SARS-CoV-2 surveillance between 2020 and 2021 of all mammalian species in two Flemish zoos (Antwerp Zoo and Planckendael Zoo)

Léa Joffrin[1*], Tine Cooreman[1*], Erik Verheyen[1,2], Francis Vercammen[3], Joachim Mariën[1], Herwig Leirs[1], Sophie Gryseels[1,2]

[1]Evolutionary Ecology Group, Department of Biology, University of Antwerp, Antwerp, Belgium
[2]OD Taxonomy and Phylogeny, Royal Belgian Institute of Natural Sciences, Brussels, Belgium
[3]Centre for Research and Conservation, Antwerp Zoo Society, Antwerp, Belgium

* These authors contributed equally to this work.

ABSTRACT

The COVID-19 pandemic has led to millions of human infections and deaths worldwide. Several other mammal species are also susceptible to SARS-CoV-2, and multiple instances of transmission from humans to pets, farmed mink, wildlife and zoo animals have been recorded. We conducted a systematic surveillance of SARS-CoV-2 in all mammal species in two zoos in Belgium between September and December 2020 and July 2021 in four sessions, and a targeted surveillance of selected mammal enclosures following SARS-CoV-2 infection in hippos in December 2021. A total of 1523 faecal samples were tested for SARS-CoV-2 via real-time PCR. None of the samples tested positive for SARS-CoV-2. Additional surrogate virus neutralization tests conducted on 50 routinely collected serum samples during the same period were all negative. This study is a first to our knowledge to conduct active SARS-CoV-2 surveillance for several months in all mammal species of a zoo. We conclude that at the time of our investigation, none of the screened animals were excreting SARS-CoV-2.

Keywords: SARS-CoV-2, surveillance, zoo, mammals, Belgium

INTRODUCTION

COVID-19 emerged in China in 2019. It is caused by an until then unknown coronavirus that causes severe acute respiratory syndrome (SARS-CoV-2). This infectious disease spread to all continents in a few months and was declared a pandemic by the World Health Organisation in March 2020. SARS-CoV-2 can be transmitted through three main routes: direct contact with infected secretions (saliva, respiratory secretions), droplet transmission (when coughing or sneezing), and aerosol transmission (1). Through experimental infections in the search for a suitable animal model, and reported infections in pets already early in the pandemic, it became clear that several animal species may be susceptible to SARS-CoV-2 infection (2–8). Experimental in vivo and in vitro infections showed that SARS-CoV-2 can infect a wide taxonomic range of mammals, including a.o. North American deer mice (*Peromyscus maniculatus*), macaques (*Macaca mulatta* and *Macaca fascicularis*), domestic cats (*Felis catus*), ferrets (*Mustela putorius furo*), American mink (*Neovison vison*), raccoon dogs (*Nyctereutes procyonoides*), Syrian hamsters (*Mesocricetus auratus*), and Egyptian fruit bats (*Rousettus aegypticus*) (3,9–18). Circulation of SARS-CoV-2 was reported in farmed American mink (*Neovison vison*) in a multitude of farms around the world, and wild white-tailed deer (*Odocoileus virginianus*) across North America (2–6).

In addition, functional, structural, and genetic analysis of viral receptor ACE2 orthologs reveal that many other species may be susceptible to SARS-CoV-2 (19,20). While these studies may appear helpful to estimate the potential host range of SARS-CoV-2, observed natural infections highlight that susceptibility based on the ACE2 receptor alone is not a sufficient proxy to estimate potential spillover risk to other species (21). For example, mink or wild white-tailed deer are not considered highly susceptible based on these in silico analysis (5).

According to the open access dataset of reported SARS-CoV-2 events in animals (data from January 2023), about 119 zoo animals have been reported with a SARS-CoV-2 infection, representing 64

report events, 17 species in 17 countries (22). The most frequently reported infected mammals in zoos are felines, followed by primates (23). However, detection of SARS-CoV-2 infections in zoo animals has relied on the observation of symptoms (cough, nasal discharge), behaviour changes (reduced appetite, lethargy), or death of these captive animals (24,25). SARS-CoV-2 infections may therefore remain undetected if animals do not show obvious symptoms.

Since infected animals have been found in zoos worldwide, and the long-term high incidence of the virus in humans, we deemed it prudent to monitor the presence of SARS-CoV-2 in zoo animals. Furthermore, the high diversity of animals in zoos, both regarding taxonomy and geographical origin, makes zoos an ideal place to (i) contribute to unravelling the potential host range of SARS-CoV-2 and (ii) evaluate the risk for the conservation of wild animal populations in captivity and *in situ*. For this study, we investigated the potential circulation of SARS-CoV-2 in zoo mammal species by sampling and screening faecal samples from all the mammals in two zoos in Belgium in four sessions between September 2020 and July 2021 via real-time polymerase chain reactions (PCR). Following the symptomatic SARS-CoV-2 infection in hippos in the Antwerp zoo in December 2021 (26), we additionally surveyed selected mammals deemed in potential indirect contact with the hippos or with expected relatively high SARS-CoV-2 susceptibility.

MATERIAL AND METHODS

Samples collection

We conducted this study at the Antwerp Zoo and Planckendael Zoo, in respectively Antwerp and Mechelen, Belgium. We collected the samples during four periods (early September 2020, mid-October 2020, mid-December 2020 and July 2021), with sampling following enclosure cleaning planning. During the first sampling period, both zoos were still open to the public; during the second sampling series both zoos were closed to the public and remained closed until after the third sampling due to government regulations. The zoos reopened in February 2021, and the fourth sampling session was conducted in July 2021. During the first three sampling sessions the original Wuhan-Hu1 variant was dominant in the human population in Belgium, during the fourth the delta variant, considered as more contagious than the previous alpha, beta, and gamma variants (27), was dominant in Belgium.

Faecal samples were collected by zookeepers in a 16.5 mL tube filled with RNAlater and then stored at -20 °C at the zoo for a few days before transport to the lab where the samples were stored at -80 °C. RNAlater is a suitable conservation medium widely used for microbiological studies (28,29). The date and freshness of each sample were documented (maximum two hours old, or not more than twelve hours old) after which the samples were stored. A maximum of five samples per species, per zoo, were collected at each sampling session. A total of 1417 faeces samples were collected from 103 different mammal species (Antwerp N= 48 and Planckendael N= 67) (**Table**). In Antwerp Zoo, the largest sampled taxonomic group was the Primates, followed by the order of the Cetartiodactyla. In Planckendael, Cetartiodactyla was sampled most often, followed by the order of the Carnivora. Additionally, 50 blood samples from 26 mammal species were available from routine collection by the zoo veterinary service, both before (14 samples/12 species) and after (36 samples/ 26 species) 2020, for animals that either moved between zoos or for those requiring a veterinary follow-up (pregnancy, injury, illness).

After our systematic surveillance was completed, two female hippopotamuses in Antwerp Zoo showed evidence of nasal discharge in late November 2021 for a few days (26,30). SARS-CoV-2 was detected by immunocytochemistry in nasal swab samples, and by PCR in nasal swab samples, faeces, and pool water (26). Serological tests also detected antibodies against SARS-CoV-2.

Following these hippo infections, we conducted in December 2021 a targeted surveillance for SARS-CoV-2, collecting samples from mammals that could have been in indirect contact with the hippo individuals (i.e. if they were managed by the same caretakers) or that were of special interest due to their known increased susceptibility and conservation status, namely primates and large felines. We

screened these samples with the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel, specifically targeting SARS-CoV-2 genes, which was also used for the diagnosis of SARS-CoV-2 in the infected hippopotamuses' faecal samples (31). Details on the 106 samples collected and tested are also available in **Table**.

Sample preparation, extraction and PCR testing

After thawing, the samples were processed under a Biosafety cabinet class II. Around 1 cm^3 of the faeces was cut off, rinsed with 200 µL of Phosphate-buffered saline (PBS), and mixed in a 1.5 mL Eppendorf tube filled with 800 µL of PBS. The tubes were briefly vortexed, centrifuged (1500g for 15 min), and for each collection date/enclosure/species, samples were pooled to extract faecal RNA using the QIAGEN QIAamp viral RNA kit (Qiagen, Valencia, CA) following the manufacturer recommendations. Overall, 420 pools were extracted. Reverse transcription was performed on 8 µL of RNA extract using the Maxima Reverse Transcriptase and Random Hexamer Primers (Thermo Fisher Scientific, Waltham, MA) on a Biometra T3000 thermocycler (Biometra, Westburg, The Netherlands). A Pan-coronavirus system suitable for the detection of alpha-, beta-, gamma- and delta-CoVs real-time PCR adapted version of the Muradrasoli et al. (2009) protocol (32,33) was used on a StepOne™ Real-Time PCR System (Applied Biosystems, Carlsbad, CA) to screen the samples for all potential coronaviruses that may occur in the zoo animals.

Validation of the PCR system for the detection of SARS-CoV-2

We conducted assays to validate the use of the Pan-CoV system for the detection of SARS-CoV-2 in our samples. We compared the limit of detection of the Pan-CoV system targeting the polymerase gene to the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel specifically targeting SARS-CoV-2 N1 gene (31). The limit of detection was determined to be the lowest dilution that still resulted with a Ct value. RNA from a SARS-CoV-2 positive clinical sample was used to conduct this assay. The CDC Real-Time RT PCR was performed on a serial dilution of the positive sample RNA ranging from 10^{-1} to 10^{-8}. The same 8-fold dilution series was reverse transcribed to cDNA (using the Maxima RT protocol described above), which was then run on the Pan-CoV Real-Time PCR.

In addition, a synthetic N1 and N2 gene positive control (2019-nCoV_N_Positive Control, Integrated DNA Technologies) with known copy number was used in the CDC panel at three concentrations: 2000 copies/ µL, 200 copies/ µL and 20 copies/ µL. The Pan-CoV Real-Time PCR was used on the cDNA synthetised following the reverse-transcription protocol described above, from the SARS-CoV-2 positive clinical sample RNA ranging from 10^{-1} to 10^{-8}. Each dilution was tested in triplicates.

For each system, the standard curve of the positive clinical sample was calculated by plotting PCR cycle threshold (Ct) to dilution number of the positive clinical sample, from which the logarithmic function ($y = -a \ln(x) + b$) was calculated. If the R^2 value was less than 0.96 all serially diluted RNA and cDNA was remade and retested.

The copy number concentration of SARS-CoV-2 N gene RNA in the SARS-CoV-2 clinical sample was inferred via the standard curve of the 2019-nCoV_N_Positive Control dilution series used in the CDC system.

Serological screening

Serum samples were tested for presence of antibodies against SARS-CoV-2 with the L00847 surrogate virus neutralization test (sVNT) (GenScript cPass™, USA) as described in Mariën et al. (34). The percentage inhibition was calculated as: ((1− OD value of sample)/ OD value of Negative control) x100%. If inhibition values were greater than 20%, serum samples were considered SARS-CoV-2 positive. Two negative serum samples, two positive serum samples and two positive serum samples from SARS-CoV-2-infected humans were used as controls. Details on samples tested are available in Supplementary material **Table S1**.

RESULTS AND DISCUSSION

None of the 1523 faecal samples across the five collection periods tested positive with the Pan-coronavirus screening system. All serum samples were seronegative for neutralizing antibodies, suggesting that the tested mammals had not experienced SARS-CoV-2 infection at the time of sample collection (Supplementary material **Figure S1**). As such, apart from the infection in two hippos in December 2021 that was discovered because of clinical symptoms and not through our active surveillance study, there was no evidence of SARS-CoV-2 or other coronavirus infection among the mammals residing in the Antwerp and Planckendael Zoos during the time span of the study.

We compared the Pan-CoV PCR system that we used to test faecal samples collected during the first four sampling sessions between September 2020-July 2021 with a golden standard test for SARS-CoV-2 detection (CDC N1/N2) to ensure the sensitivity of the detection system was not an issue. We inferred the copy number per µL of a positive control SARS-CoV-2 RNA from a patient through comparing with known copy numbers of the N1 synthetic control of the CDC SARS-CoV-2 system. In both systems the template was detectable up to a 10^{-5} dilution, corresponding to 2.42 N1-gene-copies/µL with Ct values of 34.76 ±0.12 (CDC) and 38.84 ±2.48 (Pan-CoV) (Supplementary material **Figure S2**). Hence, the detection limit and sensitivity of the CDC and the Pan-CoV system were very comparable, making it unlikely the choice of a Pan-coronavirus RT-PCR system instead of a SARS-CoV-2-specific detection system caused false-negative results. The added advantage of the Pan-CoV system is that with one PCR test, we could also determine the possible presence of other coronaviruses. While perhaps not the entire range of coronaviruses can be detected with the same sensitivity with this Pan-CoV system, it has been validated to detect SARS-CoV-2 polymerase gene.

Virus survival or successful detection of viral RNA depends on the type of virus, the medium in which it is present and environmental conditions (temperature, pH, moisture content, organic matter, light, etc.) (35). Although the SARS-CoV-2 virus is stable on most indoor surfaces (36–38), other factors in outdoor environments may reduce its survival (39). Studies on the effect of temperature on SARS-CoV-2 survival showed that it may survive from 5 to 10 days at 20°C and from 1 to 4 days at 30°C depending on the surface type (40). Even if no studies on SARS-CoV-2 stability in faeces in outdoor environments have been conducted, a comprehensive study on the survival of several other coronaviruses in faeces concluded that SARS-CoV-2 could survive from 1 hour to 4 days in human faeces, depending on the type and pH of the stool samples (35). In our study, the delay between excretion and collection, and other environmental factors might have influenced the quality of the samples. However, we tried to limit these issues by collecting the samples as fresh as possible (less than 12h after excretion). In addition, we made sure to collect the central part of the faeces and to use the central part of the sample in the laboratory to limit the effect of environmental factors on the degradation of viral RNA. Finally, the mean temperature ranged from 0 to 22°C during the whole sampling campaign. We therefore assume that the impact of temperature on the preservation of faeces on the enclosures ground will be minimal.

The non-detection of SARS-CoV-2 RNA in this study might be related to the study sampling design. Faecal samples are suitable material for the detection of SARS-CoV-2 RNA, even if there is no consensus about which sample type (i.e., nasopharyngeal swabs, oropharyngeal swabs, faeces, or rectal swabs) is best suited to detect SARS-CoV-2 RNA, especially in non-human animals (41–43). Moreover, we cannot exclude we missed a potential SARS-CoV-2 infection in zoo mammals in our study because of the duration of SARS-CoV-2 RNA in faeces after the acute infection. Zhang et al. (2021) conducted a systematic review and meta-analysis on 14 studies on the faecal shedding of SARS-CoV-2 RNA in human patients (N = 620) with COVID-19 infection (42). On average, viral RNA could be detected up to 21.8 days after infection, while nasopharyngeal swabs could only detect RNA 14.7 days after infection. The sampling sessions had on average 6 weeks apart, with over 6 months between the two subsequent sessions. We therefore cannot exclude that SARS-CoV-2 infections

occurred between the sampled sessions. However, due to logistical reasons, more frequent sampling was not feasible. Nevertheless, longitudinal faecal screening of infected tigers and lions in the USA and hippos in Belgium showed that SARS-CoV-2 RNA could be detected up to 35 days after symptom onset (25,26). Viral RNA shedding in these animals' faeces may be more apparent than what is observed in humans, where only about half of the patients have detectable SARS-CoV-2 RNA in faeces at any point during infection, and if they do, viral RNA remains detectable for 3-4 weeks.

Also, a systematic blood sampling of all the animals to conduct serology testing and look for past infection rather than ongoing infection could have helped unravel this bias related to the time windows. In humans, IgG antibodies can be detected at least 3 months after SARS-CoV-2 infection (44–47). However, little is known about the persistence of antibodies in wild mammals after infection (48). In our case, systematic blood sampling would have involved heavy logistic organisation and animal stress. We therefore relied on 50 collected serum samples from 26 species that were collected for other purposes, representing about 10% of the mammals from the zoo. Their seronegativity suggests that at least up until 2021 there has been no widespread multi-species SARS-CoV-2 epidemic in the zoos.

Previously reported cases of SARS-CoV-2 infections in zoo animals have been traced back to asymptomatic COVID-19 infected zookeepers that were in contact with these animals (23–25). The close contact of zookeepers when preparing food, veterinary consultation of animals, or enclosure cleaning represents an important risk of transmission. Since the summer of 2020 and throughout the time period of our study, face masks have been worn throughout in the Antwerp and Planckendael zoos, both by zookeepers and visitors, in addition to the already extensive hygiene measures when preparing food and entering the facilities. It is likely that the hygiene measures implemented in both Antwerp and Planckendael zoos at the beginning of the pandemic have helped to avoid the transmission of SARS-CoV-2 from humans to animals during most of the pandemic.
The origin of the infection of the two hippos at Antwerp Zoo in November 2021 is not known. The caretakers had no known infection, did not have any COVID-19 symptoms prior to the hippo's infection, and were wearing surgical masks during their work (26). While several meters distance is kept from the visitors, as the hippos are housed indoors aerosol transmission from an infected visitor without perfect masking could have taken place. The genome sequence of the Delta variant with which the hippos were infected was indeed closely related to strains commonly circulating in Belgium at the time (26).

The infection of precisely two hippopotamuses in the Antwerp Zoo was unexpected in the sense that other mammal species have been predicted to be much more susceptible to SARS-CoV-2 based on *in silico* models of the molecular interaction between the virus Spike protein and the host receptor ACE2.
The predictions of SARS-CoV-2 binding propensity to the hippopotamus viral receptor ACE2 classified the hippopotamus only at medium risk to be infected with SARS-CoV-2 while other taxa such as primates were classified as high risk (19,20). However, no primates have been reported infected in Antwerp and Planckendael zoos. The fact that the hippos were housed in an indoor complex where also visitors enter, could have contributed to an elevated infection risk of these species. Other species including bongo, tapir and nutria that were kept in the vicinity of the infected hippos were negative when sampled right after the reported hippopotamus infections. Visitors did not have access to their indoor enclosure. The hippopotamus infections emphasize that the structural analysis of the SARS-CoV-2 cellular receptor alone is insufficient to estimate the relative spillover risk of SARS-CoV-2 to other animal species (19–21). Monitoring of zoonotic infections remains the main key in controlling and limiting the spread of zoonotic pathogens.

AUTHOR CONTRIBUTIONS

LJ, EV, FV, SG, JM and HL developed the research methodology. TC and LJ conducted the lab work. LJ drafted the article. LJ, EV, FV, SG, TC, JM, and HL reviewed the article. All authors read and approved the final manuscript and its submission for publication.

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Table. Number of faecal samples collected per order, family and species in the two zoos for each sampling session.

			Antwerp						Planckendael					
Order	**Family**	**Species**	**Session 1**	**Session 2**	**Session 3**	**Session 4**	**Session 5**	**TOTAL**	**Session 1**	**Session 2**	**Session 3**	**Session 4**	**Session 5**	**TOTAL**
Cetartiodactyla	Bovidae	*Addax nasomaculatus*							5	5	5	4		**19**
		Bison bison							5	5	5	5		**20**
		Bison bonasus							3	3	3	3		**12**
		Bos taurus							3	3	3	3		**12**
		Budorcas taxicolor	2	2	2	2		**8**						
		Capra hircus							5	5	5	5		**20**
		Cephalophus natalensis	2	2	2	2		**8**						
		Gazella leptoceros							3	3	3	1		**10**
		Madoqua kirkii	4	4	4			**12**	5	5	5	3		**18**
		Nanger dama							3	3	3	5		**14**
		Oryx dammah							1	1	1	1		**4**
		Oryx leucoryx							1	1	1	1		**4**
		Ovis aries	3	3	3	2		**11**	5	5	5	3		**18**
		Ovis aries laticaudatus							2	2	2			**6**
		Syncerus caffer	5	5	5	5		**20**						
		Tragelaphus eurycerus	3	3	3	3	4	**16**	3	3	3	1		**10**
	Camelidae	*Camelus bactrianus*							5	5	5	5		**20**
		Lama guanicoe							5	5	5	3		**18**
		Vicugna pacos							5	5	5	10		**25**
		Vicugna vicugna							5	5	5	4		**19**
	Cervidae	*Cervus canadensis*							5	5	5	5		**20**
		Muntiacus reevesi							5	5	5	5		**20**

			Antwerp						Planckendael					
Order	**Family**	**Species**	**Session 1**	**Session 2**	**Session 3**	**Session 4**	**Session 5**	**TOTAL**	**Session 1**	**Session 2**	**Session 3**	**Session 4**	**Session 5**	**TOTAL**
	Equidae	*Equus asinus*							2	2	2	2		**8**
		Equus caballus							4	4	4			**12**
		Equus ferus przewalskii										**4**		**4**
		Equus grevyi							5	5	5	5		**20**
		Equus zebra	4	4	4	4		**16**						
	Giraffidae	*Giraffa camelopardalis*	3	3	3	3		**12**	5	5	5	5		**20**
		Okapia johnstoni	5	5	5	4		**19**						
	Hippopotamidae	*Hippopotamus amphibius*	2	2	2	2		**8**						
	Suidae	*Sus cebifrons*							4	4	4	4		**16**
		Sus scrofa							3	3	3	3		**12**
	Tayassuidae	*Catagonus wagneri*							5	5	5	5		**20**
		TOTAL	**33**	**33**	**33**	**27**	**4**	**130**	**102**	**102**	**102**	**95**		**401**
Carnivora	Canidae	*Crocuta crocuta*							2	2	2	3		**9**
		Speothos venaticus							5	5	5	5		**20**
	Felidae	*Acinonyx jubatus*							2	2	2	2	2	**10**
		Panthera leo	3	3	3	3	6	**18**	3	3	3	3	3	**15**
		Panthera onca							1	1	1	1	2	**6**
		Panthera pardus							1	1	1			**3**
		Panthera uncia							2	2	2	2		**8**
	Herpestidae	*Cynictis penicillata*	5	5	5	5		**20**						
		Mungos mungo							5	5	5	5		**20**
		Suricata suricatta	5	5	5	3		**18**						

			Antwerp						Planckendael					
Order	**Family**	**Species**	**Session 1**	**Session 2**	**Session 3**	**Session 4**	**Session 5**	**TOTAL**	**Session 1**	**Session 2**	**Session 3**	**Session 4**	**Session 5**	**TOTAL**
	Mustelidae	*Aonyx cinereus*	1	1	1			**3**	4	4	4			**12**
		Meles meles							1	1	1	1		**4**
	Otariidae	*Phoca vitulina*	7	7	7	2		**23**						
		Zalophus californianus	4	4	4	2		**14**						
	Procyonidae	*Nasua narica*							1	1	1	2		**5**
		Nasua nasua							3	3	3			**9**
		Procyon lotor							2	2	2			**6**
	Ursidae	*Ailurus fulgens*							2	2	2	2		**8**
		Tremarctos ornatus							2	2	2	2		**8**
		TOTAL	**25**	**25**	**25**	**15**	**6**	**96**	**36**	**36**	**36**	**28**	**7**	**143**
Chiroptera	Pteropodidae	*Rousettus aegyptiacus*							5	5	5	2		**17**
		TOTAL							**5**	**5**	**5**	**2**		**17**
Dasyuromorphia	Dasyuridae	*Sarcophilus harrisii*							3	3	3	3		**12**
		TOTAL							**3**	**3**	**3**	**3**		**12**
Diprotodontia	Macropodidae	*Dendrolagus goodfellowi*	1	1	1			**3**						
		Macropus giganteus	4	4	4	3		**15**						
		Macropus parma	3	3	3			**9**						
		Macropus rufus							1	1		2		**4**
		Thylogale brunii	1	1	1			**3**	1	1	1	1		**4**
		Wallabia bicolor							5	5	5	5		**20**
	Phascolarctidae	*Phascolarctos cinereus*	1	1	1	2		**5**	2	2	2	1		**7**

			Antwerp						Planckendael					
Order	**Family**	**Species**	**Session 1**	**Session 2**	**Session 3**	**Session 4**	**Session 5**	**TOTAL**	**Session 1**	**Session 2**	**Session 3**	**Session 4**	**Session 5**	**TOTAL**
	Potoroidae	*Bettongia penicillata*				**1**		**1**						
		TOTAL	**10**	**10**	**10**	**6**		**36**	**9**	**9**	**8**	**9**		**35**
Lagomorpha	Leporidae	*Oryctolagus cuniculus*							5	5	5	5		**20**
		TOTAL							**5**	**5**	**5**	**5**		**20**
Macroscelidea	Macroscelididae	*Rhynchocyon petersi*	3	3	3	2		**11**						
		TOTAL	**3**	**3**	**3**	**2**		**11**						
Monotremata	Tachyglossidae	*Tachyglossus aculeatus*							2	2	2	2		**8**
		TOTAL							2	2	2	2		8
Perissodactyla	Rhinocerotidae	*Ceratotherium simum simum*	2	2	2	2		**8**						
		Rhinoceros unicornis							2	2	2	3		**9**
	Tapiridae	*Tapirus indicus*	3	3	3	2	4	**15**						
		TOTAL	**5**	**5**	**5**	**4**	**4**	**23**	**2**	**2**	**2**	**3**		**9**
Pilosa	Myrmecophagidae	*Myrmecophaga tridactyla*							2	2	2	2	2	**10**
		Tamandua tetradactyla	1	1	1			**3**						
		TOTAL	**1**	**1**	**1**			**3**	**2**	**2**	**2**	**2**	**2**	**10**

			Antwerp						Planckendael					
Order	**Family**	**Species**	**Session 1**	**Session 2**	**Session 3**	**Session 4**	**Session 5**	**TOTAL**	**Session 1**	**Session 2**	**Session 3**	**Session 4**	**Session 5**	**TOTAL**
Primates	Aotidae	*Aotus trivirgatus*	1	1	1			**3**						
	Atelidae	*Ateles fusciceps*	5	5	5	5	6	**26**						
	Callitrichidae	*Callimico goeldii*	5	5	5	5	2	**22**						
		Callithrix geoffroyi							5	5	5	5	2	**22**
		Cebuella pygmaea	1	1	1	1	2	**6**						
		Leontopithecus chrysomelas	3	3	3			**9**	4	4	4	3	5	**20**
		Saguinus imperator	2	2	2	2	1	**9**						
	Cebidae	*Saimiri boliviensis*							3	3	3			**9**
	Cercopithecidae	*Cercopithecus hamlyni*	3	3	3	4	3	**16**						
		Colobus guereza	4	4	4	4	2	**18**						
		Macaca nigra							5	5	5	5	3	**23**
		Macaca sylvanus							5	5	5	5	3	**23**
		Mandrillus sphinx	5	5	5	5	4	**24**						
	Hominidae	*Gorilla beringei*	1	1	1	1	1	**5**						
		Gorilla gorilla	5	5	5	5	5	**25**						
		Pan paniscus							5	5	5	5	18	**38**
		Pan troglodytes	5	5	5	5	11	**31**						
	Hylobatidae	*Nomascus leucogenys*							2	2	2	2	2	**10**
	Lemuridae	*Eulemur macaco*							2	2	2	3	2	**11**
		Lemur catta	2	2	2	2		**8**	5	5	5	5	5	**25**
		Varecia rubra	1	1	1			**3**						
	Loridae	*Loris lydekkerianus*	5	5	5	5	2	**22**						
	Lorisidae	*Nycticebus pygmaeus*	5	5	5	2		**17**						

			Antwerp						Planckendael					
Order	**Family**	**Species**	**Session 1**	**Session 2**	**Session 3**	**Session 4**	**Session 5**	**TOTAL**	**Session 1**	**Session 2**	**Session 3**	**Session 4**	**Session 5**	**TOTAL**
		TOTAL	**53**	**53**	**53**	**46**	**39**	**244**	**36**	**36**	**36**	**33**	**40**	**181**
Proboscidea	Elephantidae	*Elephas maximus*	2	2	2			**6**	5	5	5	5		**20**
		TOTAL	**2**	**2**	**2**			**6**	**5**	**5**	**5**	**5**		**20**
Rodentia	Castoridae	*Castor fiber*							1	1	1			**3**
	Caviidae	*Dolichotis patagonum*							5	5	5	5		**20**
		Hydrochoerus hydrochaeris							3	3	3	2		**11**
	Dasyproctidae	*Dasyprocta prymnolopha*							1	1	1	1		**4**
	Echimidae	*Myocastor coypus*				5	3	**8**						
	Erethizontidae	*Erethizon dorsatum*	4	4	4	4		**16**						
	Hystricidae	*Hystrix africaeaustralis*				3		**3**	3	3	3	5		**14**
	Murinae	*Lemniscomys barbarus*	5	5	5	5		**20**						
		Phleomys padillus	5	5	5	3	1	**19**						
		TOTAL	**14**	**14**	**14**	**20**	**4**	**66**	**13**	**13**	**13**	**13**		**52**
TOTAL			146	146	146	120	57	**615**	220	220	219	200	49	**908**

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