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[illegible]

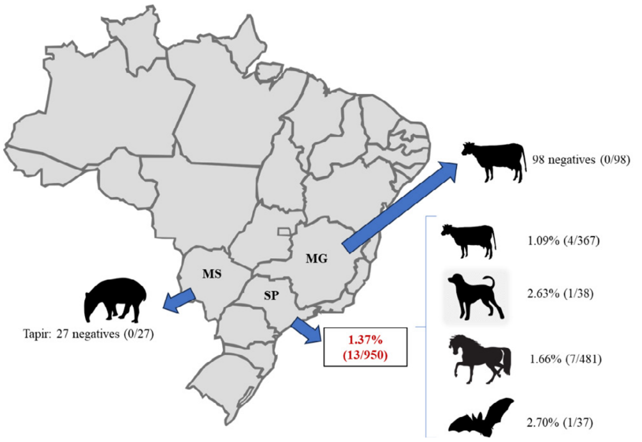
result.

Results

[illegible]

[illegible] Brazilian States evaluated for SARS-CoV-2, according year.

Brazilian States	Year			Total analyzed
	2020	2021	2022	
São Paulo	215	40	570	825
Minas Gerais	60	38	0	98
Mato Grosso do Sul	0	0	27	27
Total analyzed	275	78	597	950



[illegible] Frequency of SARS-CoV-2 according to animal species and Brazilian States.

MG = Minas Gerais, MS = Mato Grosso do Sul, SP = São Paulo

[illegible]

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Table 3: Frequency of antibodies anti-SARS-CoV-2 according to species and gender.

Gender	Equine		Bovine		Canine		Bat		Tapir		Total analyzed
	Reactor	Non reactor	Reactor	Non reactor	Reactor	Non reactor	Reactor	Non reactor	Reactor	Non reactor	
Male	6	316	2	300	0	25	0	9	0	15	673
Female	1	157	1	53	1	13	1	27	0	12	266
Not informed	n/a	n/a	1	10	n/a	n/a	n/a	n/a	n/a	n/a	11
Total	7	473	4	363	1	38	1	36	0	27	950

n/a not applicable

All seropositive samples were retested in triplicate to confirm the initial diagnosis.

Equine Seroprevalence

The horses were analyzed in 2022 to 2023. Of the 481 equine serum samples, 321 were males and 160 females, which seven animals seropositive were identified (six male and one female) and six of them were monitored for the persistence of anti-SARS-CoV-2 antibodies over time (Figure 2). The horses 1 and 2 only presented antibodies in the first analysis on May 2022. The horses 3 and 4 showed a persistence of antibodies until January 2023. The horse 6 was analyzed on June 2022 and presented antibodies anti-SARS-CoV-2, however on September 2022 was considered seronegative in ELISA test. Considering that horses may moved up to another Military Police unit, the same animal was not tested on January 2023.

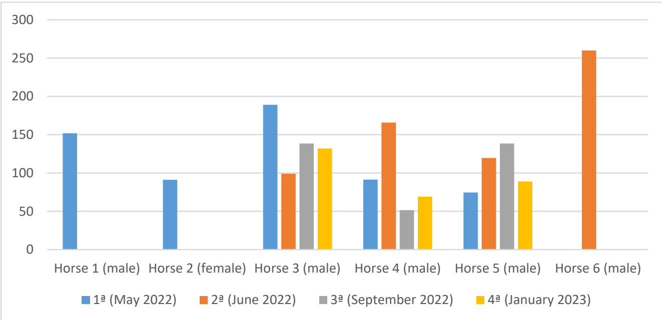

Figure 2: Evaluation of persistence of antibodies anti-SARS-CoV-2 in horses.

Canine Seroprevalence

Of the 38 dog samples analyzed, only one animal was seropositive, and its analysis was repeated in triplicate to confirm the laboratory diagnosis. A new serum was collected from this animal one month after the first analysis. Both serum samples were tested in triplicate, in the same reaction, with a reactive result of 172.52% (1st analysis) and inconclusive (2nd analysis), as it presented 58.69% (inconclusive 50-60%), as shown in Figure 3. When comparing the two results, it was found that the level of antibodies decreased between the first and second analyses.

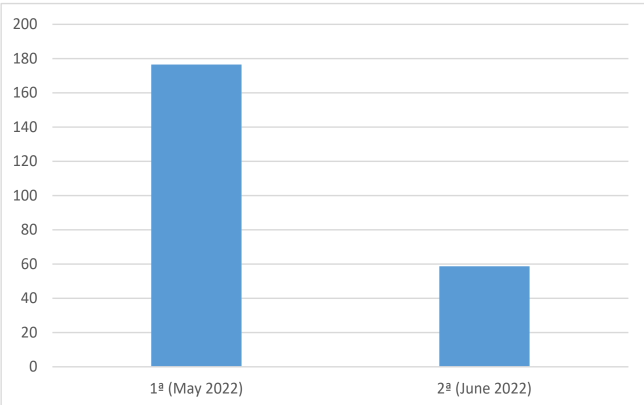

Figure 3: Evaluation of persistence of antibodies anti-SARS-CoV-2 in only one dog seropositive.

Table 2: Frequency of cattle reactive for SARS-CoV-2, classified according to respiratory symptoms and gender.

Respiratory symptoms	Total number of samples analysed	Number of animals reactive to Covid-19	Gender	Frequency (%)
Presence	61	1	Female	3.28
		1	not informed	
Absence	306	2	Male	0.65

Tapir (Tapirus terrestris)

Of the 27 tapir serum samples, all were seronegative for COVID-19.

Bat (Desmodus rotundus)

A total of 37 bats of the hematophagous species, *Desmodus rotundus*, were analyzed, and one male animal was identified with antibodies against SARS CoV-2 (S/P% 64.1).

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Discussion

The COVID-19 pandemic has brought reflections on the one-health approach, the importance of integrated action to prevent emerging and re-emerging diseases [40]. The World Organization for Animal Health quickly positioned itself for institutions working in animal health to propose to conduct studies on covid-19 in animals [41]. Brazil has reported more than 37 million cases of COVID-19, according to WHO data. With the high prevalence of cases among humans, human-animal transmission has become proportionally increased [26-29]. The present study in domestic and wild animals comprised the pandemic period from 2020 to 2022 and post-pandemic, represented by the oscillation of SARS CoV-2 waves in humans in the State of São Paulo and its different variants detected over time.

This randomized study identified the ability of SARS CoV-2 to infect different animal species, whether domestic or wild, with or without clinical symptoms compatible with respiratory diseases. The frequency of anti-SARS-CoV-2 antibodies was higher in domestic animals, although the sample number was lower for wild animals. In the literature consulted, the main way SARS-CoV-2 is transmitted to animals is through close contact with humans. Thus, it was evidenced that horses and dogs belonging to the Military Police of São Paulo, because they carry out policing rounds and have intense contact with other humans and with the police officers themselves, explain a higher prevalence of anti-SARS-CoV-2 antibodies in relation to other animal species, such as cattle, tapir and bats.

Few cases of SARS-CoV-2 infection in horses are reported, however, studies indicate that the equine ACE2 receptor (eqACE2) has an affinity for the RBD of the virus, similar to what occurs in humans [32-33]. The exposure of horses to SARS-CoV-2 occurs, however, it is not always detectable by RT-PCR and, for this reason, studies that show the presence of anti-SARS-CoV-2 antibodies in these animals indicate that there was a previous infection [34]. In the present study, when monitoring the horses, it was found that in two animals, the presence of antibodies was detected for up to eight months, approximately, which corroborates findings in the literature, which detected horses for at least 21 days [33]. In humans, it was evidenced that neutralizing antibodies to SARS-CoV-2 lasted up to 10 months [35].

In the bovine species, the frequency of animals carrying anti-SARS-CoV-2 antibodies, with or without respiratory symptoms, was similar to the findings of the literature. Wernike et. al, detected a seroprevalence of 1.1% (11/1000) in cattle from different farms [31]. The absence of severe symptoms related to covid-19 in cattle can be explained by immunological cross-protection, possibly between SARS-CoV-2 and the bovine coronavirus, due to the high homology observed between the epitopes of the spike protein of these two viruses, given that the latter already affects cattle [30]. In Brazil, there are cases of bovine coronavirus associated with diarrhea and associated with respiratory problems, called winter dysentery [38-39], and a vaccine is adopted to control it. In the present study, we did not obtain information on whether the animals received vaccine for bovine coronavirus and whether there would be cross-reactivity in the ELISA test for SARS CoV-2.

Similar to what happens with cattle, the high genetic similarity between SARS-CoV-2 and the canine respiratory coronavirus (CRCoV) can promote some immune protection, preventing them from contracting the severe form of covid-19 [30]. In the present study, only one animal seropositive for Covid-19 was detected, however, the persistence time is unknown, since only two samples were evaluated, and the last one was considered inconclusive, which denotes a gradual drop in antibodies. Decaro *et al.*, 2022 detected antibodies in the serum of dogs around 10 months after contact with the SARS-CoV-2 virus, where the animals were monitored soon after their exposure and infection by the virus, confirmed by RT-PCR [36]. The fact that the Military Police dogs received a vaccine against coronavirus disease may have promoted cross-protection and, consequently, a more efficient response to SARS CoV-2 infection, since the animal did not present, during the study period, any respiratory or enteric condition. Further studies should be conducted to evaluate these issues.

The serological results of covid-19 in wild animals corroborate the need for human contact to promote a higher rate of infection in these species. In the literature, cases of wild animals with anti-SARS-CoV-2 antibodies have been described, but it is believed that these animals became infected by foraging in regions close to human contact [37]. In the present study, the fact that the seropositive bat came into contact with humans, although remote, is possible since this animal belonged to a region that carried out anthropogenic activity. Another possibility is a cross-reactivity with other bat coronaviruses. The antigen used in the ELISA kit has high similarity with other coronaviruses such as SARS and MERS natural to bats.

Conclusions

This research showed the susceptibility of domestic and wild animals to the SARS-CoV-2 virus, through the identification of animals with anti-SARS-CoV-2 antibodies. The persistence of antibodies in animals was observed in horses, similar to what occurs in humans. This study contributed to the understanding of SARS-CoV-2 in domestic and wild animals, in line with what was proposed by the World Organization for Animal Health to promote one health.

Author Contributions

All the authors contribute equally to develop this research

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Institutional Review Board Statement

The animal study protocol was approved by the Institutional Review Board (or Ethics Committee) of the Biological Institute of São Paulo (protocol code 181/22, 05/10/2022).

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Conflicts of Interest

The authors declare no conflict of interest.

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