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MOLECULAR DETECTION OF CORONAVIRIDAE IN RODENTS FROM BAHAWALPUR

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Abstract

Coronaviruses are associated with severe illness, severe epidemics, and death in humans and animals belonging to the subfamily Coronavirinae of the family Coronaviridae. Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS) were important examples of zoonotic coronaviruses. Rodents are the largest order of mammals composed of more than 2000 species, representing more than 40% of all mammalian species. Rodents are the major zoonotic source of human infectious diseases; they often live at high densities and hence may harbor high levels of microbial diversity. This study aimed to assess the prevalence of coronaviruses in rodent populations in Bahawalpur, Pakistan using improved detection methods through reverse transcription PCR (RT-PCR). Between February and June 2024, we humanely captured 59 rats and collected the fecal swabs for analysis. We specifically targeted the RNA-dependent RNA polymerase gene with RT-PCR to increase sensitivity. According to our results, eleven of the sampled pools tested positive for coronaviruses. Results imply the prevalence of coronaviruses in the population of rats in the Bahawalpur and their significant role in zoonotic transmission. These results indicate addressing wildlife surveillance to monitor coronaviruses prior so that the possibility to occur unknown outbreaks due to coronavirus can be reduced, but also infectious diseases as well.

INTRODUCTION

The family of viruses, called *Coronaviridae (Payne, 2017)*, has an outer, protective layer as well as a single strand of RNA containing its genetic material. This category of virus can infect huge numbers of different mammals. Suborder Cornidovirineae falls under the family *Coronaviridae*, which then consists of Tornidovirineae under the order Nidovirales. It is from a family of positive-stranded RNA-enveloped viruses. Genomic classification lists an order called Nidovirales under it and four families are recognized. These are *Arteriviridae*, *Coronaviridae*, *Mesoniviridae,* and *Roniviridae*. There are Alpha-coronavirus and Beta-coronavirus which could infect a wide range of mammals; Gamma-coronavirus and Delta-coronavirus which largely infect birds(Poudel et al., 2020).

Coronavirus is classified as an RNA virus with single-stranded positive-sense genomes(Sánchez-Duque et al., 2020). The viruses are enveloped, and non-segmented, and they have the N-phosphoprotein surrounded in a helical formation by the nucleocapsid. They are known to be causative agents of a wide range of animal infections. They are involved in various diseases, such as respiratory, gastrointestinal, hepatic, and neurological infections of different severities(Gandhi et al., 2023). The family of *Coronaviridae* is divided by the International Committee on Taxonomy of Viruses into two subfamilies: Letovirinae and Ortho-coronavirinae. Letovirinae subfamily comprises one genus, Milecovirus, which infects frogs and a sea hare. These latter are further divided into four genera based on antigenic and genetic characteristics(Lang et al., 2020). From the structural perspective, coronaviruses usually consist of three main proteins: spike (S), membrane (M), and envelope (E) proteins. The other species additionally contain another protein, hemagglutinin esterase (HE). M and E proteins will be involved in forming the virus, in particular, the S protein helps in binding to the receptor, while it is also the principal site for neutralizing antibodies(Murin et al., 2019).

The Orthocoronavirinae is categorized into four distinct genera named Alpha, Beta, Gamma, and Delta coronaviruses (Zmasek et al., 2022).This order has been named Nidovirales due to a unique feature of viruses within this order where the replication process produces a nested set of sub-genomic mRNAs. This "nesting" explains why the group is named after the Latin word "nidus," meaning nest. Coronaviruses have large RNA genomes, together with the N protein, which form a helical nucleocapsid. Their envelope results from the cell membrane of the host and consists of viral proteins such as M, E, and S glycoproteins. The typical diameter of the CoV particles is about 100 nm. The genomic RNA inside these particles is one of the largest RNA genomes in viruses and varies between 27 to 32 kilobases across the different CoVs. The attachment of the virus to the receptors of host cells and the fusion of the viral envelope with the host cell membrane largely depends on the spike glycoprotein (Deng & Baker, 2021).

Rodents are the order Rodentia and represent the largest number of mammals distributed worldwide. Many zoonotic viruses have major reservoirs in them. Beta-coronavirus subgroup of coronavirus includes murine hepatitis virus strain 1, or MHV-1 (Zappulli, 2021). It was first isolated from mice in 1949. MHV-1 has emerged as one of the most common animal models to study multiple sclerosis. Replication and transcription in coronaviruses are relatively very complex. This is done in replication, a constant RNA template that is the same as the viral genome. The transcription occurs by a mechanism of discontinuous transcription which leads to the production of a pool of sub-genomic-length mRNAs (Lee et al., 2020).

The closely related coronaviruses in animals, such as SARS-CoV-1, MERS-CoV, and SARS-CoV-2 present in animals such as civets, camels, and bats indicated that these animals were the vectors through which the virus had been transmitted to humans or had zoonotic transmission. It has been claimed that human coronaviruses HCoV-229E and HCoV-NL63 are of animal origin, probably bats, whereas human coronaviruses OC43 and HKU-1, which cause the common cold, have been claimed to have originated from rodents (Samudrala et al., 2020).

This diversity makes the susceptibility of rodent species against SARS-CoV-2 vary. Mostly the non-transgenic laboratory mice (*Mus musculus*) show resistance to infection, whereas humanized mice and

some hamster species such as Syrian and dwarf, have shown potential susceptibility. Syrian hamsters (*Mesocricetus auratus*) being one of them are prevalent in SARS-Co2 research due to the superficial resemblance in disease pathology to that of humans (Yuan et al., 2020). Bosco-Lauth and Yuan have used Syrian hamsters in their research (Li et al., 2021).

The infrared sensors and thermal imaging have been well utilized in the identification of individuals, whose body temperatures are elevated, thus constituting the first line of screening likely viral infections. The thermal cameras were first fitted at the entry points of the hospital and emergency rooms because they capture infrared radiation emitted as heat and convert it into visual data. Since then, they have been applied widely in public buildings including airports, railway stations, and schools. Thermal cameras, with longer infrared wavelengths, are much more efficient in mass screening compared to single infrared scanners because they can cover a larger area in a much shorter time (Li et al., 2021).

The Nucleic Acid Amplification Test, in the present time, is mostly used for the determination of COVID-19 by nasal swabs or blood samples. The test is based on the RT-PCR-based test (Meng et al., 2020).Due to the false negativity, the test might miss the virus when the viral load is low enough. Recently, the FDA has sanctioned the use of some RT-PCR-based diagnostic kits for emergency cases like the Cobas SARS-CoV-2 test; however, there are several reasons why RT-PCR tests are inapplicable: preparation of samples leads to many errors, problems related to specificity and sensitivity, and to provide valid and reliable results, frequent calibrations are required. Further studies have even revealed that CT scanning may also be used in combination with RT-PCR, especially in low-resource settings or communities in which NAAT is either unavailable or unachievable(Hermes et al., 2020).

This study focuses on the detection of coronaviruses in rat populations in Pakistan, highlighting their role as potential reservoirs for these viruses. It offers a comprehensive overview of the ecological and evolutionary aspects of coronaviruses, detailing their divergence and the knowledge surrounding their host reservoirs. Additionally, the research examines cultural practices at the interface between humans and wildlife that may heighten the risk of disease transmission. The study also outlines the transmission cycles of these viruses, providing insights into how they may spread between different species and potentially infect humans.

Methodology

Sample Collection:

A total of 59 rat samples were collected from six locations across District Bahawalpur, Punjab, Pakistan, between February 27, 2024, and June 12, 2024. Bait stations containing peanut butter, tomato, potato, and guava were strategically placed near hostels, shops, and canteens to capture rats with minimal stress or injury. Ethical guidelines were followed to ensure humane handling.

Collection Interval:

Sampling was conducted at regular intervals to represent rat populations over time. Bait stations were monitored and replenished to maintain effectiveness.

Sample Size:

The sample size of 59 rats was determined based on statistical requirements and ethical considerations.

Table 01:
Sample Collection Details

Rats Sampling Data									
ID	Date	Time	Location Name	Gender M/F	Animal Contact	Swabs/Organ			
						Oral	Fecal	Urine	Lungs

1	27-02-24	3:00 p.m.	Mela Gali	M	×	R1 Or	R1 Fe	R1 Ur	R1 Lu
2	27-02-24	3:00 p.m.	Mela Gali	F	×	R2 Or	R2 Fe	R2 Ur	R2 Lu
3	27-02-24	4:25 p.m.	Mela Gali	M	×	R3 Or	R3 Fe	R3 Ur	R3 Lu
4	27-02-24	4:50 p.m.	Mela Gali	F	×	R4 Or	R4 Fe	R4 Ur	R4 Lu
5	29-02-24	3:26 p.m.	Mela Gali	M	×	R5 Or	R5 Fe	R5 Ur	R5 Lu
6	29-02-24	3:45 p.m.	Mela Gali	F	×	R6 Or	R6 Fe	R6 Ur	R6 Lu
7	01-03-24	12:45 p.m.	One Unit Chowk	M	×	R7 Or	R7 Fe	R7 Ur	R7 Lu
8	01-03-24	1:15 p.m.	One Unit Chowk	F	×	R8 Or	R8 Fe	R8 Ur	R8 Lu
9	01-03-24	1:30 p.m.	One Unit Chowk	F	×	R9 Or	R9 Fe	R9 Ur	R9 Lu
10	01-03-24	1:50 p.m.	One Unit Chowk	F	×	R10 Or	R10 Fe	R10 Ur	R10 Lu
11	01-03-24	2:00 p.m.	One Unit Chowk	F	×	R11 Or	R11 Fe	R11 Ur	R11 Lu
12	04-03-24	2:23 p.m.	One Unit Chowk	F	×	R12 Or	R12 Fe	R12 Ur	R12 Lu
13	04-03-24	2:45 p.m.	One Unit Chowk	F	×	R13 Or	R13 Fe	R13 Ur	R13 Lu
14	04-03-24	3:14 p.m.	One Unit Chowk	M	×	R14 Or	R14 Fe	R14 Ur	R14 Lu
15	05-03-24	1:46 p.m.	One Unit Chowk	M	×	R15 Or	R15 Fe	R15 Ur	R15 Lu
16	11-03-24	2:51 p.m.	One Unit Chowk	M	×	R16 Or	R16 Fe	R16 Ur	R16 Lu
17	11-03-24	3:32 p.m.	One Unit Chowk	F	×	R17 Or	R17 Fe	R17 Ur	R17 Lu
18	12-03-24	12:30 p.m.	One Unit Chowk	F	×	R18 Or	R18 Fe	R18 Ur	R18 Lu
19	13-03-24	11:15 a.m.	Gala Mandi	M	×	R19 Or	R19 Fe	R19 Ur	R19 Lu
20	13-03-24	11:50 a.m.	Gala Mandi	F	×	R20 Or	R20 Fe	R20 Ur	R20 Lu
21	18-03-24	12:50 p.m.	Gala Mandi	F	×	R21 Or	R21 Fe	R21 Ur	R21 Lu

22	18-03-24	1:18 p.m.	Gala Mandi	F	×	R22 Or	R22 Fe	R22 Ur	R22 Lu
23	18-03-24	2:00 p.m.	Gala Mandi	F	×	R23 Or	R23 Fe	R23 Ur	R23 Lu
24	20-03-24	1:35 p.m.	Gala Mandi	M	×	R24 Or	R24 Fe	R24 Ur	R24 Lu
25	20-03-24	1:53 p.m.	Gala Mandi	F	×	R25 Or	R25 Fe	R25 Ur	R25 Lu
26	20-03-24	2:00 p.m.	Gala Mandi	M	×	R26 Or	R26 Fe	R26 Ur	R26 Lu
27	20-03-24	2:30 p.m.	Gala Mandi	F (Pregnant)	×	R27 Or	R27 Fe	R27 Ur	R27 Lu
28	21-03-24	12:51 p.m.	Qasim Town	M	×	R28 Or	R28 Fe	R28 Ur	R28 Lu
29	21-03-24	1:30 p.m.	Qasim Town	F	×	R29 Or	R29 Fe	R29 Ur	R29 Lu
30	21-03-24	1:55 p.m.	Qasim Town	M	×	R30 Or	R30 Fe	R30 Ur	R30 Lu
31	21-03-24	2:20 p.m.	Qasim Town	M	×	R31 Or	R31 Fe	R31 Ur	R31 Lu
32	21-03-24	2:45 p.m.	Qasim Town	F	×	R32 Or	R32 Fe	R32 Ur	R32 Lu
33	25-03-24	1:50 p.m.	Qasim Town	F	×	R33 Or	R33 Fe	R33 Ur	R33 Lu
34	25-03-24	1:56 p.m.	Qasim Town	M	×	R34 Or	R34 Fe	R34 Ur	R34 Lu
35	25-03-24	2:05 p.m.	Qasim Town	F	×	R35 Or	R35 Fe	R35 Ur	R35 Lu
36	25-03-24	2:25 p.m.	Qasim Town	M	×	R36 Or	R36 Fe	R36 Ur	R36 Lu
37	26-03-24	2:15 p.m.	Qasim Town	F	×	R37 Or	R37 Fe	R37 Ur	R37 Lu
38	01-04-24	1:00 p.m.	Qasim Town	F	×	R38 Or	R38 Fe	R38 Ur	R38 Lu
39	19-04-24	12:10 p.m.	Qasim Town	M	×	R39 Or	R39 Fe	R39 Ur	R39 Lu
40	24-04-24	1:40 p.m.	Faisal Colony	M	×	R40 Or	R40 Fe	R40 Ur	R40 Lu
41	25-04-24	2:50 p.m.	Faisal Colony	F	×	R41 Or	R41 Fe	R41 Ur	R41 Lu
42	25-04-24	3:09 p.m.	Faisal Colony	F	×	R42 Or	R42 Fe	R42 Ur	R42 Lu

43	25-04-24	3:12 p.m.	Faisal Colony	F	×	R43 Or	R43 Fe	R43 Ur	R43 Lu
44	25-04-24	3:16 p.m.	Faisal Colony	M	×	R44 Or	R44 Fe	R44 Ur	R44 Lu
45	25-04-24	3:25 p.m.	Faisal Colony	F	×	R45 Or	R45 Fe	R45 Ur	R45 Lu
46	02-05-24	1:20 p.m.	Faisal Colony	F	×	R46 Or	R46 Fe	R46 Ur	R46 Lu
47	02-05-24	1:30 p.m.	Faisal Colony	F	×	R47 Or	R47 Fe	R47 Ur	R47 Lu
48	02-05-24	1:45 p.m.	Faisal Colony	M	×	R48 Or	R48 Fe	R48 Ur	R48 Lu
49	06-05-24	1:35 p.m.	IUB Cafeteria	F	×	R49 Or	R49 Fe	R49 Ur	R49 Lu
50	06-05-24	1:57 p.m.	IUB Cafeteria	M	×	R50 Or	R50 Fe	R50 Ur	R50 Lu
51	16-05-24	4:05 p.m.	IUB Cafeteria	F	×	R51 Or	R51 Fe	R51 Ur	R51 Lu
52	16-05-24	4:20 p.m.	IUB Cafeteria	M	×	R52 Or	R52 Fe	R52 Ur	R52 Lu
53	16-05-24	4:40 p.m.	IUB Cafeteria	M	×	R53 Or	R53 Fe	R53 Ur	R53 Lu
54	16-05-24	5:00 p.m.	IUB Cafeteria	F	×	R54 Or	R54 Fe	R54 Ur	R54 Lu
55	11-06-24	3:01 p.m.	IUB Cafeteria	F	×	R55 Or	R55 Fe	R55 Ur	R55 Lu
56	11-06-24	4:00 p.m.	IUB Cafeteria	F	×	R56 Or	R56 Fe	R56 Ur	R56 Lu
57	12-06-24	1:37 p.m.	IUB Cafeteria	M	×	R57 Or	R57 Fe	R57 Ur	R57 Lu
58	12-06-24	1:50 p.m.	IUB Cafeteria	F	×	R58 Or	R58 Fe	R58 Ur	R58 Lu
59	12-06-24	2:00 p.m.	IUB Cafeteria	F	×	R59 Or	R59 Fe	R59 Ur	R59 Lu

*__Animal Contact:__ Rats contact with other animals

Restraining and Handling:

Captured rats were handled gently to avoid stress and injury. Data, including the sex of each rat, was recorded.

Zoonoses and Safety Precautions:

Strict biosafety measures were followed to protect against zoonotic diseases, including the use of gloves, masks, and lab coats. Personnel underwent specific training, and disinfection protocols were enforced.

Dissection:
Preparation: Dissecting tools and workspaces were sanitized. Rats were anesthetized with chloroform.
Procedure: A mid-ventral cut exposed internal organs, which were carefully removed and preserved for molecular tests. Dissections took place under biosafety conditions.

Sample Preservation:
Organ and swab samples were stored in cryovials at -40°C.

Post-Dissection:
Rat remains were disposed of following biosafety regulations, and all tools were sterilized.

RNA Extraction: The Quick-RNA™ Viral Kit was used for RNA extraction, following these steps:

- **Buffer Preparation:** Viral RNA Buffer and ethanol solutions were prepared.
- **Sample Preparation:** Swab and organ samples were pooled and processed at 4°C.
- **RNA Purification:** Samples underwent centrifugation and washing steps to obtain high-quality RNA.

cDNA Preparation: RevertAid Kit was used to synthesize cDNA. The RNA samples were combined with primers, buffers, and reverse transcriptase, and then incubated to form cDNA.

Polymerase Chain Reaction (PCR):

- **Primer Preparation:** Stock primers were diluted and centrifuged.
- **Primer Optimization:** RT-PCR was performed with 35 cycles of denaturation, annealing, and extension to amplify target viral DNA.

Gel Electrophoresis:
PCR products were analyzed on 2% agarose gel with TAE buffer. Electrophoresis was conducted at 100-130 volts, and amplicons were visualized under UV light to confirm viral presence.

RESULTS

In this research study, a comprehensive analysis was conducted using 59 rectal swabs collected from a population of rats. These swabs were systematically organized into 20 distinct pools, with each pool comprising a set of three rats. Upon conducting viral testing on these pooled samples, it was determined that 11 out of the 20 pools—equating to a positivity rate of 55%—tested positive for the presence of coronavirus.
Importantly, a further examination of the positive pools revealed that all 11 pools contained rectal swabs, indicating a 100% positivity rate among the rectal samples analyzed. This finding underscores the predominance of rectal swabs in detecting the virus within this specific population.

$$\textbf{Positivity Rate} = \frac{\text{No. of Positive Pools}}{\text{Total No. of Pools}} = \frac{11}{20} \times 100 = \mathbf{55\%}$$

The details of these positive 11 pools and those 20 positive rats are given below in Table 02.

Table 02:
Result Details (the positive pools, corresponding rats, their location and gender)

Sr#	Pool ID	P/N Pool	Rats ID	Capturing Location	Gender
1	S1	P	R1	Mela Gali	M
			R2	Mela Gali	F
			R3	Mela Gali	M
2	S2	N	R4	Mela Gali	F

			R5	Mela Gali	M
			R6	Mela Gali	F
3	S3	**N**	R7	One Unit Chowk	M
			R8	One Unit Chowk	F
			R9	One Unit Chowk	F
4	S4	**P**	R10	One Unit Chowk	F
			R11	One Unit Chowk	F
			R12	One Unit Chowk	F
5	S5	**N**	R13	One Unit Chowk	F
			R14	One Unit Chowk	M
			R15	One Unit Chowk	M
6	S6	**N**	R16	One Unit Chowk	M
			R17	One Unit Chowk	F
			R18	One Unit Chowk	F
7	S7	**N**	R19	Gala Mandi	M
			R20	Gala Mandi	F
			R21	Gala Mandi	F
8	S8	**P**	R22	Gala Mandi	F
			R23	Gala Mandi	F
			R24	Gala Mandi	M
9	S9	**P**	R25	Gala Mandi	F
			R26	Gala Mandi	M
			R27	Gala Mandi	F
10	S10	**P**	R28	Qasim Town	M
			R29	Qasim Town	F
			R30	Qasim Town	M
11	S11	**P**	R31	Qasim Town	M
			R32	Qasim Town	F
			R33	Qasim Town	F
12	S12	**N**	R34	Qasim Town	M
			R35	Qasim Town	F
			R36	Qasim Town	M
13	S13	**N**	R37	Qasim Town	F
			R38	Qasim Town	F

			R39	Qasim Town	M
14	S14	**N**	R40	Faisal Colony	M
			R41	Faisal Colony	F
			R42	Faisal Colony	F
15	S15	**P**	R43	Faisal Colony	F
			R44	Faisal Colony	M
			R45	Faisal Colony	F
16	S16	**P**	R46	Faisal Colony	F
			R47	Faisal Colony	F
			R48	Faisal Colony	M
17	S17	**P**	R49	IUB Cafeteria	F
			R50	IUB Cafeteria	M
			R51	IUB Cafeteria	F
18	S18	**N**	R52	IUB Cafeteria	M
			R53	IUB Cafeteria	M
			R54	IUB Cafeteria	F
19	S19	**P**	R55	IUB Cafeteria	F
			R56	IUB Cafeteria	F
			R57	IUB Cafeteria	M
20	S20	**P**	R58	IUB Cafeteria	F
			R59	IUB Cafeteria	F

***P:** Positive ***N:** Negative ***R:** Rat ***M:** Male ***F:** Female

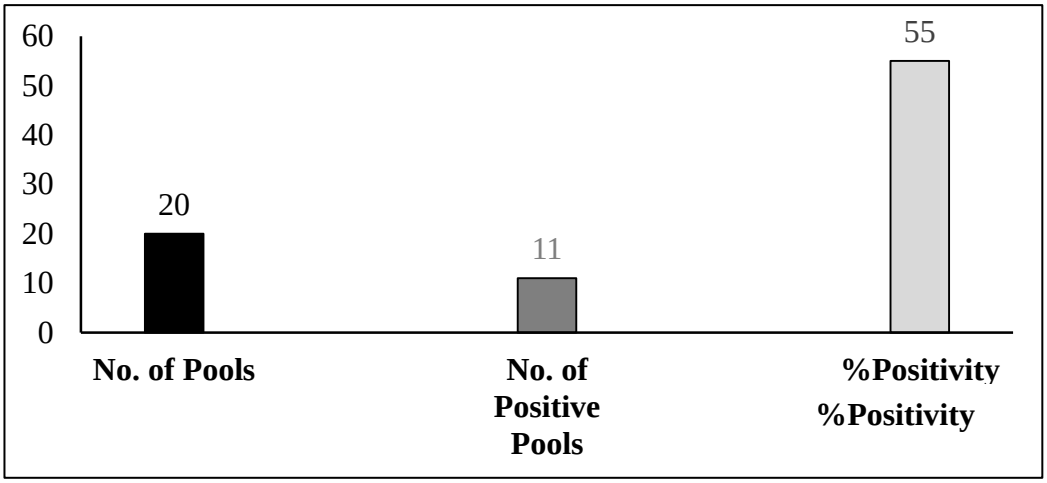


Figure 1: Graph representing the prevalence in numbers.

PCR-Detection (Pool-wise):
In this study, a gel electrophoresis analysis was performed to confirm the presence of coronavirus in the rectal swabs collected from the rat population. The gel image provides a visual representation of the PCR amplification results from the pooled samples, allowing for the identification of viral RNA.

Description of the Gel
The gel image (Figure 4.2) displays several distinct bands corresponding to the PCR products of the coronavirus target sequence. Each lane on the gel represents an individual pool, with lanes labeled according to their respective pool numbers. The expected band size for the coronavirus target is approximately [Band Size: 434 bp], as indicated by the molecular weight marker included in the first lane.

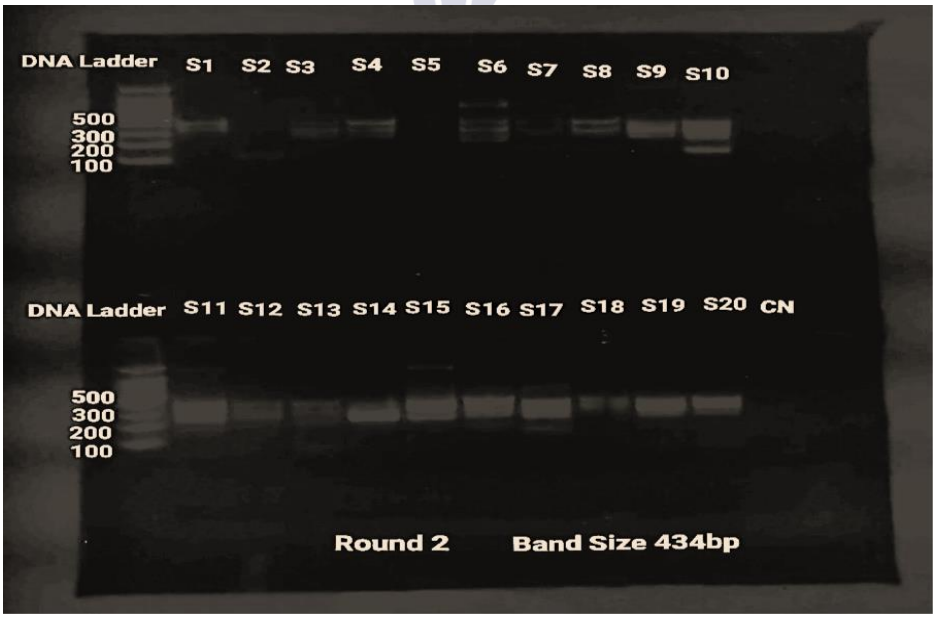
Interpretation of the Results
Upon examination of the gel:

Positive Pools: Lanes corresponding to the 11 coronavirus-positive pools show clear bands at the expected size, confirming the presence of viral RNA. The intensity of these bands suggests varying levels of viral load among the pools.

Negative Pools:
In contrast, the lanes representing the negative control pools exhibit no bands, further validating the specificity of the PCR assay.

Significance of Findings
The gel electrophoresis results corroborate the findings of the viral testing, reinforcing the conclusion that coronavirus is present in the tested rat populations. The presence of distinct bands in the positive pools aligns with the high positivity rate observed in the pooled samples (55%). The Coronaviridae family has typically 6 to 7 core genes and we targeted the RdRP gene, a conserved region in the Coronaviridae family.

Figure 2: Gel Image representing the positive bands



Overall, the gel image (Figure 4.2) serves as a critical validation tool for the findings, demonstrating the reliability of the PCR method used in detecting coronavirus within the rectal swabs of the rat population. This visual confirmation strengthens the overall conclusions of the study regarding the prevalence of the virus and its potential implicationpublic health monitoring.

Location-Based Trend of Positivity Rate in Pools

Figure 3:
Graph representing the identified trend against the localized prevalence of coronavirus

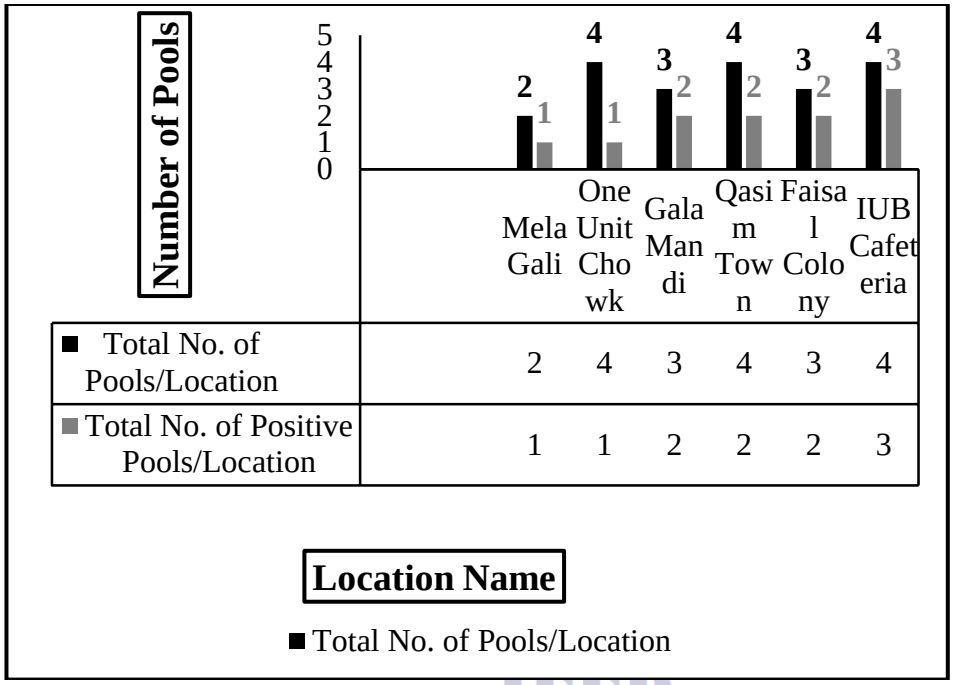


Table 3:
Location-based Statistical Data Interpretation

Location Name / Distribution of Rats	Mela Gali	One Unit Chowk	Gala Mandi	Qasim Town	Faisal Colony	IUB Cafeteria
No. of Male Rats	3	4	3	6	2	4
No. of Female Rats	3	8	6	6	7	7
Total No. of Rats	6	12	9	12	9	11
Total No. of Pools	2	4	3	4	3	4
Total No. of	1	1	2	2	2	3

Positive Pools						
%Positive Pools	50.00	25.00	66.67	50.00	66.67	75.00

DISCUSSION

This research may suggest that rats have coronaviruses. Therefore, it may indicate that they are typical coronavirus sources or reservoirs. Coronaviruses are spherical, enclosed viruses that are members of the Nidovirales order's enormous *Coronaviridae* family of single-stranded RNA viruses(Shereen et al., 2020)causing acute or persistent infection in a wide variety of mammals and birds (Weiss & Navas-Martin, 2005). The first study on coronaviruses in Canary Island mouse populations was just published. Only the house mouse (*Mus musculus*), out of the three rodent species examined, tested positive for coronavirus RNA(Goncu Ayhan et al., 2021). Despite their best efforts, they could not identify any coronaviruses in the house rats (*Rattus rattus*) under investigation. The findings imply that coronavirus circulation is little or nonexistent in the populations of house rats (R. rattus) under investigation. They examined 150 samples of house mice (M. musculus), 109 fecal samples of house rats (*R. rattus*), and one sample of brown rats (*Rattus norvegicus*). Only house mice (M. musculus) from the islands of El Hierro, Tenerife, and Lanzarote have coronavirus RNA. We analyzed 59 stool swabs from Mela Gali, one unit Chowk, Gala Mandi, Qasim Town, Faisal Colony, and IUB cafeteria, and 11 pools out of 20 were positive. Rats are widely distributed across Bahawalpur, Pakistan, according to our research. In our sample sites, we also discovered a kind of CoV circulating in house rats. Unfortunately, it is impossible to tell whether recombination is present since the detection zone is tiny and exclusive to this investigation. The presence of coronaviruses in rats was confirmed by the positive coronavirus test results in about 55% of rat pool samples.

W. Wang screened 1465 rodents from 10 species obtained in three sites in Zhejiang province, southeastern China, for coronaviruses by using RT-PCR targeting a conserved sequence of the viral RdRp (RNA-dependent RNA polymerase) gene (Wang et al., 2015). PCR products of the expected size were obtained and about 2% of the rodents were CoV positive. In the present study, we utilized RT-PCR (Reverse transcriptase) for the detection of coronaviruses. Out of 20 pools, 11 were reported positive. It's interesting to note that a significant amount of the genetic diversity seen in CoVs of the genus Betacoronavirus is found in rodent-associated CoVs. Human coronaviruses HKU1 and OC43, as well as viruses that cause respiratory and intestinal pathogenicity in domestic animals, such as hemagglutinating encephalomyelitis in pigs, are members of this lineage. Unlike gamma and delta CoVs, which are mostly carried by birds, alpha and beta CoVs are closely associated with mammals (Members et al., 2012).

The latest findings, however, indicate that the recently discovered COVID-19 variations (VOCs) have successfully infected and multiplied in the lungs of ordinary laboratory mice at large titer(Woolhouse & Gowtage-Sequeria, 2005). According to estimates, between 70 and 75 percent of the re-emerging epidemics in recent decades have been caused by zoonotic spillovers, which are the spread of infections from animals to people and vice versa(Wang & Crameri, 2014). Numerous animal species and captive fauna that come into close contact with human COVID-19 cases have been shown to have SARS-CoV-2. Additionally, it has been shown that, in laboratory settings, this virus may infect wild animals. At this time, coronavirus has been isolated from a variety of animals, including dogs, horses, ferrets, bats, rats, and cattle (Ng & Hiscox, 2020). According to a WHO report, much more intense transmission has occurred in SARS-CoV-2, where more than 138.68 million people became infected across 223 countries, with a mortality rate of 2.15% (Sánchez-Duque et al., 2020)

In March 2020, RT–PCR detected SARS-CoV-2 RNA in the content of feces and vomit from a cat in Belgium. The cat's owner had tested positive for SARS-CoV-2- A week after the owner started showing

signs of COVID-19, the cat had a brief bout of respiratory and gastrointestinal illness. It is challenging to associate the clinical indications of a cat with an active SARS-CoV-2 infection since, as of the date of this report, no information on viral isolation or blood findings was available. Positive for SARS-CoV-2 with no clinical disease manifestations was reported in a domestic cat on 1 April 2020 in Hong Kong after confirmation of the owner's COVID-19(Tiwari et al., 2020). These zoo animals got the virus from an asymptomatic zoo employee. Two domesticated cats tested positive for SARS-CoV-2 on April 22, 2020. While the second cat most likely became infected by a person who was secretly infected, the first cat received the illness from an owner who was afflicted with the virus. As of August 14, 2020, RT-PCR or virus-neutralization antibody testing has shown that 14 dogs and 13 domestic cats in the United States are infected with SARS-CoV-2. Every companion animal was exposed to either secretly infected or verified COVID-19-positive people. It has been shown that domestic cats are very vulnerable to SARS-CoV-2 after experimental exposure and that they are also adept at spreading the virus to other cats over short distances via droplets or aerosols (Halfmann et al., 2024; Halfmann et al., 2020) .

We published the first study on coronaviruses in rat populations from several Bahawalpur locations. We examined the house mouse's (M. musculus) fecal swabs and discovered that they contained coronavirus RNA. The present study underlines the need for adequate prevention against coronaviruses. To prevent zoonotic coronavirus from entering human health and a new outbreak of COVID hygiene control must be implemented. The current study would prove to be a clear insight into the prevalence of coronaviruses from rats in Bahawalpur, Punjab, Pakistan. As many possibilities are there for the outbreak of coronavirus, there also exists a particular need for epidemiological studies on the spread of coronaviruses.

Conclusion

The rapid spread of infectious agents has also something to do with our globalized and interconnected human society. It speeds up global commerce and population migration at an ever-increasing rate, creating a plethora of new links between various host populations and novel infectious agents. Few things in human history have altered global epidemiology so drastically in such a short amount of time. The fact that coronaviruses propagate so well is only evidence of that. Data from our findings points to a very recent introduction of CoV in rats. In this way, it serves as a reminder of the significance of keeping an eye on and managing the entry of species that may serve as disease reservoirs in Bahawalpur, Pakistan, as well as in other insular habitats.

REFERENCES

Deng, X., & Baker, S. (2021). Coronaviruses: molecular biology (Coronaviridae). *Encyclopedia of Virology*, 198.

Gandhi, R. T., Bedimo, R., Hoy, J. F., Landovitz, R. J., Smith, D. M., Eaton, E. F., Lehmann, C., Springer, S. A., Sax, P. E., & Thompson, M. A. (2023). Antiretroviral drugs for treatment and prevention of HIV infection in adults: 2022 recommendations of the International Antiviral Society–USA panel. *Jama*, *329*(1), 63-84.

Goncu Ayhan, S., Oluklu, D., Atalay, A., Menekse Beser, D., Tanacan, A., Moraloglu Tekin, O., & Sahin, D. (2021). COVID-19 vaccine acceptance in pregnant women. *International Journal of Gynecology & Obstetrics*, *154*(2), 291-296.

Halfmann, M., Castioni, N., Wetzel, L., Koopmann, A., König, S., & Schmieder, A. (2024). Effects of the COVID-19 pandemic on the mental health of medical students and young physicians in Germany: Gender-specific results of an online survey. *Heliyon*, *10*(1).

Halfmann, P. J., Hatta, M., Chiba, S., Maemura, T., Fan, S., Takeda, M., Kinoshita, N., Hattori, S.-i., Sakai-Tagawa, Y., & Iwatsuki-Horimoto, K. (2020). Transmission of SARS-CoV-2 in domestic cats. *New England Journal of Medicine*, *383*(6), 592-594.

Hermes, D. J., Jacobs, I. R., Key, M. C., League, A. F., Yadav-Samudrala, B. J., Xu, C., McLane, V. D., Nass, S. R., Jiang, W., & Meeker, R. B. (2020). Escalating morphine dosing in

HIV-1 Tat transgenic mice with sustained Tat exposure reveals an allostatic shift in neuroinflammatory regulation accompanied by increased neuroprotective non-endocannabinoid lipid signaling molecules and amino acids. *Journal of Neuroinflammation*, *17*, 1-26.

Lang, Y., Li, W., Li, Z., Koerhuis, D., Van Den Burg, A. C., Rozemuller, E., Bosch, B.-J., van Kuppeveld, F. J., Boons, G.-J., & Huizinga, E. G. (2020). Coronavirus hemagglutinin-esterase and spike proteins coevolve for functional balance and optimal virion avidity. *Proceedings of the National Academy of Sciences*, *117*(41), 25759-25770.

Lee, I., Wang, C., Lin, M., Kung, C., Lan, K., & Lee, C. (2020). Effective strategies to prevent coronavirus disease-2019 (COVID-19) outbreak in hospital. *The Journal of hospital infection*, *105*(1), 102.

Li, X., Wang, L., Liu, P., Li, H., Huo, S., Zong, K., Zhu, S., Guo, Y., Zhang, L., & Hu, B. (2021). A novel potentially recombinant rodent coronavirus with a polybasic cleavage site in the spike protein. *Journal of Virology*, *95*(22), 10.1128/jvi. 01173-01121.

Members, A. T. F., Camm, A. J., Lip, G. Y., De Caterina, R., Savelieva, I., Atar, D., Hohnloser, S. H., Hindricks, G., Kirchhof, P., & Guidelines, E. C. f. P. (2012). 2012 focused update of the ESC guidelines for the management of atrial fibrillation: an update of the 2010 ESC guidelines for the management of atrial fibrillation developed with the special contribution of the European Heart Rhythm Association. *European heart journal*, *33*(21), 2719-2747.

Meng, Y., Wu, P., Lu, W., Liu, K., Ma, K., Huang, L., Cai, J., Zhang, H., Qin, Y., & Sun, H. (2020). Sex-specific clinical characteristics and prognosis of coronavirus disease-19 infection in Wuhan, China: A retrospective study of 168 severe patients. *PLoS pathogens*, *16*(4), e1008520.

Murin, C. D., Wilson, I. A., & Ward, A. B. (2019). Antibody responses to viral infections: a structural perspective across three different enveloped viruses. *Nature microbiology*, *4*(5), 734-747.

Ng, L. F., & Hiscox, J. A. (2020). Coronaviruses in animals and humans. In (Vol. 368): British Medical Journal Publishing Group.

Payne, S. (2017). Family coronaviridae. *Viruses*, 149.

Poudel, U., Subedi, D., Pantha, S., & Dhakal, S. (2020). Animal coronaviruses and coronavirus disease 2019: Lesson for one health approach. *Open veterinary journal*, *10*(3), 239–251-239–251.

Samudrala, P. K., Kumar, P., Choudhary, K., Thakur, N., Wadekar, G. S., Dayaramani, R., Agrawal, M., & Alexander, A. (2020). Virology, pathogenesis, diagnosis and in-line treatment of COVID-19. *European journal of pharmacology*, *883*, 173375.

Sánchez-Duque, J. A., Arce-Villalobos, L. R., & Rodríguez-Morales, A. J. (2020). Enfermedad por coronavirus 2019 (COVID-19) en América Latina: papel de la atención primaria en la preparación y respuesta. *Atención primaria*, *52*(6), 369.

Shereen, M. A., Khan, S., Kazmi, A., Bashir, N., & Siddique, R. (2020). COVID-19 infection: Emergence, transmission, and characteristics of human coronaviruses. *Journal of advanced research*, *24*, 91-98.

Tiwari, S., Kumar, S., & Guleria, K. (2020). Outbreak trends of coronavirus disease–2019 in India: a prediction. *Disaster medicine and public health preparedness*, *14*(5), e33-e38.

Wang, L., & Crameri, G. (2014). Emerging zoonotic viral diseases. *Rev Sci Tech*, *33*(2), 569-581.

Wang, W., Lin, X.-D., Guo, W.-P., Zhou, R.-H., Wang, M.-R., Wang, C.-Q., Ge, S., Mei, S.-H., Li, M.-H., & Shi, M. (2015). Discovery, diversity and evolution of novel coronaviruses sampled from rodents in China. *Virology*, *474*, 19-27.

Weiss, S. R., & Navas-Martin, S. (2005). Coronavirus pathogenesis and the emerging pathogen severe acute respiratory syndrome coronavirus. *Microbiology and molecular biology reviews*, *69*(4), 635-664.

Woolhouse, M. E., & Gowtage-Sequeria, S. (2005). Host range and emerging and reemerging

pathogens. *Emerging infectious diseases*, *11*(12), 1842.

Yuan, S., Jiang, S.-C., & Li, Z.-L. (2020). Analysis of possible intermediate hosts of the new coronavirus SARS-CoV-2. *Frontiers in Veterinary Science*, *7*, 379.

Zappulli, D. A. (2021). Ingegneria concettuale.

Zmasek, C. M., Lefkowitz, E. J., Niewiadomska, A., & Scheuermann, R. H. (2022). Genomic evolution of the Coronaviridae family. *Virology*, *570*, 123-133.

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