (2023) proved the existence of immunity formed in dogs and cats that entered Indonesia through Soekarno Hatta airport. These dogs and cats were from the Netherlands and Russia, respectively. The formation of immunity in the dogs and cats tested raises new questions as to whether this immunity can protect or be protective in these animals, which is then associated with no transmission to other susceptible animals.

Various studies in several countries have shown that dogs and cats can be infected by COVID-19 and form antibodies. The high importation of dogs and cats into Indonesia raises the question of whether these animals still carry the virus in their bodies and can spread it, or have formed antibodies that can neutralize the presence of this virus, so as not to spread it during the process of traveling to Indonesia and within the area in Indonesia. This study aimed to determine whether the immunity formed in dogs and cats entering through the Soekarno Hatta Airport is protective. It is expected that the findings of this study will furnish information and serve as a foundation for formulating additional guidelines pertaining to the surveillance of COVID-19 in animals that have entered Indonesia. The protocol from the government can prevent the spread of COVID-19 from imported to naive animals in Indonesia.

MATERIALS AND METHODS

A total of 128 cat sera and 53 dog sera were obtained from the vena cephalica antibrachii or vena saphena magna using a 3 mL syringe. These 181 samples were tested using the

Table 1: Countries exporting dogs and cats to Indonesia.

Country	2020		2021		2022		2023	
	Dog	Cat	Dog	Cat	Dog	Cat	Dog	Cat
Australia	9	3	6	0	63	13	65	16
Austria	0	2	0	0	1	0	3	2
Belarusia	1	4	0	7	4	0	2	0
Belgium	28	0	0	0	3	3	6	12
Brazil	7	0	0	3	15	3	10	1
Bulgaria	7	4	0	2	6	8	2	4
Cambodia	2	1	0	0	9	2	18	4
Canada	7	2	0	0	16	0	6	4
Checko	8	5	0	3	21	54	9	18
Denmark	2	2	1	0	0	0	4	0
Egypt	1	0	0	0	0	5	4	4
France	4	3	0	4	17	22	19	16
Germany	23	6	8	7	37	13	23	20
Hong Kong	2	2	0	0	0	0	0	0
Hungary	13	0	5	3	17	22	15	3
India	3	3	0	0	14	11	9	3
Italy	3	4	1	1	5	10	4	12
Japan	9	5	4	2	25	31	27	25
Jordan	0	2	0	0	1	1	1	1
Kenya	1	0	0	0	0	3	2	0
Malaysia	15	41	7	27	53	87	37	41
Mexico	1	0	0	0	4	2	0	0
Myanmar	0	1	0	0	1	5	0	1
Netherlands	74	2	3	2	31	16	17	5
New Zealand	0	1	0		2	0	1	2
Oman	0	2	0	1	1	2	0	4
Philippines	18	2	11	0	53	7	34	7
Poland	6	11	0	5	21	39	14	34
Portugal	7	0	0	0	0	0	2	1
Qatar	1	8	0	3	0	7	3	8
Romania	4	3	0	0	6	8	10	0
Russian	59	634	11	357	189	558	122	337
Saudi Arabia	0	4	0	0	1	7	3	10
Serbia	5	0	6	0	81	1	56	2
Singapore	17	19	2	3	43	22	48	26
South Africa	6	27	2	0	14	64	5	8
South Korea	59	5	26	7	145	32	134	29
Spain	6	3	0	0	26	5	24	3
Switzerland	3	3	0	1	3	1	14	2
Taiwan	5	1	0	0	0	0	12	9
Thailand	12	13	0	1	31	43	126	45
Turkey	2	9	2	0	20	41	18	18
Ukraine	9	377	1	183	13	154	13	22
United Arab	10	6	0	11	10	20	12	20
United Kingdom	3	4	5	0	15	3	16	9
USA	67	15	17	1	103	32	106	33
Vietnam	3	1	1	0	96	353	52	342

ELISA method for the presence of antibodies. A total of eight out of 181 sera (4.42%) were seropositive for SARS-CoV-2, namely two dogs (3.8%) and six cats (4.7%). Sampling of dogs and cats was carried out at the Soekarno Hatta Agricultural Quarantine Center and two animal clinics in Bogor. We then tested these samples using sVNT to determine whether the antibodies formed could neutralize SARS-CoV-2. The samples used in this research were accompanied by informed consent from the relevant parties (owner/institution). Sample testing was performed at the Medical Microbiology Laboratory, University of Malaysia. The tools used in this study were a 96 plate microplate, Eppendorf tube, coolbox, freezer, centrifuge, well plate, multi-channel micropipette, single-channel micropipette, disposable tips, and reader. The materials used in this study were 70% alcohol, gloves, masks, the cPass™ SARS-COV-2 Neutralization Antibody Detection Kit, stationery, paraffin film, and distilled water.

SAMPLES

A total of 12 plasma serum samples were obtained from previous studies, comprising 8 cat sera and 4 dog sera. The samples were taken from the vena chepalica antibrachii or vena saphena magna using a 3 mL syringe. The samples were then transferred to the laboratory for testing for the presence of antibodies and genetic material. Samples that were positive for antibodies were then tested in this study. All serum samples were handled as if capable of transmitting infectious agents. Samples were collected according to standard laboratory protocols, which typically involve aseptically collecting them through venipuncture or finger-prick. Clotted, contaminated, and viscous specimens cannot be used. The centrifugation of the serum was carried out before conducting any tests. If the serum has not yet been tested and needs to be stored, it should be kept at a temperature of -20°C or lower. Strive to prevent repeated freeze-thaw cycles. This handling and storage advice is derived from reliable sources provided by the manufacturer. The serum remains stable for up to 16 days when stored at a temperature ranging from 2°C to 8°C.

NEUTRALIZATION ANTIBODY DETECTION

The sVNT cPASS™ (a product of Genscript, located in Nanjing, China) is employed to assess the quantity of SARS-CoV-2 neutralizing antibodies present in the plasma serum obtained, according to the manufacturer’s instructions. Diluted positive control, diluted negative control, and diluted samples were combined with diluted HRP-RBD at a 1:1 ratio in tubes and then incubated at 37°C for 30 minutes. Following this, 100 µL of each of the positive control mixture, negative control mixture, and sample mixture were added to their respective wells and incubated at 37°C for 15 minutes. The plate was then washed four times with 260 µL of 1x Wash solution per well. After this, 100 µL of TMB Solution was added to each well, and the plate was left in

the dark at 20-25°C for 15 minutes. The Stop Solution (50 µL) was added to each well to terminate the reaction, and the plate was examined immediately. The Optical Density (OD) at 450 nm was determined using the SpectraMax ID3 device (Molecular Devices, California, US). The formula for calculating percent inhibition (%) was applied, which is $(1 - \text{OD sample value} / \text{OD negative control}) \times 100\%$. A manufacturer's positive cutoff value of $\geq 30\%$ inhibition was utilized.

cPass™ technology mimics the interaction between a virus and a host cell to rapidly detect the total neutralizing antibodies (NAbs) present in a sample. This is achieved by simulating the process of a viral receptor binding protein (RBD) binding to the host cell's membrane receptor protein (ACE2); cat (*Felis catus*) has a similarity of ACE2 receptors by 85.2% while dog (*Canis lupus familiaris*) by 83.4% (Stout *et al.*, 2020) it can be said to be homologous. The sVNT assay is an ideal choice for standardization purposes because of its lack of species bias, elimination of the need for biocontainment facilities, and absence of cross-reactivity with antibodies that target other well-known coronaviruses, except for SARS-CoV (Tan *et al.*, 2020).

Table 2: SARS-CoV-2 Antibody Neutralization Detection Test Results (cPass™).

No	Country	Animal	Result
1	Russia	Cat	Positive
2	Dutch	Dog	Positive
3	Belarus	Dog	Negative
4	Russia	Cat	Positive
5	Indonesia	Cat	Negative
6	Indonesia	Cat	Negative
7	Indonesia	Cat	Negative
8	Russia	Cat	Negative
9	Indonesia	Cat	Negative
10	Indonesia	Cat	Negative
11	Russia	Dog	Negative
12	Russia	Dog	Positive

RESULTS AND DISCUSSION

Neutralizing antibodies to SARS-CoV-2 were detected in 33.3% of animals in this study (Table 2), with the seroprevalence found in dogs (50%) and cats (25%) (Table 3). The majority of neutralizing antibodies are capable of preventing the interaction between the spike protein of SARS-CoV-2 (RBD) and the human ACE2 receptor (hACE2) by 31-32% (dogs) and 81-89% (cats). There were two cats whose inhibition levels almost reached the cut off, namely 25% and 26%. The highest inhibition occurred in two cats from Russia, while in dogs, one came from the Neth-

erlands (32%) and followed by a dog from Russia (31%) (Figure 2). During the initial stages of the COVID-19 pandemic, it was commonly thought that domestic dogs and cats were not vulnerable to SARS-CoV-2. Nevertheless, this notion shifted in February 2020 when the first known case of human-to-animal transmission occurred in Hong Kong. In this case, an elderly Pomeranian dog with multiple pre-existing health problems and a 2-year-old male German shepherd tested positive for SARS-CoV-2 through serological and molecular testing methods, such as RT-PCR (Tan *et al.*, 2022).

Table 3: Seropositivity of dogs and cats against SARS-CoV-2 in Indonesia.

Results	Animal		Total no (%)
	Dog no (%)	Cat no (%)	
Positive	2 (50)	2 (25)	4 (33,3)
Negative	2 (50)	6 (75)	8 (66,6)
Total	4	8	12

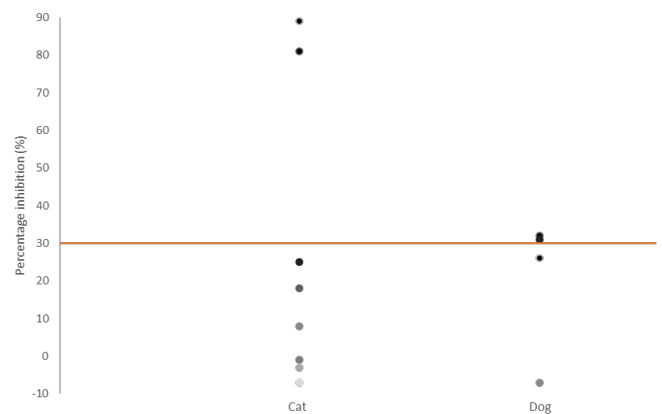


Figure 2: The measurement of neutralizing antibodies in dogs and cats' plasma represented as percentage inhibition. Horizontal dashed line represents the positive cut off of $\geq 30\%$ inhibition.

As per certain researchers, there is a possibility that cats, if infected with SARS-CoV-2, could act as a biological dead-end for the virus (Shi *et al.*, 2020). In order to have a comprehensive discussion on the transmission of SARS-CoV-2 by cats, it is necessary to conduct a large-scale, blinded, and randomized cohort clinical study that removes the influence of human factors on the outcomes. This type of study is essential to eliminate potential biases and ensure accurate results. Inaccurate interpretation could have severe implications that cannot be undone (Tazerji *et al.*, 2020).

We found that 33.3% of the animals had neutralizing antibodies, with a seroprevalence rate of 50% in dogs and 24.2% in cats. By 30.0-32.0% in dogs and 30.0-89.9% in cats, neutralizing antibodies can prevent SARS-CoV-2's spike protein from binding to human ACE2 (hACE2), with the highest inhibition observed in cats. It is interesting to note

that numerous countries have observed higher seropositivity rates in cats than in dogs, which may be attributed to the stronger affinity of the RBD (receptor binding domain) of SARS-CoV-2 for cat ACE2 (cACE2) compared to dog ACE2 (dACE2) (Schoeman dan Fielding, 2019).

The outcomes of this test are reported on a qualitative basis as either positive or negative. A positive result, where the indicator is greater than 30%, signifies the presence of SARS-CoV-2 neutralizing antibodies. Conversely, a negative result, where the indicator is below 30%, suggests the absence or insufficient levels of SARS-CoV-2 neutralizing antibodies below the detection limit of this particular test. It is important to note that a negative result may also occur in samples taken during the acute phase of infection before seroconversion, particularly if the antibody titer against SARS-CoV-2 in the sample is below the sensitivity of the assay kit.

The relationship between virus variants and the extent of infectiousness and peak viral load is not always consistent. While viral load is often used as an indicator of infectiousness, it has not yet been conclusively demonstrated that there is a direct link between the two (Owusu *et al.*, 2021). These are the animal cases (dogs and cats) notified by the WOA (2020) (Animal Health Organization) by country in some countries: Dog in Hong Kong on February, cat in Belgium on March, bigcat in USA on March, cat in France on May, cat in Spain on April, cat in Germany on April, cat in Russia on June, cat in United Kingdom on July, dog in Japan on July, puma in South Africa on July, cat in Italy on December, cat in Chile on May, dog in Canada on October, cat and dog in Brazil on October, cat and dog in Argentina on September, and cat in Switzerland on December.

According to the available epidemiological data, pets have consistently been found to be associated with humans infected with COVID-19. Among domestic pets, cats are more commonly infected than dogs. Cats tend to exhibit mild symptoms when infected with the virus, with sneezing being the most commonly reported sign. Additional symptoms may include weakness, apathy, and loss of appetite. In contrast to other animals, dogs have demonstrated a relatively low vulnerability to the virus, with just 22 reported cases worldwide and a solitary symptomatic case documented outside of the United States (Bonilauri and Rugna, 2021).

Human activities play a significant part in the emergence of novel coronaviruses by disrupting natural habitats, displacing wild animals, and promoting interactions between species that would not occur under normal circumstances. Ultimately, this creates new pathways for the transmission of coronaviruses between wild animals and humans (McNeely, 2021; Morens and Fauci, 2020). According to the

World Organization for Animal Health (OIE) expert panels, there is currently no scientific evidence to support the transmission of the virus responsible for COVID-19 from animals to humans. Individuals who are infected should minimize their interactions with animals and relocate their pets to relatives' homes or designate a separate room for isolation, all while diligently following personal hygiene guidelines and preventing their pets from leaving the house or coming into contact with other animals (CDC, 2022).

The Federal Center for Animal Health in Russia is primarily responsible for addressing infections in animals. This includes conducting monitoring studies among high-risk animal groups, studying virus properties upon detection (such as antigenicity, immunogenicity, virulence, and tropism for various cultures), improving laboratory diagnostics and specific prevention methods, undertaking educational pursuits and creating methodological resources, while simultaneously participating in international endeavors that concentrate on investigating coronaviruses, such as the IAEA/FAO collaborative efforts (Donnik *et al.*, 2022).

Coronavirus infections are the most significant emerging threat due to their unpredictable mutations and capacity for cross-species transmission (Ji *et al.*, 2020). At present, the World Organization for Animal Health (WOAH) reports no evidence of human infection with COVID-19 in animals (WOAH, 2020). As a preventive measure, people who have contracted coronavirus are recommended to refrain from both human and pet interaction to minimize the risk of inter-species infection. Vaccination and veterinary control are crucial in combating the spread of emerging infectious diseases, such as coronaviruses. By administering vaccine preparations, animals can build immunity against these diseases, thereby reducing the likelihood of outbreaks (Donnik *et al.*, 2022).

SARS-CoV-2 has demonstrated the capability to evolve and acclimate to animals, which may result in viral traits that could potentially influence public health. However, at this time, there is no concrete evidence to back up this claim (Zhou *et al.*, 2022). Furthermore, it is conceivable that the SARS-CoV-2 variant virus could persist among the animal population for an extended duration without any significant correlation to the human population's corresponding virus variant (Madhuzoodanan, 2022). Various animal species may experience evolutionary changes within their hosts, potentially resulting in the transmission of more susceptible human populations with the evolved variants, which could then be circulated among the general public (Tan *et al.*, 2022). This could potentially impact vaccine efficiency, the severity of the disease, and the spread of the virus.

Animals that have been infected with SARS-CoV-2 pose

a risk to individuals who come into direct, unprotected contact with them. This threat may pertain to individual members of a population, such as those residing in a household, or to specific groups with occupational exposure in the animal sector, including but not limited to mink farm workers, rangers, veterinarians, zoo or pet shop employees, quarantine officers, and others (Fang *et al.*, 2024). Not only individuals, but also groups of people can be recognized as occupationally exposed to the animal species that have the potential to transmit diseases.

When examining cats and dogs, hamsters, cats, and ferrets are among the animals most susceptible to contracting and transmitting the virus to their own species as well as to other animals and humans (Munnink *et al.*, 2021). Since the emergence of the Omicron variant, rodents have also demonstrated susceptibility to infection and potential for further transmission. Dogs can become infected with the virus, but they do not consistently transmit it, suggesting a lower risk associated with this species (WOAH, 2022). An outbreak that affected both humans and their pet animals was only recorded in a pet store in Hong Kong where hamsters were sold. Companion animals typically exhibit limited clustering and sporadic transmission to humans, with the viruses showing a lack of distinct evolution that is specific to species (Kok *et al.*, 2022).

Indonesia itself must issue regulations regarding the movement of dogs and cats from abroad. The requirements that are required to be met to avoid the transmission of COVID-19 from animals to other animals are of course important. Of the many animals that have antibodies against COVID-19, only a few can protect them. The time of animal shipment is an important moment, where if the animal's body has not formed antibodies, there may be a risk of transmission to surrounding or transported animals. The aim of the study was to obtain data provided to the government (policy maker), thus emphasizing the need to carry out a better risk analysis for COVID-19 on pet animals due to importation and regulation as well. After the regulation is established, it will be given to all related parties so that it can become a protocol for examining animals (dogs and cats) related to COVID-19. For example, in the method of detecting and testing COVID-19 in dogs and cats. One of the government programs is to conduct continuous monitoring of dogs and cats entering Indonesia because these animals will spread throughout Indonesia.

Although not many, with the discovery of antibodies that can neutralize COVID-19 in dogs and cats, it is known that dogs and cats have been infected with COVID-19 and produce antibodies, and some animals have managed to produce antibodies that can neutralize the presence of this virus. It is likely that the virus has been eliminated before it can be transmitted to other naive animals. Animal owners

can be given an explanation not to panic and continue to carry out animal health protocols, considering that during the pandemic, many animal owners were frightened and threw away their dogs and cats because of fears of transmission of this disease from animals to humans. To date, no studies have shown the transmission of COVID-19 from animals to humans (zoonosis), but transmission from animals to other naive animals can occur.

CONCLUSIONS AND RECOMMENDATIONS

There is a specific antibodies against COVID-19 found in dogs and cats from Russia and the Netherlands. To mitigate the risk of infecting pets, there must be rules regarding the examination of dogs and cats from countries with high COVID-19 status in animals, which are enforced as monitoring, not only detecting the presence of genetic material or antibodies but also protective antibodies.

The government must make a regulation based on a risk analysis related to the importation of animals (dogs and cats) into Indonesia, which must be enforced when there is an import. This rule will become a standard protocol and a requirement for the importation of pet animals and must be socialized throughout Indonesia. This research emphasizes the importance of setting up extensive, long-term surveillance programs that monitor both serological and molecular data to keep track of SARS-CoV-2's prevalence and evolutionary patterns in companion animals. Introducing these programs will provide crucial information for public health initiatives and guarantee the well-being of both humans and their animal companions in the face of ongoing transmission trends. These data must be provided to the government (policy makers) so that a better analysis of the risk of COVID-19 in companion animals imported into Indonesia can be made immediately. Regulations for monitoring and inspecting imports of cats and dogs must continue to be enforced to prevent the spread of COVID-19 between companion animals (dogs and cats).

The following limitations should be acknowledged in this study: a sample size that is relatively small could lead to either an underestimation or overestimation of the true seropositivity neutralization antibody rate. To obtain a more accurate seroprevalence, it is crucial to conduct further serological tests on a larger number of samples from dogs and cats in clinics and households that have had COVID-19 disease. Additionally, developing vaccines for animals should be made a requirement for the traffic of dogs and cats to Indonesia, especially from countries with cases of COVID-19.

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NOVELTY STATEMENT

The presence of antibody responses and neutralization events against SARS-CoV-2 virus in companion animals that were transshipped into Indonesia

AUTHOR'S CONTRIBUTIONS

YPA wrote the article. YPA, JP, IWTW, and DI critically revised the article. All authors contributed to the article and approved the submitted version

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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