

# Identification of the delta variant of SARS-CoV-2 in lions with respiratory signs and their keepers in a zoo in Johannesburg, South Africa

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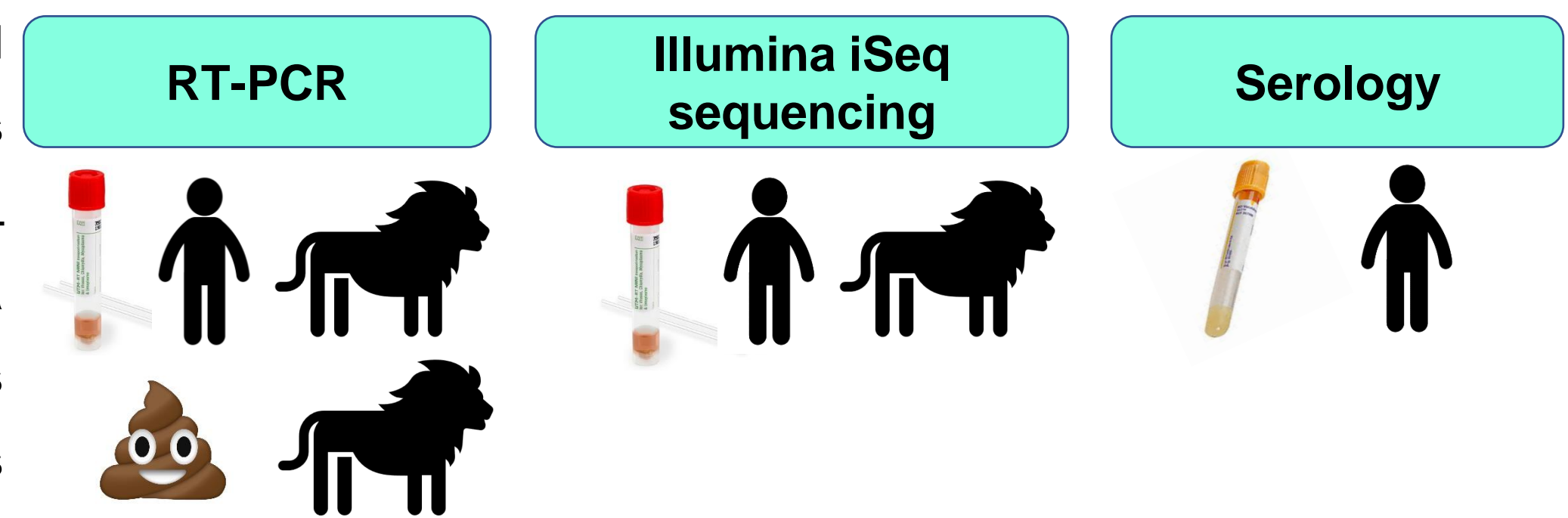
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## Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of the disease COVID-19(1). Due to the high level of sequence similarity to a virus isolated from *Rhinolophus affinis* bats, RaTG13, it is believed that SARS-Cov-2 is descended from an animal virus(2, 3). Since the initial transmission to humans, the epidemiology of SARSCOV2 was related to human to human transmission with limited role for animals, however the risk for reverse zoonotic spill-over and the potential transmission dynamics in animal hosts remains important to identify the emergence of new variants. Animal surveillance programs have discovered reverse zoonotic transmission from humans to animals in several domestic species and captured wildlife, including domestic cats, dogs, mink, otter, domestic ferrets, lions, tigers, pumas, snow leopards and gorillas(4). With the emergence of SARS-CoV-2 variants of concern, the question as to whether evolution of the virus will favour reverse-zoonoses and animal transmission is important. Here we describe the natural infection of the SARS-CoV-2 delta variant in 3 lions in at least 2 transmission events from their human keepers in a zoo in South Africa.

## Materials and Methods

Three lions (ZRU125/21, ZRU127/21, ZRU128/21) with respiratory symptoms as well as zoo staff (n = 12) with direct or indirect contact were tested for the presence of SARS-CoV-2. Multiple samples were collected from 22 June 2021 to 27 July 2021 to monitor the outbreak. RNA extraction was done with the Qiamp Viral Mini Kit (Qiagen) and the SARS-CoV-2 E and RdRp genes was tested with the LightMix SarbecoV RT-PCR kits (TIP MOL – BIOL). Presence of IgG was determined with Anti-SARS-CoV-2 ELISA (Euroimmun) in humans. Whole genomes were determined on an Illumina iSeq platform using Superscript IV (Invitrogen) to synthesize cDNA. SARS-CoV-2 amplification was done using the V2 and V3 ARTIC primers (Inqaba) and libraries were created with Illumina DNA prep kit (Illumina). The paired end reads were assembled in Galaxy and consensus sequences were analysed in Nextclade.



## Results

### Timeline of SARS-CoV-2 outbreak in South African lions indicating RT-PCR positive samples



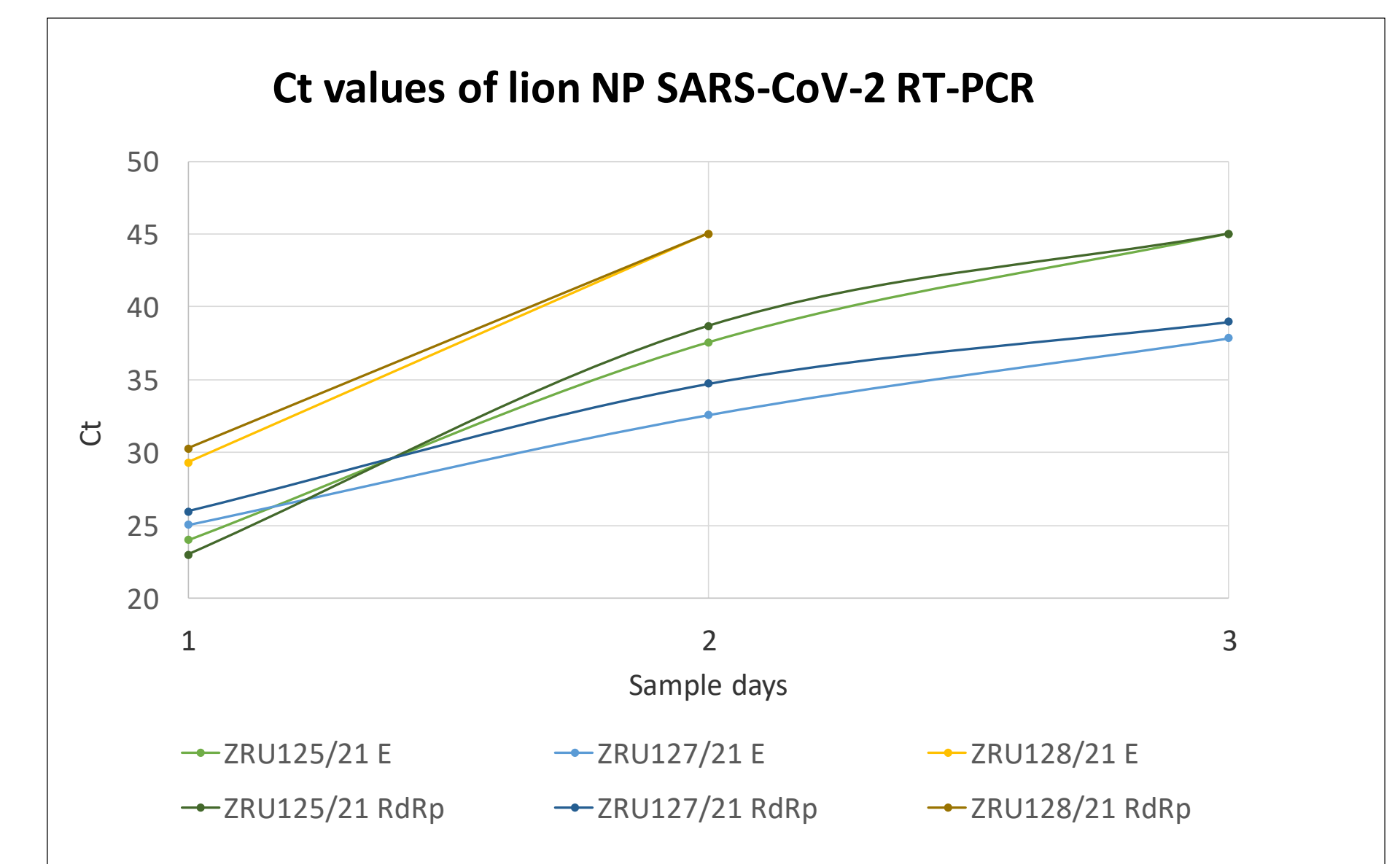
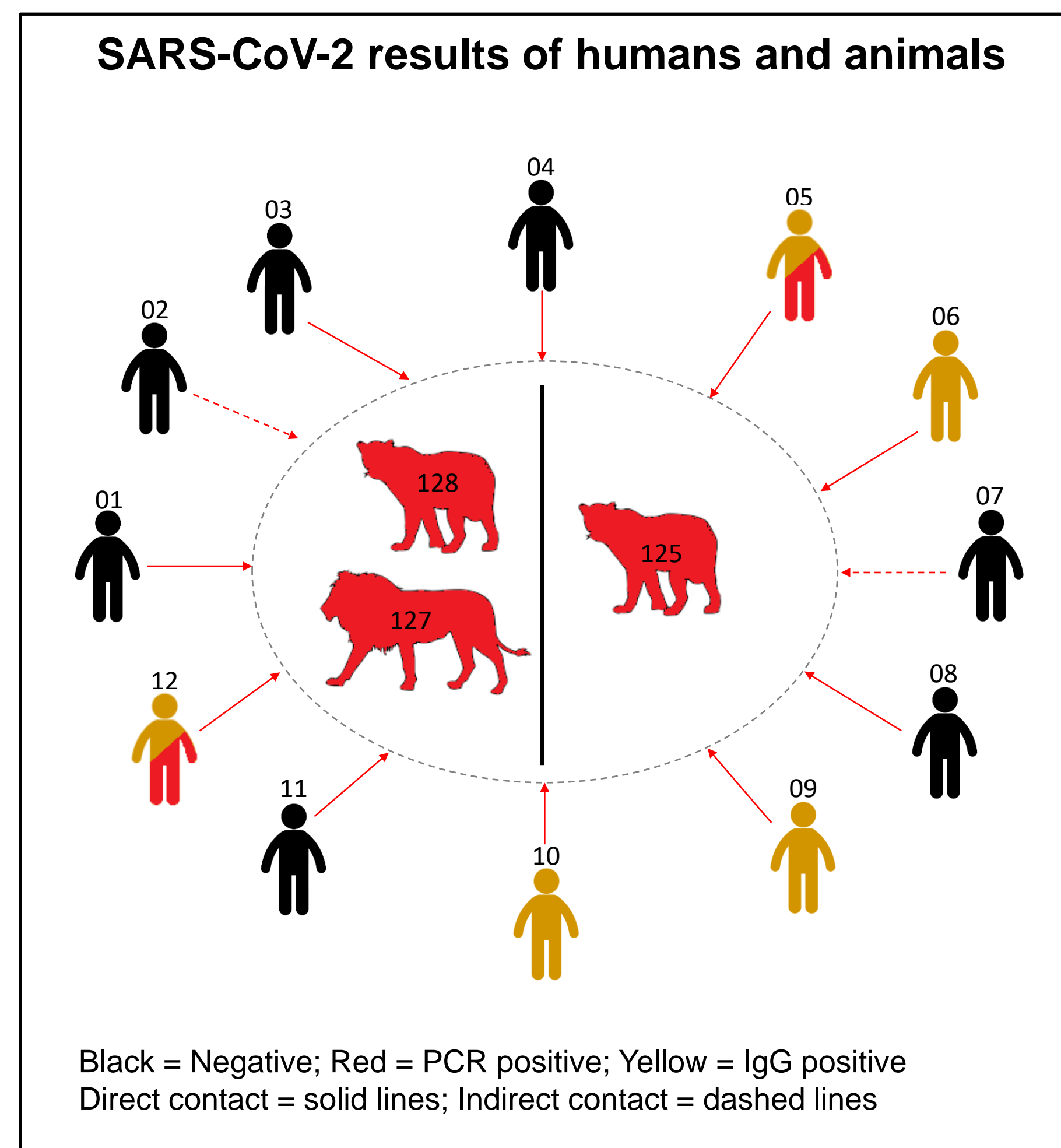
### Surveillance

All three lions and two of the zoo staff tested positive for SARS-CoV-2 on respiratory swabs at the first sample collection point in June. The lions also tested positive on stool samples. Ct values ranged from 19-21 suggesting high RNA levels were present. Five staff members tested positive for IgG on serum indicating previous infections for three of them.

One female lion (ZRU128/21) cleared her infection by 12 July and by 27 July only the male lion (ZRU127/21) still tested positive for SARS-CoV-2. However, these ct values were very high (37,8) and literature on SARS-CoV-2 infections suggests that when the PCR value is over 30, very few active viral particles are still present in the nasopharynx.

### SARS-CoV-2 variant analyses

Whole genomes were determined for the three lions as well as ZRUCWL005. Genome coverage was between 96.8 and 97.6 % and average depth coverage was between 397.2 and 969.6. All four genomes were identified as the delta variant that has been circulating in South Africa since May 2021. The four genomes have the same protein mutations apart from a I60V substitution in ORF6 in the human sample. Amino acid changes A222V and T250S in the spike protein is commonly seen in South African delta variants.



Detection of SARS-CoV-2 E and RdRp genes from lion faecal samples

Target	25-Jun-21	07-Jul-21	10-Jul-21	12-Jul-21
<b>ZRU125/21</b> E	No sample	33,5	-	-
<b>ZRU125/21</b> RdRp	No sample	-	-	-
<b>ZRU127/21</b> E	37,53	-	-	-
<b>ZRU127/21</b> RdRp	-	-	-	-
<b>ZRU128/21</b> E	32,73	-	-	-
<b>ZRU128/21</b> RdRp	34,7	-	-	-

- Negative for SARS-CoV-2

### Spike mutations of SARS-CoV-2 variants and study strains

	18	19	20	26	69	70	80	138	142	144	156	157	158	190	222	241	242	243	250	417	452	478	484	494	501	570	614	655	681	701	716	950	982	1118	1191	1027
Alpha					del	del				del													E/K	S/P	N/Y	A/D	D/G		P/H		T/I		S/A	D/H	K/N	
Beta							D/A									D/G	del	del		K/N			E/K		N/Y		D/G			A/V						
Delta		T/R							G/D		del	del	R/G									L/R	T/K				D/G		P/R			D/N				
Gamma	L/F		T/N	P/S				D/Y						R/S						K/T					N/Y	D/G	H/Y									T/I
<b>ZRU125/21</b>		T/R													A/V				T/S			L/R	T/K				D/G		P/R			D/N				
<b>ZRU127/21</b>		T/R									del	del	R/G		A/V				T/S			L/R	T/K				D/G		P/R			D/N				
<b>ZRU128/21</b>		T/R													A/V				T/S			L/R	T/K				D/G		P/R			D/N				
<b>ZRUCWL005</b>		T/R									del	del	R/G		A/V				T/S			L/R	T/K				D/G		P/R			D/N				

Grey amino acids: missing data

## Conclusions

SARS-CoV-2 was detected in South African lions and their keepers. Surveillance data indicated that lions and humans were clearing their infections. Whole genome sequencing of the SARS-CoV-2 present in the lions and one of the keepers indicated that the same virus was circulating in the zoo. The presence of the SARS-CoV-2 delta variant in the lions indicate that at least two reverse zoonotic events took place where a human infected the lions. Of the 3 lions, one presented with pneumonia (lower respiratory tract infection) while the other 2 only had upper respiratory tract infection. The zoo keepers were asymptomatic at the time of collection of the initial sample but both developed mild symptoms in the next week. This study emphasises the need for surveillance of SARS-CoV-2 in wildlife kept in captivity during the pandemic and use of adequate PPE to protect these animals from infection by humans with direct contact.

## Acknowledgements

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## References

1. P. J. Walker *Arch. Virol.* **165**, 2737–2748 (2020). 3. P. Zhou. *Nature.* **579**, 270–273 (2020). 2. K. G. Andersen. *Nat. Med.* **26**, 450–452 (2020). 4. OIE situation report 30 June 2021