Journal of Ideas in Health

e ISSN: 2645-9248 Journal homepage: www.jidhealth.com Open Access

Original Article

Metagenomics screening of viruses in Arusha, north Tanzania: a one health perspective across wild rodents, domestic dogs and humans

Amina Ramadhani Issae[1,2,3*], Abdul Ahmed Selemani Katakweba[1,3], Rose Peter Kicheleri[2], Augustino Alfred Chengula[4], Christopher Jacob Kasanga[4]

Abstract

Background: One of the most significant and perilous challenges faced by African societies, including Tanzania, is the public health threat posed by zoonotic viruses. This study aimed to ascertain the presence of viruses in wild rodents, domestic dogs, and humans who shared the same environment.

Methods: A cross-sectional study was conducted in the Ngorongoro district from January to September 2022. Metagenomics next-generation sequencing technologies, including Illumina (MiSeq) and Oxford Nanopore (MinION), were employed to identify viruses in 530 blood samples collected from 200 humans, 230 wild rodents, and 100 domestic dogs. The captured wild rodents belonged to various species: Mastomys spp (n=87), Ratus spp (n=45), Avicanthis spp (n=40), Arcomys spp (n=28), Mus spp (n=16), and Field mice (n=14). The taxonomic classification of viruses was carried out using the Kraken2 program.

Results: A total of 20 RNA and 20 DNA viral families were detected. Zoonotic RNA families identified in rodents included Peribunyaviridae, Hantaviridae, Flaviviridae, Coronaviridae, Orthomyxoviridae, Paramyxoviridae, Retroviridae, Picornaviridae, Arenaviridae, Togaviridae, and Tobaniviridae. The zoonotic DNA families detected in rodents were Adenoviridae, Poxviridae, Herpesviridae, Anelloviridae, and Circoviridae. Peribunyaviridae and Hataviridae were identified in both humans and rodents. Unclassified +ssRNA viruses were found in dogs and humans. Herpesviridae was present in all three hosts. When compared to dogs and humans, rodents harbored zoonotic viruses that pose a significant public health safety concern.

Conclusion: The study unveiled a noteworthy pattern where the majority of RNA and DNA viruses with zoonotic potential were detected in wild rodents, as opposed to dogs and humans. These findings underscore the pivotal role played by wild rodent populations in the transmission and maintenance of such viruses.

Keywords: Metagenomics Next-Generation Sequencing, Zoonotic Viruses, Wild Rodents, Domestic Dogs, Humans, Ngorongoro, Tanzania

Background

Zoonotic infections exert a considerable strain on healthcare systems globally, particularly in less developed countries [1]. Diseases stemming from rodents have always been a global public health issue [2]. Many rodent species, such as rats and mice, are renowned carriers of multiple viruses [2]. Research in sub-Saharan Africa has highlighted the presence of zoonotic viruses like Hantaviruses and Lassa fever viruses in wild rodents [3-4]. Furthermore, domestic dogs have been implicated in transmitting zoonotic viruses to humans and other animals [4], with instances of rabies in domestic dogs being reported throughout Tanzania [4]. Both rodents and dogs can transfer pathogenic and zoonotic viruses to humans and other animals via bites, or by contact with infected surfaces, food, or water [3-5]. Research from around the world indicates that over half of human pathogens are zoonotic, and a significant portion of emerging infectious diseases arise from zoonotic viruses [6-7]. Historically, global pandemics have often been traced back to

*Correspondence: amina.issae@sua.ac.tz

[1]Institute of Pest Management, Sokoine University of Agriculture, P.O. Box 3110, Morogoro Tanzania

A full list of author information is available at the end of the article

pathogens from wildlife [6-10]. Including rodents, dogs, and humans in virus surveillance adheres to the One Health model, which emphasizes the interconnectedness of human, animal, and environmental health [10]. Many infectious diseases involve intricate ecological interactions spanning multiple species, making it essential to comprehend these relationships for effective disease prevention [10]. Concurrent monitoring of infections in animals and humans offers a holistic view of disease patterns, transmission dynamics, and associated risk factors, ultimately promoting improved public health outcomes.

Tanzania boasts a diverse range of small mammal species scattered across various regions [11]. With its plethora of Wildlife National Parks and conservation areas, Tanzania has seen increased human intrusion into these zones due to expanding agricultural practices, hunting, tourism, and the quest for herbal remedies [11]. Such activities heighten the risk of infectious pathogen transmission from wildlife to humans and animals. Although Tanzania is abundant in areas where wildlife, livestock, and humans coexist, there is a dearth of data on rodent-related zoonotic viruses. The Ngorongoro district, with its rich wildlife, domestic animals, and migratory species, is a potential hotspot for the spread of rodent-borne viruses to animals and humans [11-12]. Past studies on livestock and human patients in the district have revealed evidence of infections like the Rift Valley fever virus [13]. A study in Arusha even found the presence of Hantaviruses in asymptomatic adults visiting hospitals [3]. However, the role of wild rodents in viral transmission within the district remains unexplored. Hence, this study aimed to screen and identify viruses in wild rodents, domestic dogs, and humans, with a focus on zoonotic variants. This research serves as foundational data, shedding light on the RNA and DNA viruses in rodents, dogs, and humans.

Methods

Study design and setting

This research was conducted in five villages within the Ngorongoro district (Fig.1). Situated in the northern part of Tanzania within the Arusha region, the district spans an area of 14,036 km2 and is geographically positioned between longitude 35’30°E and 36’23°E, and latitude 02’45°S and 4’0°S [13]. It's bordered by Kenya to the north, Serengeti National Park to the west, and the Longido and Moduli Districts to the east, while Karatu District lies to the south. The district is divided into three divisions, comprising 28 wards and 65 villages, with an estimated human population of 174,278 [14].

Rodent trapping protocol

Wild rodents were trapped using Sherman LFA live traps (HB Sherman Traps, Inc., Tallahassee, FL) and wire cage traps, baited with a mix of peanut butter, maize bran, and sardines [15]. Habitats like homes, human settlements' peripheries, livestock pens, and nearby agricultural plots served as trapping grounds. After capture, rodents were identified morphologically to genus level following the Happold manual [16]. Depending on a home's size, 2 to 4 wire cage traps were placed indoors, while Sherman traps were strategically placed around pathways and burrows for peri-domestic rodents. Each village had 40 modified wire cage traps and 80 Sherman traps designated for indoor and surrounding trapping, respectively. Each village's trapping lasted seven days, setting traps between 4-5 p.m. and collecting them the next morning, around 8-10 a.m.

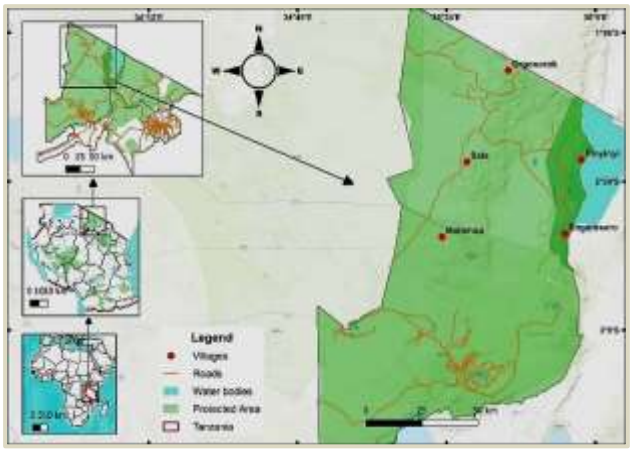

Figure 1: A map that displays Tanzania and studied villages within the Ngorongoro district created by Issae et al. [15]

Sample size and pooling procedures

Human blood samples (n=200) were pooled into 22 pools based on their sex [15]. Rodent samples (n=230) were pooled into 16 groups according to genera, while dog samples (n=100) were divided into 10 pools based on sex.

Sample collection and handling procedures

From Rodents: After being humanely anesthetized and euthanized using Isoflurane [15], rodents were prepared for sampling. Their ventral surface was disinfected with 70% alcohol, and researchers collected approximately 0.5 ml of blood using hypodermic needles and syringes [17]. This blood, obtained using the cardiac puncture method, was mixed with 1 ml of DNA/RNA shield reagents in cryovials to preserve nucleic acids [15]. The samples were kept in liquid nitrogen and transported to the Sokoine University of Agriculture where they were stored at -80°C until molecular analysis.

From Humans and Dogs: Expert personnel from the Ngorongoro district collected blood samples, ensuring sanitary precautions. After disinfecting the skin with 70% alcohol, 1 ml of blood was drawn from the cephalic veins using a 21-gauge needle [17]. The blood was mixed with DNA/RNA shield reagents in cryovials to prevent and stored at -80°C until analysis [15]. Molecular studies were conducted at the Kilimanjaro Clinical Research Institute (KCRI) biotechnology laboratory at the Kilimanjaro Christian Medical Centre (KCMC) in Moshi.

Extraction of RNA and DNA, cDNA synthesis, and sequencing library preparation

The QIAamp RNA Blood Mini Kit was used for RNA extraction and the QIAamp DNA Blood Kit for DNA extraction, both by the manufacturer's instructions (Qiagen, Valencia, CA, USA). The extracted RNA was then reverse transcribed into complementary DNA (cDNA) using the Omniscript Reverse Transcription Kit (Qiagen, Valencia, CA, USA), following the manufacturers' guidelines. Two Next-Generation Sequencing techniques were applied: Oxford Nanopore with the MinION sequencer and Illumina with the MiSeq sequencer. For the MiSeq platform, libraries were

prepared using the Illumina Nextera XT DNA Library Prep Kit (Illumina, San Diego, CA), based on the supplier's recommended procedures. For the MinION platform, the PCR-cDNA sequencing-barcoding kit (SQK-PCB109) from Oxford Nanopore Technologies was followed as per the manufacturer's instructions. The concentration of nucleic acids at various stages of library preparation was determined using Thermo Fisher Scientific's Qubit high-sensitivity RNA and DNA assays. Subsequently, equimolar quantities of nucleic acids were combined, and the libraries were sequenced in a single lane on an Illumina MiSeq sequencer (Illumina, San Diego, CA).

Statistical analysis

The MiSeq reads were processed for base calling and demultiplexing using Illumina BCL Convert software, version 3.9.3. For the MinION reads, base calling was performed using the Guppy Software version 6.4.2 from Oxford Nanopore Technologies (ONT), with the 9.4.1_450bps_SUP model. Segregation of reads based on their specific barcodes was achieved using ONT Guppy barcoder, version 6.4.2. Reads that were not classified were maintained as separate pseudo-samples. Following this, the reads were scanned for contamination from humans and vectors using FastQ Screen version 0.14.1, with GRCh38 and UniVec Core as the reference sources [18]. The reads were then trimmed with fastp, using default parameters as well as trimming the front (-5) and tail (-3) ends. Basic quality metrics were generated using fast-stats from the fastq-util tool, version 1.3.0 [19]. For taxonomic classification, Kraken2 version 2.1.2 was used [20], leveraging standard Kraken2 databases augmented with fungi. The databases were formulated using reference sequences from the National Center for Biotechnology Information (NCBI), gathered between November 15th and 18th, 2022. The quality-filtered and trimmed MiSeq and MinION reads were then analyzed in Kraken2's paired-end mode. Notably, Kraken2 is a bioinformatics package renowned for its high accuracy and sensitivity in microbial classification, even detecting those in minimal abundance [20].

Results

This study identified 34 viral families in rodents, 5 in dogs, and 7 in humans. Notably, the study pinpointed 20 RNA and 20 DNA viral families, with rodents demonstrating the highest viral prevalence (Figure 2).

Table 1: Inclusive explanation of rodent genera, size of the sample, and formation of blood pools

Category of samples	Species	Sex	Samples counts	Pools counts	Samples count per pool	Volume (µl) pooled per sample	Final volume (ml) per pool
Human	NA	F	130	13	10	100	1
	NA	M	70	9	7-8	100	0.7-0.8
	Total samples		**200**	**22**			
Rodents	*Mastomys* spp	F	67	6	11-12	100	1.1-1.2
		M	20	1	20	80	1.6
	Ratus spp	F	27	2	13 and14	100	1.3-1.4
		M	18	1	18	80	1.44
	Avicanthis spp	F	20	1	20	80	1.6
		M	20	1	20	80	1.6
	Arcomys spp	F	15	1	15	100	1.5
		M	13	1	13	100	1.3
	Mus spp	M & F	16	1	16	80	1.3
	Field mice spp	M & F	14	1	14	100	1.4
	Total samples		**230**	**16**			
Dogs	Mongrels	F	57	5	11-12	100	1.1-1.2
	Mongrels	M	43	5	8-10	100	0.8-1
	Total samples		**100**	**10**			

RNA Viral families in rodents

From the wild rodents sampled, 14 RNA families were identified. Notably, the Retroviridae family was prevalent in 9 out of 16 pools, accounting for 56.25% of the pools. The remaining viral families each appeared in a single pool, constituting a 6.35% representation. Of all rodent species, Mastomys spp showed the most RNA viral presence (Table 2).

DNA viral families in rodents

This study identified five DNA viral families within the rodent samples (Table 3). It's noteworthy that some viruses outlined in Table 3 have zoonotic implications.

DNA and RNA viruses in domestic dogs

The findings in domestic dogs included three distinct viral families and an array of unclassified +ssRNA viruses (Table 4). Within these, the Retroviridae family was present in 3 out of 10 pools, translating to a 30% occurrence. Each of the other viral families was detected in a single pool, each with a 6.25% representation.

DNA and RNA viruses in humans

In the human samples, three viral families and unclassified +ssRNA viruses were detected in 5 out of the 22 pools (Table 5). Specifically, Peribunyaviridae was observed in two out of the 22 pools, equating to a 9.09% presence. All other viral families appeared once, each having a 4.54% proportion.

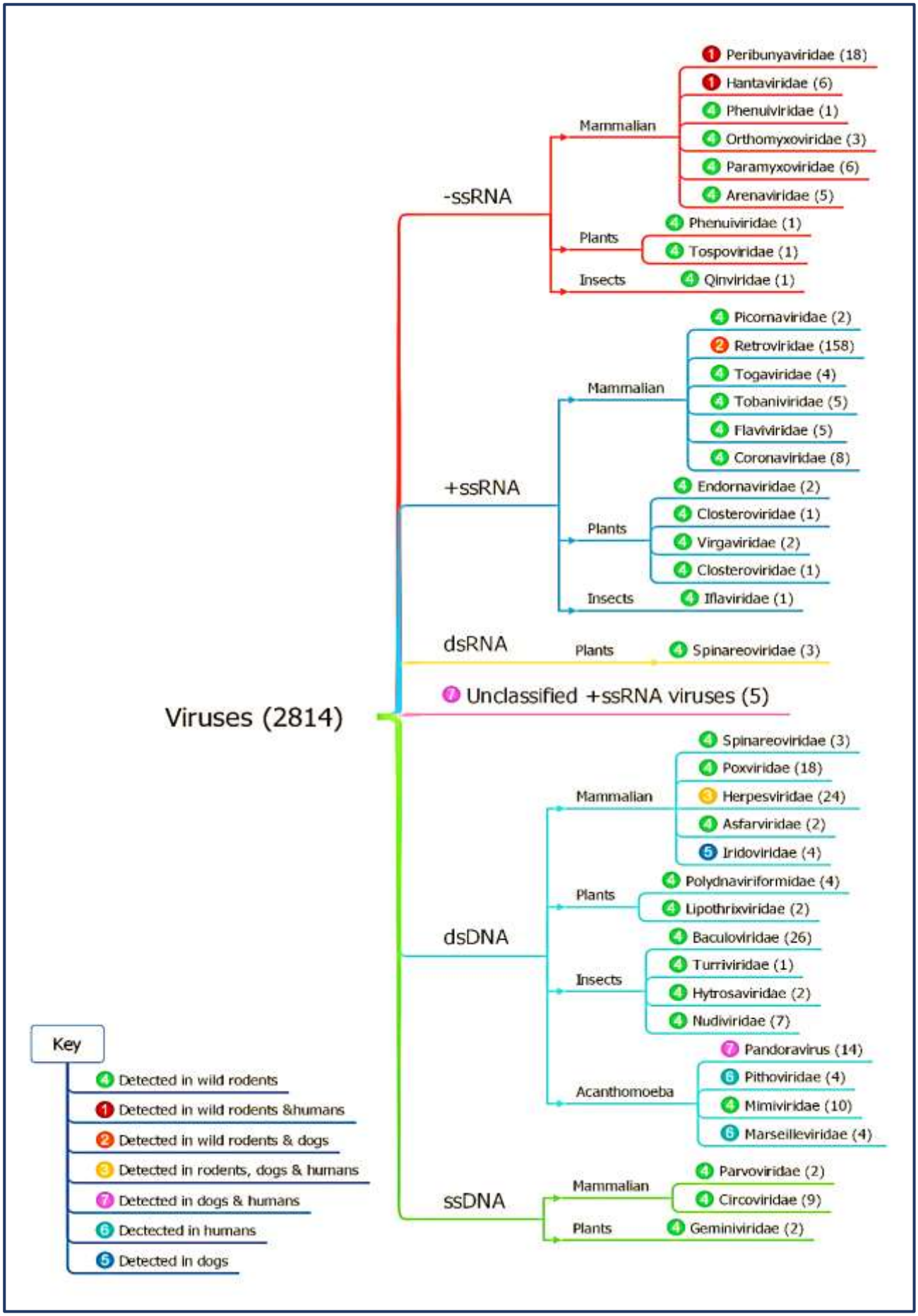

Figure 2: Distribution of viral families in wild rodents, domestic dogs, and humans, with their respective read counts indicated in brackets

Table 2: RNA viruses present in rodent blood samples from Ngorongoro District

Host	Family	Genus	Species and strains	Reads	Genome
Mastomys spp	*Peribunyaviridae*	*Orthobunyavirus*	*California encephalitis orthobunyavirus*	7	-ssRNA
			MPoko orthobunyavirus	5	-ssRNA
		Herbevirus	*Tai herbevirus*	2	-ssRNA
	Hantaviridae	*Orthohantavirus*	*Hantavirus Z10*	4	-ssRNA
			Choclo orthohantavirus	4	-ssRNA
			Seoul orthohantavirus	4	-ssRNA
Mastomys spp	*Picornaviridae*	*Sicinivirus*	*Sicinivirus A*	2	+ssRNA
		Kunsagivirus	*Kunsagivirus A*	2	+ssRNA
Mastomys spp	*Satellites*	*RNA satellites*	*Olive viral satellite RNA*	3	
Ratus spp	*Orthomyxoviridae*	*Alphainfluenzavirus*	*Influenza A virus*	2	-ssRNA
	Paramyxoviridae	*Orthorubulavirus*	*Human orthorubulavirus 4*	3	-ssRNA
		Jeilongvirus	*Tailam jeilongvirus (Tailam virus)*	2	-ssRNA
Mastomys spp	*Retroviridae*	*Betaretrovirus*	*Mason-Pfizer monkey virus*	23	-ssRNA
			Ovine enzootic nasal tumor virus	15	-ssRNA
			Simian retrovirus 8	6	-ssRNA
			Simian retrovirus 4	4	+ssRNA
Avicanthis spp			*Enzootic nasal tumor virus of goats*	1	+ssRNA
			Desmodus rotundus endogenous retrovirus	10	+ssRNA
			Mouse mammary tumor virus	6	+ssRNA
Ratus spp		*Alpharetrovirus*	*Avian myelocytomatosis virus*	3	+ssRNA
		Gamma retrovirus	*Murine leukemia virus*	2	+ssRNA
			Abelson murine leukemia virus	5	-ssRNA
			XMRV-related retroviruses	5	+ssRNA
Mus spp	*Arenaviridae*	*Reptarenavirus*	*Giessen reptarenavirus (University of Giessen virus)*	3	+ssRNA
	Togaviridae	*Alphavirus*	*Middelburg virus*	4	+ssRNA
Arvicathis spp	*Tobaniviridae*	*Torovirus*	*Hainan hebius popei torovirus*	3	+ssRNA
Field mice	*Flaviviridae*	*Flavivirus*	*T'Ho virus*	5	+ssRNA
	Coronaviridae	*Alphacoronavirus*	*Porcine epidemic diarrhea virus*	3	+ssRNA
			Rat coronavirus	2	+ssRNA
			Feline coronavirus	2	+ssRNA

Table 3: DNA viruses found in rodent blood samples from Ngorongoro District

Host	**Family**	**Genus**	**Species and strains**	**Reads**	**Genomes**
Ratus spp	*Spinareoviridae*	*Mastadenovirus*	*Human mastadenovirus* C *Bat mastadenovirus* F *Bat mastadenovirus* WIV17	4 2 2	dsDNA
Arcomys spp	*Poxviridae*	*Centapoxvirus*	*Yokapox virus*	1	dsDNA
			Murmansk microtuspox virus	1	dsDNA
			Murmansk poxvirus	1	dsDNA
		Avipoxvirus	*Pigeonpox virus*	1	dsDNA
			Canarypox virus	1	dsDNA
		Oryzopoxvirus	*Cotia virus* SPAn232	1	dsDNA
		Cervidpoxvirus	*Mule deerpox virus*	1	dsDNA
			Deerpox virus W-848-83	1	dsDNA
		Yatapoxvirus	*Yaba monkey tumor virus*	1	dsDNA
		Capripoxvirus	*Sheeppox virus*	1	dsDNA
		Orthopoxvirus	*Raccoonpox virus*	1	dsDNA
***Mastomys* spp**	*Herpesviridae*	*Percavirus*	*Equid gammaherpesvirus 5*	2	dsDNA
		Muromegalovirus	*Murid betaherpesvirus 8*	1	dsDNA
		Proboscivirus	*Elephant endotheliotropic herpesvirus*	1	dsDNA
		Quwivirus	*Tupaiid betaherpesvirus 1*	1	dsDNA
		Macavirus	*Bovine gammaherpesvirus 6*	1	dsDNA
		Rhadinovirus	*Saimiriine gammaherpesvirus 2*	1	dsDNA
			Rhinolophus gammaherpesvirus 1	1	dsDNA
		Varicellovirus	*Felid alphaherpesvirus 1*	1	dsDNA
			Cercopithecine alphaherpesvirus 9	1	dsDNA
Field mice spp	*Anelloviridae*	*Alphatorquevirus*	*Torque teno virus 22*	2	ssDNA
	Circoviridae	*Circovirus*	*Rodent-associated circovirus 7*	4	ssDNA
			Rodent-associated circovirus 5	2	ssDNA

Table 4: List of RNA and DNA viruses in domestic dogs from the Ngorongoro district

Viral family	Genus	Species	Reads	Genome
Retroviridae	*Gammaretrovirus*	*Baboon endogenous virus* *(Baboon endogenous virus strain M7)*	2 2	+ssRNA
Unclassified Riboviria	unclassified RNA viruses ShiM-2016	*Sanxia water strider virus 19*	1	+ssRNA
		Wenzhou tombus-like virus 15	1	+ssRNA
		Wenzhou picorna-like virus 5	1	+ssRNA
		Wuhan house centipede virus 2	1	+ssRNA
		Hubei picorna-like virus 52	1	+ssRNA
		Hubei picorna-like virus 34	1	+ssRNA
		Wenzhou picorna-like virus 39	1	+ssRNA
		Beihai Charybdis crab virus 1	1	+ssRNA
Herpesviridae	*Simplexvirus*	*Ateline alphaherpesvirus 1*	4	dsDNA
Iridoviridae	*Lymphocystivirus*	*Lymphocystis disease virus Sa*	2	dsDNA
		Lymphocystis disease virus 4	2	dsDNA

Table 5: Catalog of RNA and DNA viruses identified in human samples from the Ngorongoro district

Viral family	Genus	Species	Reads	Genome
Peribunyaviridae	*Orthobunyavirus*	*Simbu orthobunyavirus*	5	-ssRNA
		Shamonda orthobunyavirus	3	-ssRNA
Hantaviridae	*Orthohantavirus*	*Oxbow orthohantavirus*	4	-ssRNA
		Oxbow virus	4	-ssRNA
Unclassified RNA viruses	Unclassified +ssRNA viruses	*Wuhan centipede virus*	3	+ssRNA
Herpesviridae	*Roseolovirus*	*Human betaherpesvirus 6B*	4	dsDNA
		Human betaherpesvirus 5	5	dsDNA
	Cytomegalovirus	*Panine betaherpesvirus 2*	3	dsDNA
		Cercopithecine betaherpesvirus 5	2	dsDNA

Discussion

In our research, we conducted an in-depth examination of the study revealed the presence of 19 RNA viral families in rodents, 2 in dogs, and 3 in humans, marking their first identification in the study area. Moreover, the majority of DNA viruses were found in rodents. This indicates that rodents host a wider variety of viruses compared to dogs and humans. One contributing factor is that most rodents, even when infected, don't necessarily die from these infections. Instead, they often become virus carriers due to non-obvious illnesses and their established role as reservoirs for various pathogens [21]. Furthermore, rodents are hosts to ectoparasites, such as ticks, mites, and fleas, which serve as vectors for disease transmission [22]. This research also offers the inaugural evidence of Orthobunyavirus in both rodents and humans in Tanzania. Specifically, the California encephalitis orthobunyavirus and MPoko orthobunyavirus were identified in Mastomys spp, while the Simbu and Shamonda orthobunyaviruses were discovered in humans. Notably, the two Orthobunyaviruses detected in rodents had previously been recognized as human pathogens [23]. The California encephalitis virus is linked to encephalitis in humans [23]. However, the natural hosts and transmission dynamics of the MPoko orthobunyavirus remain unclear, necessitating more research to decipher its potential ramifications on both human and animal health. Meanwhile, Simbu Orthobunyavirus infections are known to cause congenital malformations and abortions in ruminants [24-25]. These discoveries carry significant implications in the context of holistic health due to the potential for these viruses to spread among various mammalian hosts, their widespread arthropod vectors, and the grave nature of the diseases they cause. The study also uncovered the presence of Tai herpesvirus in Mastomys spp. Previously, this virus was isolated from Culex sp. mosquitoes in Côte d'Ivoire and was later detected in the same mosquito species in Ghana [25]. It was then categorized as an insect-specific virus [25]. However, its detection solely in rodents raises questions: Does Tai herbevirus naturally infect rodents, or did the rodents ingest insects carrying the virus? A deeper understanding of its epidemiology and potential implications for public health necessitates further research and surveillance. This research marked the inaugural identification of Orthohantavirus in both rodents and humans in Tanzania. Specifically, the Choclo orthohantavirus and Seoul orthohantavirus species were identified in wild rodents (Mastomys spp), while the Oxbow orthohantavirus (or Oxbow virus) was found in humans. Seoul orthohantavirus is linked with hemorrhagic fever with renal syndrome (HFRS) in Eurasia and hantavirus pulmonary syndrome (HPS) in America [26-27]. The Oxbow virus was first detected in the American shrew mole in 2003, with its genome analysis revealing a low sequence similarity to rodent-borne hantaviruses [28]. These discoveries represent the first records of Choclo and Seoul orthohantaviruses in wild rodents, and the Oxbow orthohantavirus in Tanzanian humans. Thus, the detection of Orthohantaviruses in both wild rodents and humans highlights the potential risk of cross-species virus transmission between wildlife and humans. Furthermore, the Picornaviridae viral family was detected in rodents. Viruses within this family are recognized as zoonotic [29]. The study identified both Sicinivirus A and Kunsagivirus A in rodents. Remarkably, this is the inaugural report of Sicinivirus A's presence in wild rodents. Nonetheless, Sicinivirus A had previously been

isolated from commercially raised chickens exhibiting severe infections in China [29]. On the other hand, Kunsagivirus A was first found in migratory bird feces and subsequently in wild yellow baboon blood [30]. This study now documents its presence in Mastomys spp. Given these discoveries, it's plausible that Sicinivirus A and Kunsagivirus A could undergo cross-species transmission between birds and mammals. This underscores the importance of further research to determine the prevalence and zoonotic potential of these viruses in mammals.

In the current study, four species of unclassified +ssRNA viruses were detected in dogs and one in humans. These viruses have been characterized previously as the unclassified RNA viruses ShiM-2016, novel Picorna-like viruses, found in Culex spp. mosquitoes from the Zambezi Valley in Mozambique [31]. These viruses were likely transmitted to dogs and humans via mosquito bites. Further investigations are essential to elucidate their potential to cause illnesses in animals and humans. The study pinpointed the presence of the Flavivirus, specifically the T'Ho virus. Members of the Flavivirus genus are known to cause severe endemic infections in humans, such as Yellow Fever, Dengue Fever, Encephalitis, and various hemorrhagic illnesses [32]. The T'Ho virus was initially isolated from Culex quinquefasciatus mosquitoes in the Yucatan Peninsula of Mexico [33]. As of now, there are no documented diseases in vertebrates attributed to this virus worldwide. This research marks the first instance of the T'Ho virus being identified in wild rodents, specifically field mice, indicating they could serve as a potential reservoir for this flavivirus. The study also recognized the presence of the Influenza A virus, part of the Orthomyxoviridae family, known to cause respiratory infections in both mammals and birds [33]. Further, the Human Parainfluenza virus 4a and Tailam jeilongvirus, members of the Paramyxoviridae family, were detected in Ratus spp. It's noteworthy that the Human parainfluenza virus 4 had earlier been identified in African bats [34]. Such findings accentuate that rodents can act as reservoirs for a plethora of viruses, posing significant threats to public health. The Retroviridae family was identified in both rodents and dogs. Retroviruses can cause an array of diseases in animals, including lymphomas, leukemias, sarcomas, immunodeficiencies, neurological ailments, and more [35]. The detection of retroviruses with origins traceable to Ovine, Caprine, Avian, and Baboons in Rodents and Dogs hints at possible cross-species transmission among animals sharing an environment. The presence of livestock, poultry, and baboons in the study area might contribute to the introduction of these viruses in dogs and rodents. It has been documented that certain viruses can be transmitted between animal hosts through aerosolized infectious particles [35]. Moreover, dogs could contract these viruses by consuming baboons and smaller mammals. This study also revealed several families of zoonotic viruses with public health significance in rodents, including Arenaviridae. Arenaviruses are associated with hemorrhagic fever in humans and are endemic to West Africa [36]. Notably, the Giessen reptarenavirus was identified in Mus spp. within this study. Previously, this virus was linked to the Boid inclusion body disease in captive snakes in Brazil, with Boid snakes identified as the primary reservoirs [36]. These findings suggest that rodents might also serve as reservoirs for this virus.

The Middelburg virus, belonging to the Alphavirus genus, was also detected in rodents. Alphaviruses, primarily transmitted by mosquitoes, are of significant public health concern and are prevalent across Africa [37]. The Middelburg virus was first isolated from Aedes mosquitoes in 1957 in South Africa and has been attributed to neurological diseases in various animals, including cattle, sheep, horses, wildlife, and humans [37]. This research marks the inaugural evidence of Middelburg virus infections in Mus spp. in Tanzania, underscoring the need for further research into its role as a source of infections in both animals and humans. Additionally, the study reported the discovery of the Hainan hebius popei torovirus, a member of the Tobaniviridae family, in wild rodents (Arvicathis spp.). Toroviruses are known to cause gastroenteritis in both animals and humans [38]. Notably, the Hainan torovirus was initially isolated from freshwater snapping turtles in Australia in 2015 and was linked to significant mortality rates among these turtles in a New South Wales coastal river [39]. The presence of this virus in Arvicathis spp. indicates potential cross-species transmission. Interestingly, the study also detected Coronaviruses in Field mice, specifically identifying the Porcine epidemic diarrhea virus and the Feline infectious peritonitis virus. These viruses traditionally cause diseases in pigs and cats, respectively [40-41]. While previous studies did not label them as zoonotic, an experimental study involving mice suggested the potential for the Porcine epidemic diarrhea virus to cause neuropathogenic effects [42]. This study thus introduces the notion that the Porcine epidemic diarrhea virus, along with the Feline infectious peritonitis virus, might possess the capability to infect a broader range of animals. Also, the research found the presence of Human mastadenovirus C and Bat mastadenovirus F in rodents (Ratus spp). Specifically, Human mastadenovirus C (HAdV-C) is known to cause respiratory infections in children [43]. Mastadenovirus is known to induce diseases across a range of animals [43]. It's plausible that rodents could act as a source, transmitting these viruses to humans and domestic animals. In a surprising turn, the Lymphocystis disease virus was found in dogs. This virus, traditionally associated with lymphocyte disease in fish, has significant implications for the global fish industry [44-45]. This research marks the first documentation of the Lymphocystis disease virus in domestic dogs within Tanzania. Further investigations are essential to understand its transmission dynamics and its broader health implications. The study also revealed the presence of various herpesviruses in rodents, dogs, and humans. The identified herpesviruses had origins traceable to both humans and animals [46]. Such findings reinforce the potential for cross-species transmission of herpesviruses. Given a recent incident in China where a death was attributed to the herpes B virus (Cercopitherin``e Herpesvirus 1) [46], it's a pressing reminder of the risks associated with this virus. Further studies are warranted to discern the transmission routes of these viruses within the study area. Notably, apart from viruses affecting animals, the study also identified plant and insect viruses. A majority of these viral families were found in wild rodents. It's well-established that rodents consume both insects and plants [47]. Additionally, viruses that infect free-living amoebae are prevalent in soil and water sources [48]. A recent Australian study indicated that these amoebae might be conduits, transferring pathogenic

viruses like Adenoviruses to humans [48]. This revelation demands more in-depth research to understand the public health ramifications of viruses associated with amoebae. While metagenomics next-generation sequencing was utilized, it had inherent drawbacks. These include sequencing host genomes, which potentially reduces the sensitivity in detecting viruses, and the absence of a standardized program for sequence analysis [49].

Conclusion

This research offers a comprehensive view of the viral families present in various animal species, with a particular focus on rodents, dogs, and humans. The data underscores rodents' pivotal role as virus carriers in contrast to dogs and humans. Notably, zoonotic RNA viral families like Peribunyaviridae and Hantaviridae were identified in both rodents and humans, hinting at cross-species transmission potential. This necessitates vigilance in monitoring these viral families across species to curb the spread of zoonotic diseases. The concurrent presence of Herpesviridae in rodents, dogs, and humans suggests mutual vulnerability to this DNA viral family, reinforcing the urgency for robust surveillance and prevention strategies. Intriguingly, Retroviridae was detected in both rodents and dogs, indicating potential viral exchanges within the study area's fauna. Moreover, the discovery of unclassified +ssRNA viruses in dogs and humans highlights the need for continued monitoring and investigation to grasp their transmission dynamics and implications for public health. In essence, these insights enrich our understanding of viral diversity, and potential zoonotic transmission pathways, and highlight the critical role of a collaborative approach encompassing both veterinary and human health sectors to address virus-associated risks. Future endeavors should intensify efforts in examining prevalence, transmission routes, and the broader impact of viruses on livestock and human populations. The ultimate goal is to architect efficient strategies to preclude the spread of zoonotic diseases.

Abbreviation

RNA: Ribonucleic acid; DNA: Deoxyribonucleic acid; cDNA: Complementary Deoxyribonucleic acid; BCL: Binary Base call; QC: Quality control; NCBI: National Center for Biotechnology Information

Declaration

I, Amina Ramadhani Issae, declare that the manuscript titled "Metagenomics screening of viruses in Arusha, north Tanzania: A One Health Perspective across wild Rodents, Domestic Dogs and Humans" contains materials related to a previously published article. I hereby acknowledge and reference the following published article titled "Exploring Pathogenic and Zoonotic Bacteria from Wild Rodents, Dogs and Humans of the Ngorongoro District in Tanzania Using Metagenomics Next-Generation Sequencing" authored by Issae et al., 2023, published by MDPI Zoonotic Diseases Journal. I confirm that the current manuscript builds upon and provides additional insights into the content of the above-published paper.

Acknowledgment

The authors are thankful to the participants, community authorities, and Arusha regional authority for support during sample collection. Also, biotechnologists at the Kilimanjaro Clinical Research Institute (KCRI) biotechnology laboratory are recognized for their pleasant assistance throughout this study.

Funding

The current study was funded by the African Centre of Excellence for Innovative Rodent Pest Management and Biosensor Technology Development (ACEII-IRPM and BTD) with credit number 5799-TZ at Sokoine University of Agriculture, Morogoro, Tanzania. The funding project didn't play a role in the designing of the study and collection, analysis, and interpretation of data and in writing the manuscript.

Availability of data and materials

Data will be available by emailing amina.issae@sua.ac.tz

Authors' contributions

Amina Ramadhani Issae (ARI) is the principal researcher who designed the study methodology, performed field work, performed data analysis, and wrote the first draft of the manuscript. Abdul Selemani Katakweba (ASK) is a co-supervisor who assisted in improving the study methodology, fund acquisition, reviewing and editing the manuscript. Rose Peter Kicheleri (RPK) is a co-supervisor, reviewing and editing the manuscript. Augustino Alfred Chengula (AAK) is a co-supervisor, reviewing and editing the manuscript. Christopher Jacob Kasanga (CJK) is the main supervisor who assisted in designing the study methodology and reviewing and editing the manuscript. All authors have read and agreed to publish the final version of the manuscript.

Ethics approval and consent to participate

The ethical clearance to perform this study was granted by the Ethical Review Committee of the Tanzania Medical Research Institute (NIMR) (Ref. No. NIMR/HQ/R.8a/Vol. IX/3676; 19th May 2021). Also, the Tanzania Commission of Science and Technology (COSTECH) granted the research authorization following approval of the proposal by the Tanzania Wildlife Research Institute (TAWIRI) (Ref. No. 2023-38-NA-2022-480). Likewise, the Sokoine University of Agriculture granted permission to conduct this study (Ref. No. SUA/ADM/R.1/8A/718; 3rd February 2021). Furthermore, the permission was issued by the Arusha Regional Authority (Ref. No. FA.132/95/01/38; 12th February 2021) and the Ngorongoro district administration (Ref. No. AB.114/354/01/134; 1st April 2021). Before rodent capturing and collection of blood samples from humans and dogs, the heads of the households provided their written agreement. All individuals participated voluntarily.

Consent for publication

Not applicable

Competing interest

The authors declare that they have no competing interests.

Author Details

[1]African Centre of Excellence for Innovative Rodent Pest Management and Biosensor Technology Development (ACEII-IRPM &BTD), Sokoine University of Agriculture, P.O. Box 3110, Morogoro Tanzania

[2]Department of Wildlife Management, Sokoine University of Agriculture, P.O. Box 3073, Morogoro Tanzania

[3]Institute of Pest Management, Sokoine University of Agriculture, P.O. Box 3110, Morogoro Tanzania

[4]Department of Veterinary Microbiology, Parasitology and Biotechnology, Sokoine University of Agriculture, P.O. Box 3019, Morogoro Tanzania

Article Info

Received: 26 July 2023

Accepted: 16 September 2023

Published: 27 September 2023

References

1. Shaheen MN. The concept of one health applied to the problem of zoonotic diseases. Reviews in Medical Virology. 2022 Jul;32(4):e2326. DOI: 10.1002/rmv.2326.

2. Günther S, Hoofd G, Charrel R, Röser C, Becker-Ziaja B, Lloyd G, Sabuni C, Verhagen R, Van Der Groen G, Kennis J, Katakweba A. Mopeia virus–related arenavirus in natal multimammate mice, Morogoro, Tanzania. Emerging infectious diseases. 2009 Dec;15(12):2008.

3. Hofinger DM, Nofchissey R, Jacobson M, Goade D. 443. Serological survey of hantavirus in adult humans living in Tanzania. Journal of Investigative Medicine. 2006;54(1)S156.

4. Kang HJ, Stanley WT, Esselstyn JA, Gu SH, Yanagihara R. Expanded host diversity and geographic distribution of hantaviruses in sub-Saharan Africa. Journal of virology. 2014 Jul 1;88(13):7663-7.

5. Cleaveland S, Fevre EM, Kaare M, Coleman PG. Estimating human rabies mortality in the United Republic of Tanzania from dog bite injuries. Bulletin of the World Health Organization. 2002;80(4):304-10.

6. Onyuok SO, Hu B, Li B, Fan Y, Kering K, Ochola GO, Zheng XS, Obanda V, Ommeh S, Yang XL, Agwanda B. Molecular detection and genetic characterization of novel RNA viruses in wild and synanthropic rodents and shrews in Kenya. Frontiers in Microbiology. 2019; 10:2696. DOI: 10.3389/fmicb.2019.02696.

7. Wang N, Yang L, Li G, Zhang X, Shao J, Ma J, Chen S, Liu Q. Molecular detection and genetic characterization of Wenzhou virus in rodents in Guangzhou, China. BMC veterinary research. 2021;17(1):1-8. DOI: 10.1186/s12917-021-03009-2.

8. Ishii A, Thomas Y, Moonga L, Nakamura I, Ohnuma A, Hang'ombe BM, Takada A, Mweene AS, Sawa H. Molecular surveillance and phylogenetic analysis of Old-World arenaviruses in Zambia. Journal of general virology. 2012;93(10):2247-51. DOI: 10.1099/vir.0.044099-0.

9. Fichet-Calvet E, Becker-Ziaja B, Koivogui L, Günther S. Lassa serology in natural populations of rodents and horizontal transmission. Vector-Borne and Zoonotic Diseases. 2014;14(9):665-74. DOI: 10.1089/vbz.2013.1484.

10. Chauhan RP, Dessie ZG, Noreddin A, El Zowalaty ME. Systematic review of important viral diseases in Africa in light of the 'one health' concept. Pathogens. 2020;9(4):301. DOI: 10.3390/pathogens9040301.

11. Salerno JD, Mulder MB, Kefauver SC. Human migration, protected areas, and conservation outreach in Tanzania. Conservation Biology. 2014;28(3):841-50. DOI: 10.1111/cobi.12237.

12. Linuma OF, Mahenge AS, Mato RR, Greenwood AD. Drivers of Human–wildlife interactions in a co-existence area: a case study of the Ngorongoro conservation area, Tanzania. Discover Sustainability. 2022;3(1):1-5. DOI: 10.1007/s43621-022-00113-7.

13. Mhina AD, Kasanga CJ, Sindato C, Karimuribo ED, Mboera LE. Rift Valley fever potential mosquito vectors and their infection status in Ngorongoro district in Northern Tanzania. Tanzania Journal of Health Research. 2015;17(4).

14. Tanzania Ministry of Finance. 2012 Population and Housing Census General Report. National Bureau of Statistics, Dar es salaam, Tanzania. 2013; 264pp.

15. Issae AR, Katakweba AS, Kicheleri RP, Chengula AA, van Zwetselaar M, Kasanga CJ. Exploring Pathogenic and Zoonotic Bacteria from Wild Rodents, Dogs, and Humans of the Ngorongoro District in Tanzania Using Metagenomics Next-Generation Sequencing. Zoonotic Diseases. 2023; 3(3):226-242. DOI: 10.3390/zoonoticdis3030019.

16. Happold DCD. Mammals of Africa Rodents, Hares and Rabbits. Bloomsbury Publishing, London. 2013; 784pp. [DOI not available].

17. Lima-Oliveira G, Lippi G, Salvagno GL, Picheth G, Guidi GC. Laboratory Diagnostics and Quality of Blood Collection. Journal of Medical Biochemistry. 2015; 34(3): 288-294. DOI: 10.2478/jomb-2014-0043.

18. Wingett SW, Andrews S. FastQ Screen: A tool for multi-genome mapping and quality control [version 2; referees: 4 approved]. F1000Research 2018; 7:1338. DOI: 10.12688/f1000research.15931.2.

19. Chen S, Zhou Y, Chen Y, Gu J. fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics. 2018;34: 884–90. DOI: 10.1093/bioinformatics/bty560.

20. Wood DE, Lu J, Langmead B. Improved metagenomic analysis with Kraken 2. Genome Biol. 2019; 20; 257. DOI: 10.1186/s13059-019-1891-0.

21. Dahmana H, Granjon L, Diagne C, Davoust B, Fenollar F, Mediannikov O. Rodents as hosts of pathogens and related zoonotic disease risk. Pathogens. 2020;9(3):202. DOI: 10.3390/pathogens9030202.

22. Theonest NO, Carter RW, Amani N, Doherty SL, Hugho E, Keyyu JD, Mable BK, Shirima GM, Tarimo R, Thomas KM, Haydon DT. Molecular detection and genetic characterization of Bartonella species from rodents and their associated ectoparasites from northern Tanzania. Plos one. 2019;14(10): e0223667. DOI: 10.1371/journal.pone.0223667.

23. Putkuri N, Kurkela S, Levanov L, Huhtamo E, Vaheri A, Sironen T, Vapalahti O. Isolation and characterization of a California encephalitis serogroup orthobunyavirus from Finnish mosquitoes. Infection, Genetics and Evolution. 2014; 22:164-73. DOI: 10.1016/j.meegid.2014.01.023.

24. Mathew C, Klevar S, Elbers AR, Van der Poel WH, Kirkland PD, Godfroid J, Mdegela RH, Mwamengele G, Stokstad M. Detection of serum neutralizing antibodies to Simbu sero-group viruses in cattle in Tanzania. BMC Veterinary Research. 2015;11(1):1-9. DOI: 10.1186/s12917-015-0526-2.

25. Kopp A, Hübner A, Zirkel F, Hobelsberger D, Estrada A, Jordan I, Gillespie TR, Drosten C, Junglen S. Detection of two highly diverse Peribunyaviruses in mosquitoes from Palenque, Mexico. Viruses. 2019;11(9):832. DOI: 10.3390/v11090832.

26. Kang C, Kim JI, Lee J, Bae S, Kim MJ, Song KJ, Song JW, Kim SH, Park MS. Human infection with Seoul orthohantavirus in Korea, 2019. PLoS neglected tropical diseases. 2021;15(2). DOI: 10.1371/journal.pntd.0009168.

27. Clement J, LeDuc JW, Lloyd G, Reynes JM, McElhinney L, Van Ranst M, Lee HW. Wild rats, laboratory rats, pet rats: Global Seoul hantavirus disease revisited. Viruses. 2019;11(7):652. DOI: 10.3390/v11070652.

28. Kang HJ, Bennett SN, Dizney L, Sumibcay L, Arai S, Ruedas LA, Song JW, Yanagihara R. Host switch during evolution of a genetically distinct hantavirus in the American shrew mole (Neurotrichus gibbsii). Virology. 2009;388(1):8-14. DOI: 10.1016/j.virol.2009.03.019.

29. Zhou H, Zhu S, Quan R, Wang J, Wei L, Yang B, Xu F, Wang J, Chen F, Liu J. Identification and genome characterization of the first

sicinivirus isolate from chickens in mainland China by using viral metagenomics. Plos one. 2015;10(10). DOI: 10.1371/journal.pone.0139668.

30. Buechler CR, Bailey AL, Lauck M, Heffron A, Johnson JC, Campos Lawson C, Rogers J, Kuhn JH, O'Connor DH. Genome sequence of a novel kunsagivirus (Picornaviridae: Kunsagivirus) from a wild baboon (Papio cynocephalus). Genome Announcements. 2017;5(18): 261-17. DOI: 10.1128/genomeA.00261-17.

31. Cholleti H, Hayer J, Fafetine J, Berg M, Blomström AL. Genetic characterization of a novel picorna-like virus in Culex spp. mosquitoes from Mozambique. Virology journal. 2018;15(1):1-0. DOI: 10.1186/s12985-018-0981-z.

32. Briese T, Loroño-Pino MA, Garcia-Rejon JE, Farfan-Ale JA, Machain-Williams C, Dorman KS, Lipkin WI, Blitvich BJ. Complete genome sequence of T'Ho virus, a novel putative flavivirus from the Yucatan Peninsula of Mexico. Virology journal. 2017;14(1):1-8. DOI: 10.1186/s12985-017-0777-6.

33. Nelson MI, Edelman L, Spiro DJ, Boyne AR, Bera J, Halpin R, Ghedin E, Miller MA, Simonsen L, Viboud C, Holmes EC. Molecular epidemiology of A/H3N2 and A/H1N1 influenza virus during a single epidemic season in the United States. PLoS pathogens. 2008;4(8). DOI: 10.1371/journal.ppat.1000133.

34. Hause BM, Nelson E, Christopher-Hennings J. Eptesicus fuscus orthorubulavirus, a close relative of human parainfluenza virus 4, discovered in a bat in South Dakota. Microbiology spectrum. 2021;9(2): 930-21. DOI: 10.1128/Spectrum.00930-21.

35. Rosenberg N, Jolicoeur P. Retroviral Pathogenesis. In: Coffin JM, Hughes SH, Varmus HE, editors. Retroviruses. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 1997. Retroviridae. Veterinary Microbiology. 2022; 698-727.

36. Argenta FF, Hepojoki J, Smura T, Szirovicza L, Hammerschmitt ME, Driemeier D, Kipar A, Hetzel U. Identification of Reptarenaviruses, Hartmaniviruses, and a Novel Chuvirus in Captive Native Brazilian Boa Constrictors with Boid Inclusion Body Disease. J Virol. 2020;94(11): 1-20. DOI: 10.1128/JVI.00001-20.

37. Fourie I, Williams J, Ismail A, Jansen van Vuren P, Stoltz A, Venter M. Detection and genome characterization of Middelburg virus strains isolated from CSF and whole blood samples of humans with neurological manifestations in South Africa. PLoS neglected tropical diseases. 2022;16(1). DOI: 10.1371/journal.pntd.0010020.

38. Pradesh U, PDDUP C, Vishwa V, CrnrK D, Izcitncigcir B. Toroviruses affecting animals and humans: A review. Asian Journal of Animal and Veterinary Advances. 2014;9(8):190-201. DOI: 10.1016/s0065-3527(08)60050-0.

39. Zhang J, Finlaison DS, Frost MJ, Gestier S, Gu X, Hall J, Jenkins C, Parrish K, Read AJ, Srivastava M, Rose K. Identification of a novel nidovirus as a potential cause of large-scale mortalities in the endangered Bellinger River snapping turtle (Myuchelys georgesi). PloS one. 2018;13(10). DOI: 10.1371/journal.pone.0205209.

40. Pedersen NC. A review of feline infectious peritonitis virus infection: 1963–2008. Journal of feline medicine and surgery. 2009;11(4):225-58. DOI: 10.1016/j.jfms.2008.09.008.

41. Duan C, Luo Y, Liang X, Wang X. A Review of Bioactive Compounds against Porcine Enteric Coronaviruses. Viruses. 2022;14(10):2217. DOI: 10.3390/v14102217.

42. Kotani O, Shirato K, Nagata N, Ikeda H, Takahashi K, Taguchi F. Neuropathogenesis of a mouse-adapted porcine epidemic diarrhea virus infection in suckling mice. Journal of General Virology. 2013;94(4):831-6. DOI: 10.1099/vir.0.047787-0.

43. Mao N, Zhu Z, Rivailler P, Yang J, Li Q, Han G, Yin J, Yu D, Sun L, Jiang H, Zhan Z, Xiang X, Mei H, Wang X, Zhang B, Yu P, Li H, Lei Z, Xu W. Multiple divergent Human mastadenovirus C co-circulating in mainland of China. Infect Genet Evol. 2019; 76:104035. doi: 10.1016/j.meegid.2019.104035.

44. Rahmati-Holasoo H, Ahmadivand S, Marandi A, Shokrpoor S, Palić D, Jahangard A. Identification and characterization of lymphocystis disease virus (LCDV) from Indian glassy fish (Parambassis ranga Hamilton, 1822) in Iran. Aquaculture International. 2022;30(5):2593-602. DOI: 10.1007/s10499-022-00922-7.

45. Borrego JJ, Valverde EJ, Labella AM, Castro D. Lymphocystis disease virus: its importance in aquaculture. Reviews in Aquaculture. 2017;9(2):179-93. DOI: 10.1111/raq.12131.

46. Hu G, Du H, Liu Y, Wu G, Han J. Herpes B virus: History, zoonotic potential, and public health implications. Biosafety and Health. 2022;4(04):213-9. DOI: 10.1016/j.bsheal.

47. Ademola OJ, Massawe AW, Mulungu LS, Makundi RH. Dietary composition of rodents across different habitats in the Ukaguru Mountains, Tanzania. African Journal of Ecology. 2022;60(4):958-68.

48. Rayamajhee B, Willcox MD, Henriquez FL, Petsoglou C, Subedi D, Carnt N. Acanthamoeba, an environmental phagocyte enhancing survival and transmission of human pathogens. Trends in Parasitology. 2022. DOI: 10.3390/microorganisms11020295.

49. Gu W, Miller S, Chiu CY. Clinical metagenomic next-generation sequencing for pathogen detection. Annual Review of Pathology: Mechanisms of Disease. 2019; 14:319-38. DOI: 10.1146/annurev-pathmechdis-012418-012751.