UNIVERSITY OF HELSINKI

Faculty of Veterinary Medicine

# REPTARENAVIRUSES IN CONSTRICTOR SNAKES: TISSUE TROPISM AND IMMUNE RESPONSES


**Yegor Korzyukov**

Department of Virology

Faculty of Veterinary Medicine

Doctoral Programme in Clinical Veterinary Medicine

University of Helsinki

Finland


ACADEMIC DISSERTATION

*To be presented for public discussion with the permission of the*

*Faculty of Veterinary Medicine*

*of the University of Helsinki, for public examination on Thursday, December 17, 2020, at 16.00 in Lecture hall 3, Biomedicum Helsinki 1, Haartmaninkatu 8, Helsinki*

*Helsinki 2020*

SUPERVISORS: JUSSI HEPOJOKI, PhD
Docent of zoonotic virology
Department of Virology
Medicum

University of Helsinki

OLLI VAPALAHTI, MD, PhD
Professor of zoonotic virology
Department of Virology
Medicum

University of Helsinki

REVIEWERS: PETRI SUSI, PhD, Docent
Adjunct Professor in molecular virology
Institute of Biomedicine

University of Turku

MAIJA VIHINEN-RANTA, PhD, Docent, Senior Lecturer
Department of biological and environmental science

University of Jyväskylä

OFFICIAL OPPONENT: MARK D. STENGLEIN, PhD, Assistant Professor
Department of Microbiology, Immunology, and Pathology

College of Veterinary Medicine and Biomedical Sciences

Colorado State University

ISBN 978-951-51-6879-5 (paperback)
ISBN 978-951-51-6880-1 (PDF, available at http://ethesis.helsinki.fi)

Unigrafia
Helsinki 2020

To my family around the world

# TIIVISTELMÄ

RNA-virukset, Arenaviridae-heimo mukaan lukien, muodostavat laajan ja monimuotoisen mikrobijoukon. Mammarenavirus- suvun virukset ovat pääosin jyrsijöiden viruksia, joista eräät ihmiseen tarttuessaan voivat aiheuttaa verenvuotokuumeita, toiset keskushermostotulehduksia. Vaikka mammarenaviruksia on tutkittu yli 90 vuoden ajan, diagnostiikkaa, rokotteita ja viruslääkkeitä on kehitetty vain joitain mammarenaviruslajeja vastaan. Toinen suku, *Reptarenavirus*, koostuu kuristajakäärmeiden viruksista, jotka on yhdistetty tautiin nimeltä Boid Inclusion Body Disease (eng.), BIBD. BIBD:tä esiintyy vankeudessa elävillä kuristajakäärmeillä ja sitä on havaittu 1970-luvulta lähtien, mutta taudinaiheuttajat tunnistettiin vasta vuonna 2012. BIBD on vaarallinen sairaus käärmeille ja se voi johtaa koko käärmekokoelman hävittämiseen. Reptarenavirusten isäntälajikirjo ja immuunivasteen kehittymisen mekanismeja tartunnan saaneilla eläimillä ei toistaiseksi tunneta yksityiskotaisesti. Tämän työn tavoitteisiin kuului reptarenaviruksen isäntäsoluspektrin tunnistaminen sekä käärmeidein immuunivasteen tutkimus.

Reptarenavirukset aiheuttavat infekoituneissa soluissa inkluusiokappaleiden (IB, inclusion body, eng.) muodostumista. Aiempien raporttien mukaan IB:eiden muodostus on tyypillinen löydös tutkittaessa infektoituneita käärmeitä. Nisäkässoluille luonnollisessa 37°C:een lämpötilassa reptarenavirusinfektoituneissa soluissa ei havaittu selkeää IB:ien muodostusta, vastaavasti käärmesolujen viljelylämpötilassa (30°C) havaittiin voimakasta IB:ien muodostusta eri niveljalkais- ja nisäkässolulinjoissa. Virusten kykyä replikoitua testattiin niveljalkais-, nisäkäs- ja matelijasolulinjoilla kahdessa lämpötilassa, 30°C ja 37°C. Virukset replikoituivat tehokkaasti 30°C:een lämpötilassa, mutta heikosti 37°C:een lämpötilassa.

Monet vaipalliset virukset hyödyntävät glykoproteiineja (GP) isäntäsolupinnan reseptoreihin sitoutumiseen sekä isäntäsolun ja viruskalvon välisen fuusion. Reptarenaviruksien GP:ien kykyä kuljettaa virus erilaisiin solutyyppeihin käytettiin geneettisesti muokattua vesikulaarista stomatiitti-virusta (rVSV, recombinant vesicular stomatitis virus, eng.), jonka pintarakenne korvattiin eri arenaviruksien GP:eilla. Kokeissa havaittiin eri arenaviruksien GP:ien kykenevän kuljettamaan reportterigeenillä varustetun pseudoviruksen useisiin eri kudoksista peräisin oleviin matelija- että nisäkässoluihin vaihtelevalla tehokkuudella.

Kokeellista reptarenavirusinfektiota kuristajakäärmeillä (Boa constrictor ja Python regius) käytettiin työkaluna BIBD:n ja reptarenavirusinfektion välisen yhteyden todistamiseksi. Reptarenavirusinfektoiduilla eläimillä havaittiin

ohimeneviä keskushermosto-oireita, mutta BIBD:een liittyvää IB:ien muodostumista ei havaittu koe-eläimillä. Käärmeet tapettiin ja kerätyistä seeruminäytteistä tutkittiin reptarenaviruksia vastaan kehittynyttä humoraalista immuunivastetta. Reptarenavirusten immuunivasteen arvioimiseksi oli välttämätöntä kehittää reagensseja, jotka kykenevät havaitsemaan immunoglobuliinit käärmeseerumista. Kehitettyjen reagenssien toimivuutta arvioitiin ensin BIBD:iä sairastavien käärmeiden seerumeilla. Seeruminäytteissä havaittiin reptarenavirusta tunnistavia IgY- ja IgM-luokan vasta-aineita, ja tämän avulla kyettiin osoittamaan luotujen reagenssien toimivan halutulla tavalla. Seuraavassa tutkimuksessa käärmeiltä löydettiin reptarenaviruksia tunnistavia IgY- ja IgM-luokan vasta-aineita sekä kokeellisen että luonnollisen reptarenavirusinfektion seurauksena. Reptarenaviruksien GP:eilla koristeltuja rVSV pseudoviruksia hyödynnettiin reptarenavirusinfektiota neutraloivien vasta-aineiden etsimiseen käärmeiden seerumista. Sekä kokeellisen että luonnollisen reptarenavirusinfektion seurauksena käärmeille oli kehittynyt reptarenavirusinfektiota neutraloivia vasta-aineita.

Tämän väitöskirjan töiden ansiosta tunnemme paremmin reptarenaviruksen kykyä hyppiä lajirajojen yli. Työssä kehitettyjen uusien reagenssien avulla voidaan jatkossa kehittää testejä reptarenavirusinfektion havaitsemiseen eläviltä käärmeiltä. Tulevaisuuden tavoitteisiin reptarenavirologian alalla kuuluu muun muassa eri reptarenaviruslajien ja BIBD:n yhteyden tutkiminen, joka puolestaan voi auttaa kehittämään tehokkaita hoitoja tai menetelmiä infektioiden ennaltaehkäisyyn.

# ABSTRACT

The family *Arenaviridae* is a well-represented clade of RNA viruses. The genus *Mammarenavirus* is dominated by rodent-borne arenaviruses, several of which have been identified as the causative agents behind hemorrhagic fevers and neurological infections in humans. Despite having been studied for more than 90 years, mammarenavirus diagnostics, vaccines and antiviral compounds are only available for some mammarenaviruses. Another genus, *Reptarenavirus*, includes viruses linked to boid inclusion body disease (BIBD) in constrictor snakes. BIBD has been reported in captive constrictor snake species since the 1970s, but the etiological agents were only identified in 2012. BIBD can lead to the eradication of the entire affected snake populations. The range of possible host spectrum and the immune response against reptarenaviruses are not well characterized. This thesis aims to define the potential reptarenavirus host cell spectrum as well as expand understanding of the boid immune response.

One of the hallmark signs of reptarenavirus infection in snakes is the formation of inclusion bodies (IB) in host cells. Snakes are poikilotherm and the replication of viruses is often susceptible to temperature variation. Reptarenavirus infection in mammalian, boid, and arthropod cells, incubated at 37 °C did not induce IB formation, whereas prominent IB formation occurred in all three phyla when incubated at 30°C. Reptarenaviruses replicated efficiently at 30°C, whereas at 37°C the replication efficiency reduced significantly. Many animal viruses take advantage of glycoproteins (GPs) to mediate binding and entry via attachment to host cell surface receptors. To study the ability of reptarenavirus GPs to mediate cell entry, a pseudovirus system based on reporter gene-bearing recombinant vesicular stomatitis virus (rVSV) was introduced. The pseudoviruses with reptarenavirus GPs served to demonstrate that the majority of arenavirus GPs could mediate entry to both mammalian and reptilian cells but at varying efficiencies.

In order to validate the link between BIBD and reptarenavirus infection, constrictor snakes (*Boa constrictor* and *Python regius*) were experimentally infected. Despite transient central nervous system signs, IB were not detected in the infected snakes. The snakes were sacrificed and sera was collected to determine the magnitude of the humoral immune response. In order to assess the antibody response against reptarenaviruses it was necessary to develop reagents capable of detecting immunoglobulins in snake sera. The generated reagents were initially tested using sera from BIBD-positive snakes. IgY and IgM class antibodies binding reptarenaviruses were detected in serum samples, validating the functionality of the reagents. In the next study, these antibodies were used to

detect IgM and IgY antibodies in experimentally and naturally infected snake populations. Extracted sera was further assayed using the rVSV-based pseudoviruses decorated with reptarenavirus GPs to show a neutralizing antibody response following reptarenavirus infection.

This thesis adds to understanding of reptarenavirus infectivity across species barriers. The generation of novel diagnostic reagents will allow generation of serodiagnostic tools for reptarenavirus infection. Future studies of reptarenaviruses should aim to establish a virus-specific link between reptarenaviruses and BIBD that could serve in the development of effective and preventive treatment strategies.

# TABLE OF CONTENTS

# LIST OF ORIGINAL PUBLICATIONS

The publications are referred to in the text by their roman numerals.
This thesis is based on the following publications:

I- Hepojoki J, Kipar A, **Korzyukov Y**, Bell-Sakyi L, Vapalahti O, Hetzel U. 2015. Replication of boid inclusion body disease-associated arenaviruses is temperature sensitive in both boid and mammalian cells. J Virol 89:1119–1128. doi:10.1128/JVI.03119-14

II- **Korzyukov Y**, Hetzel U, Kipar A, Vapalahti O, Hepojoki J. 2016. Generation of anti-boa immunoglobulin antibodies for serodiagnostic applications, and their use to detect anti-reptarenavirus antibodies in boa constrictor. PLoS One 11:e0158417. doi:10.1371/journal.pone.0158417.

III- **Korzyukov Y**, Iheozor-Ejiofor R, Levanov L, Smura T, Hetzel U, Szirovicza L, de la Torre JC, Martinez-Sobrido L, Kipar A, Vapalahti O, Hepojoki J. Differences in tissue and species tropism of reptarenavirus species studied by vesicular stomatitis virus pseudotypes. *Viruses*.2020;12(4):395. doi:10.3390/v12040395

IV- Hetzel U, **Korzyukov Y**, Keller S, Szirovicza L, Jelinek C, Pesch T, Vapalahti O, Kipar A, Hepojoki J. Experimental reptarenavirus infection on *Boa constrictor* and *Python regius*. *Submitted*.

# ABBREVIATIONS

ABV-1- Aurora borealis virus 1

ABV-2- Aurora borealis virus 2

A-DG- Alpha-dystroglycan

BIBD- Boid Inclusion Body Disease

CASV-1- CAS Virus 1

GGV-1- Golden Gate virus 1

GPC- Glycoprotein complex

HISV-1- Haartman institute snake virus 1

IB- Inclusion bodies

JUNV- Junin virus

LCMV- Lymphocytic choriomeningitis virus

NP- Nucleoprotein

OW- Old World

NW- New World

RdRp- RNA-dependent RNA polymerase

S-5- S-5 like virus

SSP- Stable signal peptide

TfR1- Transferrin 1 receptor

TSMV-2- Tavallinen suomalainen mies virus 2

UGV-1- University of Giessen virus 1

UHV-1- University of Helsinki virus 1

UHV-2- University of Helsinki virus 2

VHF- Viral hemorrhagic fever

VSV- Vesicular stomatitis virus

ZP- Z-RING matrix protein

# 1 INTRODUCTION

## 1.1 Origin of arenaviruses

Arenaviruses are characterized as enveloped, negative-sense RNA viruses with either a bi- or tri-segmented genome. The first arenavirus was isolated in 1933 from a patient with suspected St. Louis encephalitis (1). The isolated virus was identified as Lymphocytic choriomeningitis virus (LCMV)(1). After the identification of LCMV, isolation of Junin (JUNV) arenavirus took place in the city of Junin (Argentina), thus giving a name to the etiological agent of Argentine hemorrhagic fever (AHF) (2). Tacaribe (TACV) was identified later in bats and insect species, which suggested the presence of arenaviruses in other than rodent species (3). Machupo virus (MACHV) was identified in Bolivia and also isolated from rodent species in 1965, the causative agent of Bolivian hemorrhagic fever (BHF) (4). Arenavirus prevalence on the African continent was firstly analyzed by studying Lassa virus (LASV), which causes infections in humans that have come into contact with the virus-carrying reservoir rodent species (5). With the increase in the number of newly identified arenaviruses, the *Arenaviridae* family was established in 1976 (6). The following years have witnessed the discovery of other virus members within *Arenaviridae* (7, 8). Since the identification of the first arenavirus infection in humans by LCMV, arenaviruses have also been isolated from rodent, fish, insect, bat and snake species (3, 5, 9-14). However, the disease associations have not always been evident. In captive constrictor snakes, arenaviruses are associated with Boid Inclusion Body Disease (BIBD), often with progressive and fatal outcomes (11-13, 15). Hence, the discovery of new arenaviruses in different animal species has led to the diversification of the taxonomy of the *Arenaviridae* family into several genera (8).

## 1.2 Taxonomy and reservoir hosts of arenaviruses

The virus order of *Bunyavirales* includes RNA viruses with segmented, linear, single-stranded, negative-sense or ambisense genome classifications (8). Overall, *Bunyavirales* is composed of nine families (8). The *Arenaviridae* family belongs to the order of *Bunyavirales* (16). According to the International Committee on the Taxonomy of Viruses (ICTV) the family of *Arenaviridae* currently consists of four genera: *Mammarenavirus*, *Reptarenavirus*, *Hartmanivirus*, and *Antennavirus* (16) (Table 1-4). The genus *Mammarenavirus* is composed of two lineages, the Old World (OW) and New World (NW) mammarenaviruses (17).

### 1.2.1 Mammarenavirus reservoir hosts

The least geographically restricted mammarenavirus is LCMV, which circulates on many continents, but is phylogenetically related to the OW mammarenaviruses (16). NW mammarenaviruses are predominantly restricted to South American regions due to the presence of virus-carrying rodent species, while most of the OW mammarenaviruses are present in African regions as a result of the presence of specific virus-carrying rodent reservoir species (18, 19). The geographical distribution of OW mammarenavirus extends outside the African regions, since Wēnzhōu (WENV) and Dandenong (DANV) viruses have been detected in China and Australia, respectively (20, 21) (Figure 1). The reservoir hosts of OW mammarenaviruses are found in the genera *Mastomys, Praomys*, and *Arvicanthis* of the family of *Muridae* (18).

NW mammarenaviruses are carried by rodent reservoir species from the family of *Cricetidae,* genera *Oryzomys, Sigmodon, Neotoma, Nephelomys, Oecomys, Calomys, Zygodontomys, Neacomys*, or *Akodon* (18). Tacaribe (TACV) is the only known exception of mammarenaviruses that has been found in bats and lone star tick species (3) although it is limited to Central American regions. The NW mammarenavirus lineage is further subdivided into three clades: A, B, and C (22). Clade A includes South American mammarenaviruses, including non-pathogenic and pathogenic to humans rodent-borne viruses (23). Clade B includes all hemorrhagic fever (HF) causing viruses; however, clade B also includes mammarenaviruses that are non-pathogenic to humans (24). Clade C mammarenaviruses have been isolated from Central American regions, and their pathogenicity to humans remains unknown (25). In addition to clades A-C, clade D has been represented as a recombinant clade of A/B, and includes mammarenaviruses isolated from North American regions (26-29).

**Table 1**. Table of the *Mammarenavirus* genus, indicating viruses classified within genus according to the ICTV. Abbreviation used in the table: NW- New World, OW- Old World.

| **Virus** | **Reservoir** | **Lineage (clade)** | **Geographic distribution** | **Other known hosts** | **Reference and year of identification** |
|---|---|---|---|---|---|
| AALV- Allpahuayo virus | *Oecomys bicolor* | NW (A) | Peru | unknown | (30) 2001 |
| BCNV- Bear Canyon virus | *Peromyscus californicus* | NW (D) | USA | *Peromyscus californicus* | (28) 2002 |
| JUNV- Junin virus | *Calomys musculinus* | NW (B) | Argentina | Human | (2, 31) 1958 |
| SBAV- Sabiá virus | unknown | NW (B) | Brazil | Human | (32) 1994 |
| PICHV- Pichindé virus | *Oryzomys albigularis* | NW (A) | Colombia | | (33) 1971 |

| | | | | | |
|---|---|---|---|---|---|
| CHAPV- Chapare virus | unknown | NW (B) | Bolivia | Human | (34) 2008 |
| CUPXV- Cupixi virus | *Oryzomys sp.* | NW (B) | Brazil | unknown | (35) 2002 |
| FLEV- Flexal virus | *Oryzomys spp.* | NW (A) | Brazil | unknown | (36) 1977 |
| GAIV- Gairo virus | *Mastomys natalensis* | OW | Central African Republic (CAR), Ethiopia, Tanzania | unknown | (37)2015 |
| GTOV- Guanarito virus | *Zygodontomys brevicauda* | NW (B) | Venezuela | Human | (38) 1994 |
| IPPYV- Ippy virus | *Arvicanthis sp.* | OW | CAR | *Arvicanthus sp.* | (39) 1985 |
| LASV- Lassa virus | *Mastomys sp.* | OW | West African regions | Human | (5, 40) 1970 |
| LATV- Latino virus | *Calomys callosus* | NW (C) | Bolivia | *Calomys callosus* | (41) 1975 |
| LORV- Loei River virus | *Rattus exulans* | OW | Southeastern Asia | unknown | (42) 2016 |
| LUJV- Lujo virus | unknown | OW | Southern Africa | Human | (43) 2009 |
| LUAV- Luna virus | *Mastomys natalensis* | OW | Zambia | unknown | (44) 2012 |
| LULV- Luli virus | *Grammomys sp.* | OW | Zambia | unknown | (8) 2018 |
| LNKV- Lunk virus | unknown | OW | Eastern Africa | unknown | (44) 2012 |
| LCMV- Lymphocytic choriomeningitis virus | *Mus musculus, Apodemus sylvaticus, Microtus arvalis, Apodemus flavicollis, Myodes glareolus* | OW | Globally spread | Human | (1, 45, 46) 1934 |
| MACV- Machupo virus | *Calomys callosus* | NW (B) | Bolivia | Human | (4, 47) 1965 |
| MRLV- Mariental virus | *Micaelamys* [*Aethomys*] *namaquensis* | OW | Namibia | unknown | (48) 2015 |
| MRWV- Merino Walk virus | unknown | OW | South Africa | *Myotomis unisulcatus* | (49) 2010 |
| MOBV- Mobala virus | *Mastomys awashensis, Stenocephalemys albipes* | OW | Ethiopia, CAR | *Praomys sp* | (50) 1983 |
| MOPV- Mopeia virus | *Mastomys natalensis* | OW | East Africa | *Mastomys natalensis* | (51) 1977 |
| MORV- Morogoro virus | *Mastomys natalensis* | OW | Tanzania | *Mastomys natalensis* | (52) 2009 |
| OKAV- Okahandja virus | *Micaelamys* [*Aethomys*] *namaquensis* | OW | Namibia | unknown | (48) 2015 |
| OLVV- Oliveros virus | *Necromys lasiurus* | NW (C) | Argentina | *Bolomys sp.* | (22, 53) 1996 |

| | | | | | |
|---|---|---|---|---|---|
| PRAV- Paraná virus | *Oryzomys angouya* | NW (A) | Paraguay | unknown | (54) 1970 |
| PIRV- Pirital virus | *Sigmodon alstoni* | NW (A) | Venezuela | *Sigmodon alstoni* | (55) 1997 |
| PINV- Pinhal virus- | *Calomys tener* | NW (C) | Brazil | *Calomys tener* | (56) 2015 |
| RYKV- Ryukyu virus | Unknown | OW | unknown | unknown | (8) 2018 |
| AMAV- Amaparí virus | *Neacomys guianae* | NW (B) | Brazil | *Oryzomys gaeldi* (rice rat); *Neacomys guianae* (bristly mouse) | (36) 1977 (57) 1966 |
| SOLV- Solwezi virus | Unknown | OW | unknown | unknown | (8) 2018 |
| SOUV- Souris virus | Unknown | OW | Unknown | unknown | (8) 2018 |
| TCRV- Tacaribe virus | *Artibeus jamaicensis trinitatis* | NW (B) | Caribbean regions | unknown | (3) 1963 |
| TMMV- Tamiami virus | *Sigmodon alstoni* | NW (D) | USA | *Sigmodon hispidus* | (26) 1970 |
| WENV- Wēnzhōu virus | *Rattus norvegicus*, *R. rattus, R. flavipectus*, *R. Losea, Niviventer* rats, *Suncus murinus* | OW | China | unknown | (20) 2015 |
| WWAV- Whitewater Arroyo virus | *Neotoma albigula* | NW (D) | USA | *Neotoma albigula* | (27) 1996 |
| BBRTV- Big Brushy Tank virus | *Neotoma albigula* | NW (D) | USA | unknown | (58) 2008 |
| CTNV- Catarina virus | *Neotoma micropus* | NW (D) | USA | *Neotoma micropus* | (59) 2007 |
| SKTV- Skinner Tank virus | *Neotoma mexicana* | NW (D) | USA | *Neotoma mexicana* | (60) 2008 |
| TTCV- Tonto Creek virus | *Neotoma albigula* | NW (B) | North America | unknown | (58) 2008 |
| DANV- Dandenong virus- | unknown | OW | Australia | human | (21) 2008 |
| Gbargoube virus | *Mus setulosus* | OW | Ivory Coast | unknown | (61) 2011 |
| Jirandogo virus | *Mus baoulei* | OW | Ghana | unknown | (62) 2013 |
| Kodoko virus- KDKV | *Mus minutoides* | OW | Guinea | unknown | (63) 2007 |
| Menekre virus | *Hylomuscus sp.* | OW | Ivory Coast | unknown | (61) 2011 |
| RCTV-Real de Catorce virus | *Neotoma leucodon* | NW (B) | Mexico | unknown | (64) 2010 |
| OCEV- Ocozocoautla de Espinosa- | *Peromyscus mexicanus* | NW (B) | Mexico | unknown | (65) 2012 |
| APOV- Aporé virus | *Oligoryzomys mattogrossae* | NW (B) | Brazil | unknown | (66) 2019 |

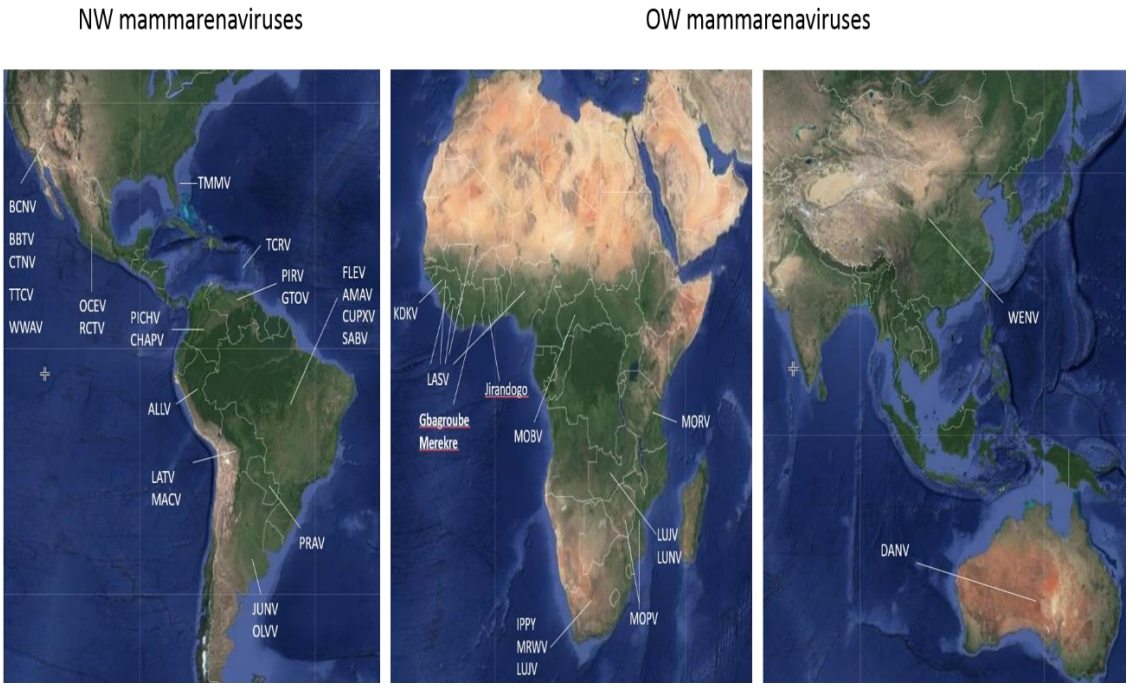



**Figure 1.** Geographical distribution of NW and OW mammarenaviruses, with the exclusion of LCMV. Mapping based on outbreaks and presence of mammarenavirus reservoir rodent species. NW mammarenaviruses in North, Central, and South Americas. OW mammarenaviruses in African regions, Oceania and Asian regions Abbreviations for the viruses: BCNV- Bear Canyon virus, TMMV- Tamiami virus, BBRTV- Big Brushy Tank virus, CTNV- Catarina virus, TTCV- Tonto Creek virus, WWAV- Whitewater Arroyo virus, OCEV- Ocozocoautla de Espinosa virus, RCTV- Real de Catorce virus, PICHV- Pichindé virus, CHAPV- Chapare virus, TCRV- Tacaribe virus, PIRV- Pirital virus, GTOV- Guanarito virus, FLEV- Flexal virus, AMAV- Amaparí virus, CUPXV- Cupixi virus, SABV- Sabiá virus, AALV- Allpahuayo virus, LATV- Latino virus, MACV- Machupo virus, PRAV- Paraná virus, JUNV- Junin virus, OLVV- Oliveros virus, KDKV- Kodoko virus, LASV- Lassa virus, MOBV- Mobala virus, MORV- Morogoro virus, LUJV- Lujo virus, LUNV- Luna virus, MOPV- Mopeia virus, IPPY-Ippy virus, MRWV- Merino Walk virus, WENV- Wēnzhōu virus, DANV- Dandenong virus

### 1.2.2 Reptarenaviruses, hartmaniviruses, and antennaviruses reservoir hosts

Reptarenaviruses have been detected in constrictor snake species from various continents (11-13, 67-72) (Table 2 and 3). Unlike OW and NW mammarenaviruses, the geographical origin of reptarenavirus infection has not been established. Isolation of reptarenaviruses from infected snakes, the presence of multiple RNA segments of different reptarenavirus species and co-infections with hartmaniviruses have provided evidence that these viruses are common in snakes (73, 74). Identification and characterization of hartmaniviruses was conducted from constrictor snakes species, and they were included into the family of *Arenaviridae*, although the establishment of the *Hartmanivirus* genus is based

on the genetically high divergence from mammarenaviruses and reptarenaviruses (74). The latest addition to the family *Arenaviridae* is the detection of novel arenaviruses in Wengling frogfish, which have been assigned to the fourth genus, *Antennavirus,* within the *Arenaviridae* family (Table 4) (14, 16). It is of note that *Antennavirus* genus representatives have been studied the least out of all arenaviruses.

**Table 2**. Table of the *Reptarenavirus* genus, indicating viruses classified within genus according to the ICTV. Reptarenaviruses unclassified by the ICTV were also included in the study.

| **Virus and abbreviation** | **Reservoir** | **Geographical distribution** | **Other known hosts** | **Reference and year of identification** |
|---|---|---|---|---|
| CAS virus -CASV | Ringed tree boa (*Corallus annulatus*) | USA | Ringed tree boa (*Corallus annulatus*) | (11, 75) 2012 |
| University of Helsinki virus 1 to 4- UHV-1,2, 3, 4 | Ringed tree boa (*Corallus annulatus*), Garden tree boa (*Corallus hortulanus*) Red tail boa (*Boa Constrictor Constrictor*) | Germany, UK, Costa Rica | Ringed tree boa (*Corallus annulatus*), Garden tree boa (*Corallus hortulanus*), Red tail boa (*Boa Constrictor Constrictor*) | (13, 67, 74, 75) 2013, 2015 |
| University of Giessen virus 1 to 3- UGV-1, 2, 3 | Red tail boa (*Boa Constrictor Constrictor*) | Unknown | Red tail boa (*Boa Constrictor Constrictor*) | (74) 2015 |
| Golden Gate virus-GGV | Red tail boa (*Boa Constrictor Constrictor*) | USA | Red tail boa (*Boa Constrictor Constrictor*) | (11) 2012 |
| Tavallinen suomalainen mies virus 2- TSMV-2 | Red tail boa (*Boa Constrictor Constrictor*) | Unknown | Red tail boa (*Boa Constrictor Constrictor*) | (74) 2015 |
| ROUT virus-ROUTV | Red tail boa (*Boa Constrictor Constrictor*), Ringed tree boa (*Corallus annulatus*) | Netherlands | Red tail boa (*Boa Constrictor Constrictor*) Ringed tree boa (*Corallus annulatus*) | (12, 75) 2013 |
| Aurora borealis viruses 1 to 3- ABV-1, 2, 3 | Red tail boa (*Boa Constrictor Constrictor*), | Unknown | Red tail boa (*Boa Constrictor Constrictor*), | (74) 2015 |
| Boa Av BL B3 | Red tail boa (*Boa Constrictor Constrictor*), Emerald tree boa (*Corallus caininus*) | Netherlands | Red tail boa (*Boa Constrictor Constrictor*), Emerald tree boa (*Corallus caininus*) | (12, 75) 2013 |

**Table 3**. Table of the *Hartmanivirus* genus. According to the ICTV, one virus has been assigned to the *Hartmanivirus* genus.

| Virus | Reservoir | Geographical distribution | Other known hosts | Reference and year of identification |
|---|---|---|---|---|
| Haartman Institute Snake Virus 1- HISV-1 | *Boa Constrictor* | Unknown | *Boa Constrictor* | (74, 76) 2015 |
| Old School Virus 1- OScV | *Boa Constrictor* | Unknown | *Boa Constrictor* | (76) 2018 |
| Veterinary Pathology Zurich Virus- VPZV | *Boa Constrictor* | Unknown | *Boa Constrictor* | (76) 2018 |
| Dante Muikkunen Virus- DAMV | *Boa Constrictor* | Unknown | *Boa Constrictor* | (76) 2018 |
| SetVetPat virus- SPVV-1 | *Boa Constrictor* | Unknown | *Boa Constrictor* | (72) 2020 |
| Andre Heimat virus-1- AHeV-1 | *Boa Constrictor* | Unknown | *Boa Constrictor* | (72) 2020 |

**Table 4**. Table of the *Antennavirus* genus, indicating two identified viruses in fish species.

| Virus | Reservoir | Geographical distribution | Other known hosts | Reference and year of identification |
|---|---|---|---|---|
| Wēnlǐng frogfish arenavirus 1- WlFV-1 | *Antenaarius sp.* | N/A | *Antenaarius sp.* | (14) 2018 |
| Wēnlǐngfrogfish arenavirus 2- WlFV-2 | *Antenaarius sp.* | N/A | *Antenaarius sp.* | (14) 2018 |

## 1.3 Structural characteristics

### 1.3.1 Virion structure

Arenaviruses have enveloped, pleomorphic virion structures with diameter ranging between 110-130 nm (77, 78). The viral envelope contains spikes formed of glycoproteins (GPs) (79, 80). Inside the virion, the nucleoprotein (NP) encapsidates two, small (S) and large (L), RNA genome segments (27, 53, 81, 82) (Figure 2). Antennavirus virions contain three distinguishable segments of viral RNA that are assigned as L, M, and S accordingly to their relative size (14). Grainy particles found within the virion have been identified as trapped ribosomes acquired from host cells, and their morphological appearance originated the name for the arenaviruses, as *arena* means sand in Latin (82).

Virions are sensitive to acidic conditions and elevated temperatures, both of which lead to rapid inactivation (83, 84). Virions are heat-inactivated after 30 minutes by temperatures above 56 °C (85). Inactivation of the virion is rapid below pH 5.5 and above 8.5 (85). Ultraviolet (UV) irradiation is also effective in the inactivation of arenaviruses, however survival rates of up to 10% for UV irradiated LASV have been reported (86). Gamma irradiation has been demonstrated to be more effective for inactivation than UV irradiation (87). Thiuram and aromatic disulfides also have virucidal and antiviral effects on the virion (88).

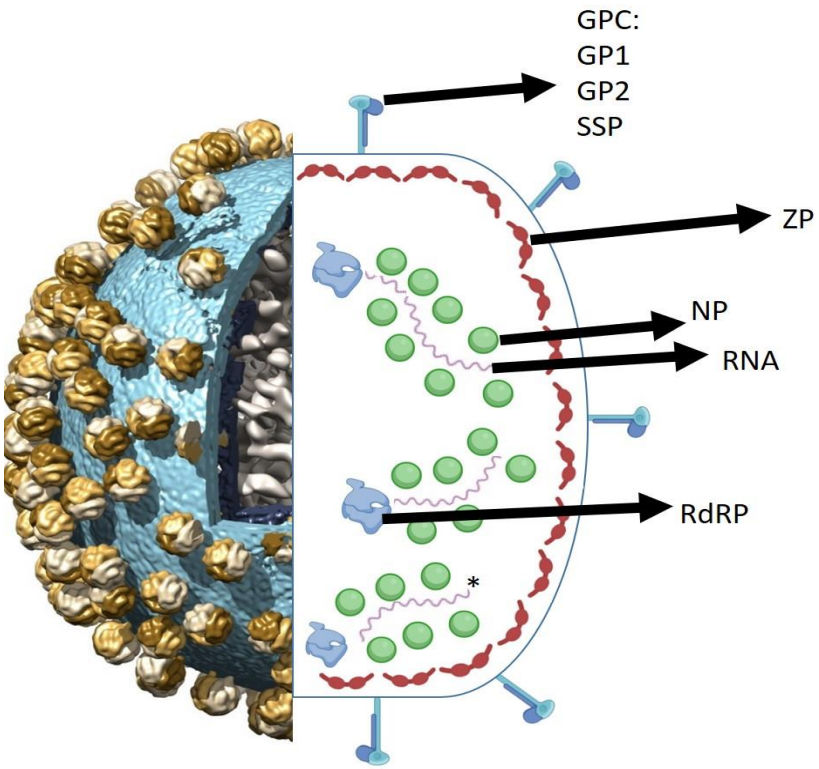



**Figure 2.** Schematic presentation of the virion structure. Abbreviations: RdRp- RNA-dependent RNA-polymerase, GPC- Glycoprotein complex, GP 1 and 2- glycoprotein 1 and 2, SSP- Stable signal peptide, ZP- Z-RING finger protein, NP- Nucleoprotein. Mammarenaviruses, reptarenaviruses, and hartmaniviruses are represented by bi-segmented RNAs, while antennaviruses are represented by tri-segmented RNAs, where the third segment is marked with an asterisk (*). SSP is absent in the reptarenavirus spike structure. ZP is absent in the hartmanivirus structure. Adopted from Li et al 2016 (89).

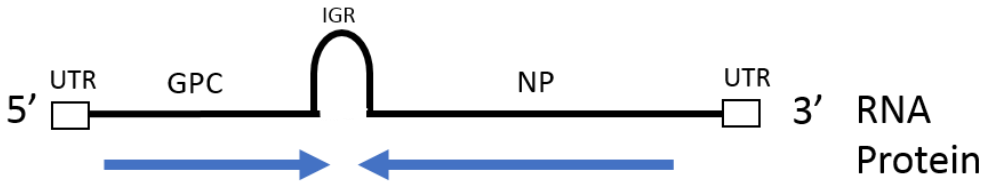
### 1.3.2 Genome structure

The genome of arenaviruses is composed of two or three single-stranded negative-sense RNA segments, using ambisense coding strategy for protein synthesis (14, 90, 91) (Figure 3). The bi-segmented genomes are represented by S and L segments, while viruses with a tri-segmented genome harbor L, M, and S segments (14). The S segment size range is approximately between 2 and 3.4kb and it encodes the glycoprotein precursor (GPC) and the NP (14, 90, 92, 93) (Figure 3). The L segment size range is approximately between 6 and 7.2Kb and it encodes the small zinc-binding protein (ZP) and viral RNA-dependent RNA-polymerase (RdRp) (94). Apart from other arenaviruses, the antennaviral genome structure is composed of three segments, which is the only distinguishable genome structure within the family of *Arenaviridae* (14). In antennaviruses NP, unidentified protein with GPC, and RdRp are encoded by S-, M-, and L segments respectively (14). Notably, the L segment of hartmaniviruses lacks the open reading frame (ORF) for ZP, while reptarenaviruses appear to lack a stable signal peptide (SSP) region in their GPC (62). ZP has not been identified in the antennavirus genome (14, 76). Thus, significantly different genome structures have been identified amongst different arenavirus genera (Figure 3).

Mammarenavirus and
reptarenavirus genome

L- segment 7.2 kb

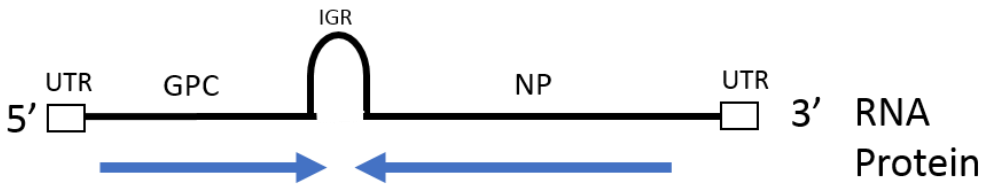

S-segment 3.4 kb




Hartmanivirus genome

L- segment 6 kb


S-segment 3.4 kb



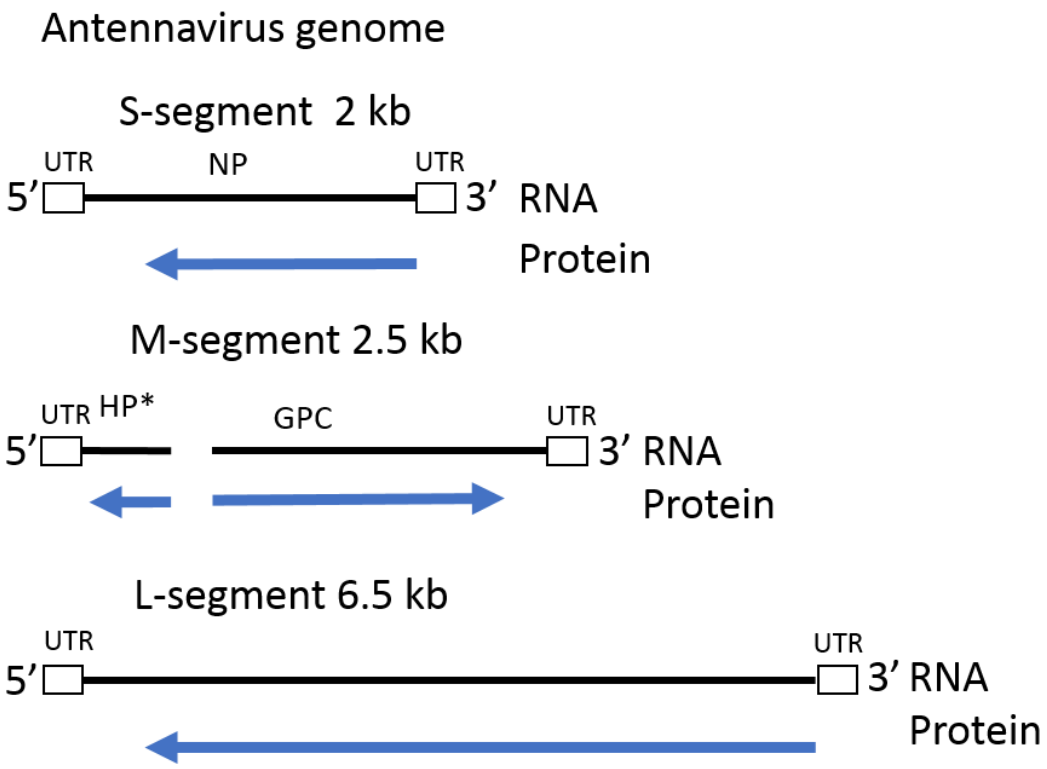



**Figure 3**. Organization of arenavirus RNA segments. Abbreviations used in the figure: UTR- untranslated region, IGR- intergenomic region, HP*- hypothetical protein, ZP- RING finger Z protein, NP- Nucleoprotein, RdRp- RNA-dependent-RNA-polymerase, GPC- Glycoprotein precursor

Non-coding regions of 5´and 3´ of the S segment play a role in virus replication and virulence (95). The 5´ and 3´ ends are conserved and complementary to each other from a region of approximately 19-30 nucleotides (nt) (96, 97). Each segment contains a non-coding intergenomic region (IGR), which separates the ORFs within each segment (92, 98). The predicted hairpin loop of IGR, represented by the stable secondary structure, provides the signal for transcription termination (99). Depending on the virus species, the IGR can range from 59 to 217 nt in size, and can form a structure with one to three stem loops in both genomic and antigenomic RNA (99). To date, no confirmed reassortant mammarenaviruses have been isolated in nature, and recombination appears to be a rare case only within phylogenetically close virus species (100-102). On the contrary, reptarenaviruses are suggested to cause widespread recombination, reassortment in infected snakes, with frequent detection of multiple L and S segments of different reptarenavirus species (73, 74). Co-infection of hartmaniviruses with reptarenaviruses lead to their identification and further assignment to a new genus within *Arenaviridae* family (74, 76).

## 1.4 Structural proteins

The *Arenaviridae* family have four structural proteins (Table 5). Structural proteins interact with both cellular and viral proteins during entry into the host cell, replication, and the host organism's immune response evasion (94). All proteins are synthesized in the cytoplasm of the host cell (94). This process depends on the host's intracellular factors that are described for each structural protein in later sections. Antennaviruses possess hypothetical protein, however it has not been identified not the function has been established (14). Antennaviruses may also lack ZP protein, just like hartmaniviruses (76), if the hypothetical protein's identification will confirm the absence of ZP properties. The arenavirus genome can vary from two to three RNA segments and encoding viral proteins, yet the final number of structural proteins has been conserved to a total of four (16). Many arenavirus proteins possess multiple functions, described in detail in sections 1.4.1 to 1.4.4.

**Table 5**. Summary of mammarenaviral protein interactions and functions in the host cell.

| Protein | Size and Segments location | Function | Known host (mammalian) cell interaction partners |
|---|---|---|---|
| RdRp | 200-250 kDa L segment | Transcription and translation of viral mRNA, viral genome replication | Not established |
| NP | 63-70 kDa S segment | Encapsidation of viral RNA, immune response suppression, role in assembly | Inhibition of IRF3 |
| ZP | 11-15 kDa L segment | RNA synthesis regulation, viral assembly and budding, interaction with the proteins of host cell, and immune response suppression | Alix/AiP1, eIF-4E, Nedd4, (Po, PML, PRH, RIG-I, Tsg101) |
| GPC | 75 kDa S segment | Attachment to the host cell surface receptors | Subtilisin Kexin Isozyme-1 (SKI-1)/Site-1 Protease (S1P) |

### 1.4.1 Nucleoprotein (NP)

The NP of arenaviruses is encoded by the S segment, and its size ranges between 64 and 68 kDa (103, 104). NP serves multiple functions and is the most abundant protein in the virions and in infected cells (105). The identified functions of NP include encapsidation of viral genome segments, interaction with RdRp in the

formation of ribonucleoprotein complex (RNP) for the transcription and replication of RNA, and suppression of innate immune responses of the host cell (106-109). Encapsidation of the viral genome by the NP generates the recognition platform template, which allows RdRp to initiate the transcription and replication (110). Structural analysis has revealed that the N-terminal domain of NP is involved in the binding and shielding of the m7GpppN cap structure, which is essential in the viral RNA transcription process (104). The C-terminal domain of NP contains 3'-5' exoribonuclease activity, which plays a role in the suppression of interferon induction (104). NP's inhibition of innate immunity is based on counteraction with the host type I interferon (IFN) response pathways through the impediment of retinoic acid-inducible gene I (RIG-I) (106, 111-113). RIG-I becomes activated upon binding to double-stranded RNA (dsRNA) during virus replication (114). The activation of RIG-I initiates the activation of molecular pathways that eventually lead to the expression of type 1 interferons (IFN) such as IFN- α and IFN- β (114). The NPs of OW and NW mammarenaviruses contain elements which inhibit the translocation and transcriptional activity of nuclear factor kappa B (NF-κB), leading to inhibition of IFN (115). In contrast, the NP of some mammarenaviruses, such as TCRV, does not inhibit NF-κB fully as those of other mammarenaviruses do (115). The NP of reptarenaviruses has been shown to induce the formation of inclusion bodies (IB) within infected cells (13).

### 1.4.2 RING finger Z protein (ZP)

RING finger Z protein (ZP) is the smallest arenavirus protein (90). The size varies between 11 and 15 kDa, and the ZP appears in monomeric and oligomeric forms (116-118). ZP is considered to be a multifunctional protein, and is involved in crucial steps of the viral life cycle including viral RNA synthesis regulation, viral assembly and budding, interaction with host cell proteins, and immune response suppression (119). The protein's structure contains several functional domains. The N-terminal myristoylation site of the protein serves in anchoring into the cell membrane, while the zinc-binding RING motif is a central core of the ZP (90, 120). C-terminal late-domain motifs play an important role in the budding process in the viral cycle (121, 122).

Amongst the studied mammarenaviruses, ZP has been found to be highly conserved (123). The ZPs of reptarenaviruses have 16% similarity at amino acid level to mammarenavirus counterparts (11). Regardless of this, the ZPs of reptarenaviruses are suggested to play a similar functional role to mammarenavirus ZPs (13). In contrast, ZP has not been identified in antennaviruses and hartmaniviruses (14, 76). Low abundance of ZP permits RNA synthesis, while high concentrations lead to the inhibition of the synthesis of the ZP-RdRp-RNA complex (124).

The interaction of ZP with the SSP of GPC and RNP presumably enhances the incorporation of the GPs into nascent virions (119). The C-terminal late-domain in ZP is responsible for interaction with the host cell proteins Tsg101 and Nedd4 that are suggested to serve a role in the cellular endosomal sorting complexes required for transport (ESCRT) machinery (119). Other known cellular factors that are known to interact with ZP are the promyelocytic leukemia protein (PML), the nuclear fraction of the ribosomal protein P0, eukaryotic translation initiation factor 4E (eIF4E), and proline-rich homeodomain protein (PRH) (119). Interaction with PML is suggested to play a role in the suppression of apoptosis, allowing arenaviruses to replicate in high yields (125, 126). ZP's interaction with host P0 indicates ribosome inclusion in the virion structure (127). Interaction with eIF4E downregulates the host cell translation machinery and suppresses interferon's regulatory factor (IRF-7), demonstrating another approach to the suppression of immune response (119). LCMV infections have revealed the role of ZP in the downregulation of proline-rich homeodomain protein (PRH) in the liver, abolishing the antiproliferative properties of the liver tissue and promoting cell division (128). The ability of ZP to bind to RIG-I, the cellular sensor of viral RNA responsible for the activation of the cellular beta interferon response, allows arenaviruses to evade cell immune responses (129).

### 1.4.3 RNA-dependent RNA polymerase (RdRp)

Arenavirus RdRp is essential for genome replication and mRNA transcription (98, 110). The size of RdRp varies between 200 and 250 kDa depending on the virus species, and represents the largest of all known arenavirus proteins (98). RdRp contains domains involved in the cap-snatching mechanism that have been identified in the N-terminus (130). Crystal structure analysis has identified N-terminus domain binding of nucleotides, with a preference for UTP, and RNA (130). The presence of type II endonuclease activity in the N-terminus is associated with the cap-snatching step (130). Endonuclease activity is essential for arenavirus RNA transcription, yet replication is not dependent on endonuclease activity (130). RdRp activity is also strongly dependent on the correct 5' RNA sequence, which directs the optimal synthesis of viral proteins (131). Furthermore, RNA ligands in the 5' termini of viral genomic RNA (vRNA) activate the polymerase in a promoter-specific manner, where the 5' vRNA ligands activate polymerase only for 3' vRNA and do not allow activation for the 3' complementary antigenomic RNA (cRNA) (131).

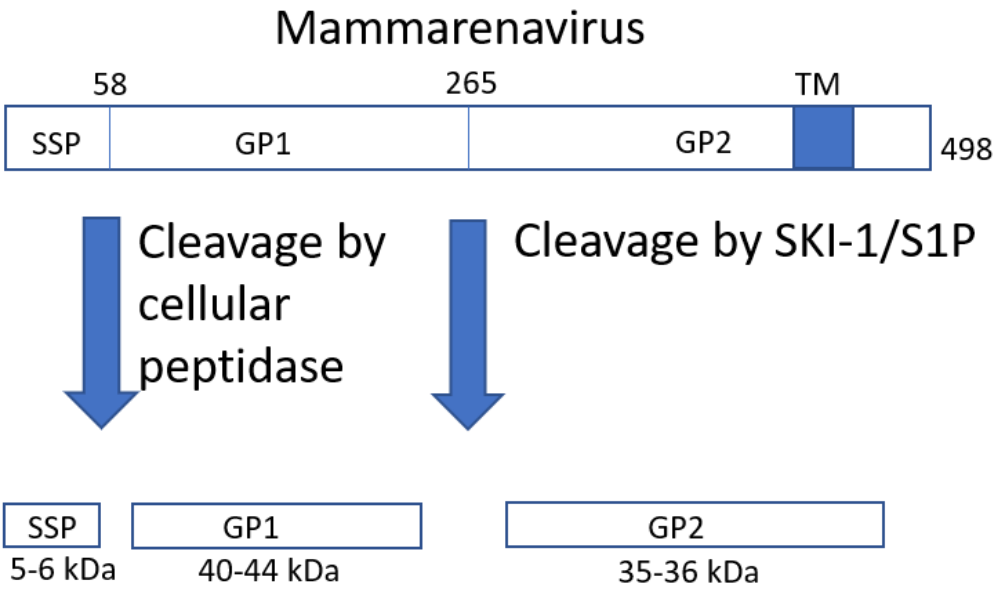
The sequences of arenaviral RdRps have high divergence, although conserved motifs have been identified within the structure of RdRp (132-134). Arenaviral RdRp domains show similarities to other negative-stranded RNA polymerases, which are characterized by the presence of conserved regions (135-138). Conserved domains have been identified and linked with active sites of RNA

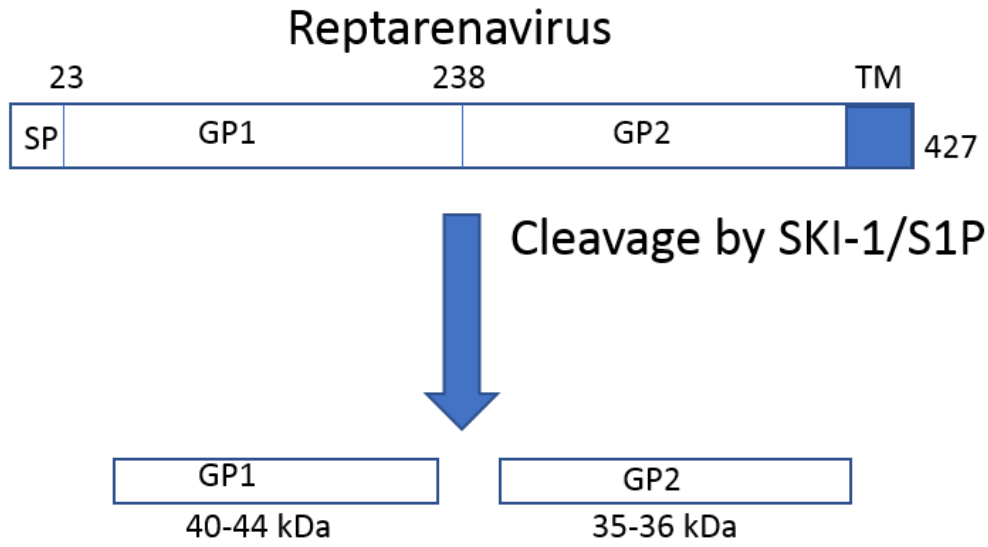
synthesis, template recognition, and polymerizing activity (133, 134). RdRp is directly involved in the synthesis of antigenomic complementary RNA (cRNA), capping viral mRNA via the cap-snatching mechanism, and the generation of genomic viral RNA (vRNA) (132).

### 1.4.4 Glycoproteins (GPs)

Arenaviral GPs are responsible for attachment to the cell surface receptors, leading to the initiation of infection in the target cells (139-144). The GPs are synthesized from the S segment as glycoprotein precursors (GPC), and they undergo a critical maturation process into fully functional subunits (93, 145, 146) (Figure 4). Prior to reaching full functionality, the GPC precursor is synthesized as a premature polypeptide with size ranging from 70 to 75 kDa. Signal peptidase and protease processing enzyme Subtilisin Kexin Isozyme-1 (SKI-1)/Site-1 Protease (S1P) are required in the maturation process of GPC (147-149). In contrast, hartmaniviruses appear to contain a furin processing site that appears as a divergent processing site from other known arenavirus GPC maturation processes (76). Proteolytic processing of GPC yields GP1, GP2 and SSP (93, 145, 146). Notably, reptarenaviruses appear to produce GP1 and GP2 without the SSP (76) (Figure 4). The molecular weight (MW) of GP1 is approximately 40-44 kDa, and the MW of GP2 is approximately 35-36 kDa (93, 145, 146, 150). SSP is 58 amino acids in length with an MW of approximately 5-6 kDa (151, 152). Computational analysis of antennavirus GPC demonstrates structural similarities with mammarenavirus and hartmanivirus GPCs, where the presence of SSP has been detected (153). Computational analysis has identified the presence of SSP, class I viral fusion protein, and an internal Zinc-binding domain in antennaviruses (153).

GP1 includes the receptor-binding domain, which mediates the binding of GP1 to a cell surface receptor (24, 154-156) (Figure 5). GP2 directs the fusion process (157) between cell and virus membranes where the process is activated by acidic pH in the endosome (158-160). SSP, when present in the native arenavirus structure, is involved in transport, maturation of GPC, and viral membrane fusion processes (161-164) (Figure 5). Table 6 gives a summary of structural characteristics of the arenavirus GP.


Mammarenavirus
58
265
TM
SSP
GP1
GP2
498
Cleavage by cellular peptidase
Cleavage by SKI-1/S1P
SSP
5-6 kDa
GP1
40-44 kDa
GP2
35-36 kDa


Reptarenavirus
23
238
TM
SP
GP1
GP2
427
Cleavage by SKI-1/S1P
GP1
40-44 kDa
GP2
35-36 kDa

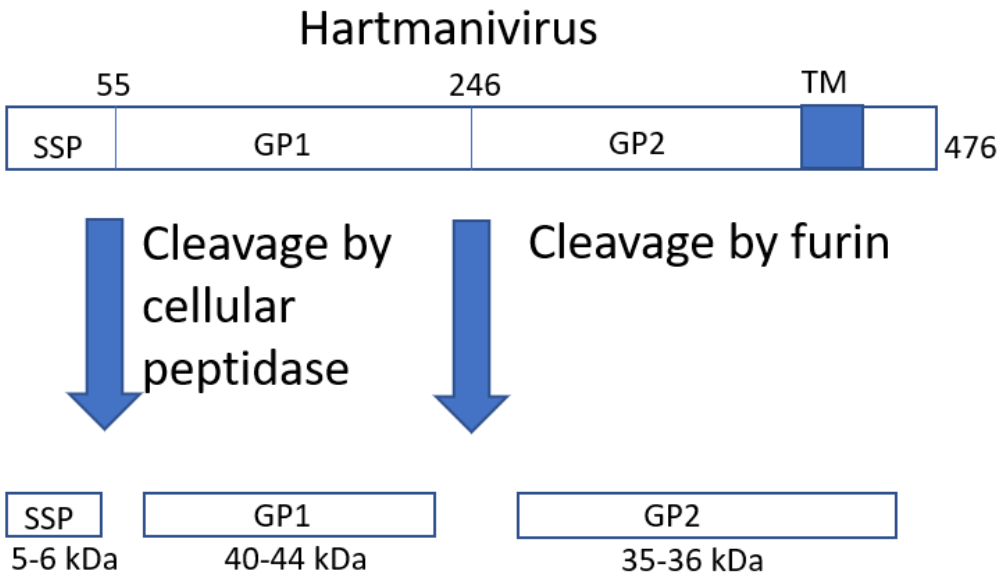



**Figure 4.** GPC processing of mammarenaviruses, reptarenaviruses, and hartmaniviruses. Abbreviations: TM- transmembrane region of GP2, SP- Signal peptide, SSP-Stable signal peptide.

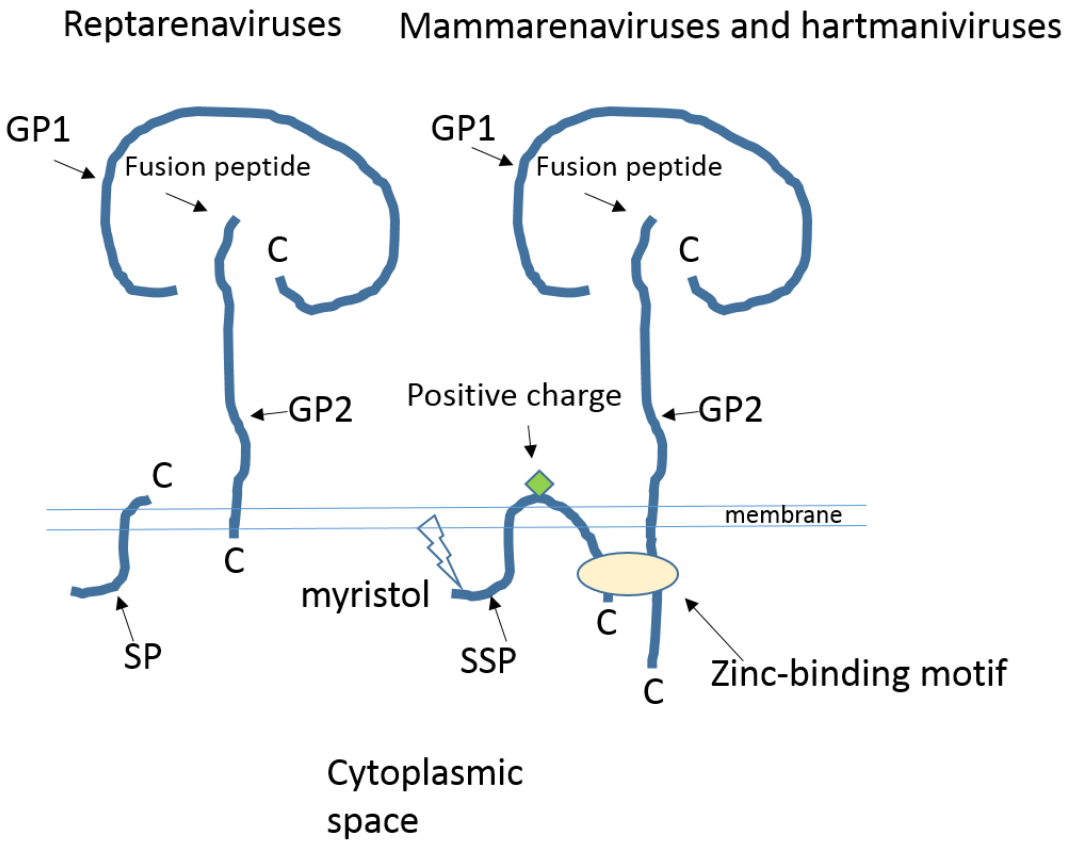



**Figure 5**. Schematic illustration of GPC structure after processing by cellular protease and signal peptides, and after trafficking to the cell membrane surface. Absence of SSP and myristoylation are indicated in reptarenaviruses. Structural illustration adopted from Hepojoki et al 2018 (76) ; Kranzusch PJ and Whelan SP 2011 (124).

**Table 6**. Structural properties of GPC within the family of *Arenaviridae*

| **Virus** | **Quantity of glycans** | **Cellular protease involved in the cleavage of GP1 and GP2** | **Myristoylation** | **SSP cleavage by signal peptidase** |
|---|---|---|---|---|
| Reptarenavirus | Up to 9 glycans (76) | SKI-1/S1P (76) | Absent (76) | Absent (76) |
| Mammarenavirus | Up to 15 glycans (165) | SKI-1/S1P (93) | Present (166) | Present (167) |
| Hartmanivirus | Up to 7 glycans (76) | Furin (76) | Present (76) | Present (76) |
| Antennavirus | Up to 7 glycans (153) | Furin (153) | Not identified | Present (153) |

## 1.5 Infection cycle

### 1.5.1 Entry

*In vitro* and *in vivo* infection studies using OW and NW mammarenaviruses have identified major cellular receptors involved in the attachment of the virus to the target cell and endocytosis pathways for internalization (155, 168, 169). Three types of receptors have been identified for mammarenaviruses. The cell surface receptor for NW mammarenaviruses is transferrin receptor 1 (TfR1) (155). For all known OW mammarenaviruses, the cell surface receptor is alpha-dystroglycan (A-DG) (168, 170), while neuropilin 2 (NRP-2) acts as a cell surface receptor for LUJV (169). There have been no studies related to the tropism of reptarena- and hartmaniviruses, and their cell surface receptors have not been identified.

The initial attachment of the arenavirus is initiated through the binding by GP1 to primary cell surface receptors. The virions are then internalized either through clathrin-independent or clathrin-dependent endocytotic pathways for OW and NW mammarenaviruses, respectively (171, 172). The entry of arenaviruses is dependent on cholesterol (150, 171). The cellular entry OW viruses such as LASV and LCMV with subsequent transport to late endosomes requires microtubular transport (172). Fusion process of the cellular and viral membranes is activated by low pH in the maturing endosome (159, 160). Upon the acidification of the endosome, GP2, enables fusion of cell and virus membranes and the release of the replication complexes with viral genome into the cell cytoplasm (173). The application of recombinant LCMV-expressing LASV GP and LCMV has also revealed dependency on phosphatidyl inositol 3-kinase (PI3K) as well as lysobisphosphatidic acid (LBPA) in the formation of intraluminal vesicles (ILV) of the multivesicular body (MVB) of the late endosome (172). The ESCRT pathway is one of the key mediators in MVB biogenesis (174). OW mammarenaviruses depend on ESCRT components, such as Hrs, Tsg101, Vps22, Vps24, and Alix (172). Cell line-generated lacking clathirin heavy chain (CHC), allowed the entry of LCMV and LASV, indicating the use of clathirin-independent cell entry by OW mammarenaviruses (172). OW mammarenaviruses, represented by LASV and LCMV, have also demonstrated independent cell entry from Rab5, a key regulator for endosomes for fusion and trafficking (143, 175). LUJV, as another representative of OW mammarenaviruses, distinguishably enables viral entry through attachment to NRP-2, with the stimulation of CD63 for the fusion process (169). NW mammarenaviruses are restricted to specific cell surface receptor domains, where entry is efficient through human, cat, *Calomys callosus*, *Calomys musculinus*, and *Zygodontomys brevicauda* TfR1, however rat and house mouse TfR1 orthologs do not permit viral entry from NW mammarenaviruses (156).

The reptarenaviral, hartmaniviral, and antennaviral GP proteins have remained poorly characterized. The target cell surface receptors for the GP of non-mammarenaviral species remain unknown, and the internalization and endocytotic pathways are also not identified. Upon the final stages of entry, viral ribonucleoprotein complex (vRNP) is released into the cytoplasm where the replication is taking place (176) (Figure 6).

### 1.5.2 Replication

Shortly after the release of vRNP into the cytoplasm, viral RdRp initiates the replication of viral RNA and the transcription of viral genes (110, 177). For the RNA template to be recognized by the RdRp, the RNA template needs to be encapsidated by viral NP (110). Prior to translation, NP and RdRp mRNAs are transcribed directly from the virion RNA segments (110). During the infection, the newly synthesized RdRp and NP are involved in the synthesis of complementary RNA. The newly synthesized RNA serves for the transcription of GPC and ZP mRNA or serves as a template for the synthesis of additional full-length virion-sense RNA (110). Full-length antigenomic- and genomic-sense RNA are generated via a "prime and align" strategy, through the initiation transcription of viral mRNA by short $m^7$G-capped oligonucleotides deriving from cellular mRNAs (178). The termination of viral mRNA synthesis is regulated by IGR, which separates the opposite-sense ORF of the viral genomic RNAs (179). Synthesis of viral proteins is performed through translation from subgenomic mRNAs, which do not possess 3'-terminal poly (A), and the 5'-cap is trailed by few non-templated bases (180-183). This feature is caused most likely by the cap-snatching mechanism, which is a known replication mechanism for other viruses (180-183).

### 1.5.3 Assembly and Budding

The assembly of the virion is mediated by viral proteins, additionally involving host cell protein interactions (176). The ZPs of arenaviruses play a central role in the assembly process, enabling the trafficking of the viral components to the cell membrane by interacting with host cell proteins (121). ZP's interactions with RdRp (124, 184), NP (185) and GPC (186, 187) have been demonstrated and implicated in playing a role in the assembly of mammarenaviruses. ZP additionally plays a role in the transportation of vRNP to the budding site (121, 188). Studies suggest that prior to the encapsidation of viral RNA, ZP initially interacts with viral NP and RdRp in order to establish a vRNP complex (176). Upon generation of a vRNP complex, ZP interaction is involved with the plasma membrane (PM), which occurs via its myristoylation followed by binding to the cellular Tsg-101 ESCRT pathway proteins (121, 176). ZP myristoylation is also

involved in interaction with the SSP of GPC, where the incorporation of processed GPC subunits into the virion structure is suggested to take place (119). Prior to becoming fully functional and incorporated into the virion, GPC is transported through the endoplasmic reticulum (ER) and Golgi compartments (189). Activation of the ESCRT-III and Vps4 cellular components leads to the progeny virus particles pinching off from the cells (190) (Figure 6). Due to the apparent absence of ZP in hartmaniviruses, an alternative model of the assembly and budding without ZP should be established.

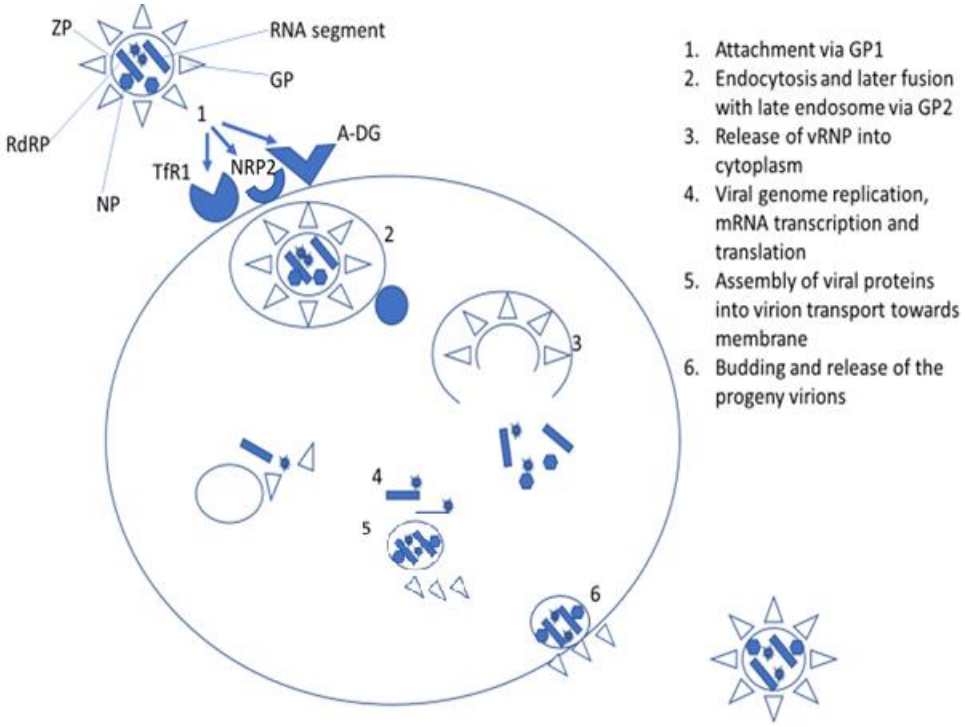



**Figure 6**. Schematic illustration of arenavirus infection cycle steps. Stages indicate entry, replication, and budding of the progeny virions from the infected cell.

## 1.6 Epidemiology and Diseases

### 1.6.1 Mammarenavirus infections and pathogenesis in rodents

Rodent reservoir species infected with mammarenaviruses do not express severe symptoms, and only immunodeficient rodent species can develop severe symptoms (17, 72, 89). LCMV infection in a mouse results in the presence of the

virus in virtually all tissues; however, efficient replication appears to be restricted to neurons (191). In murine models, LCMV can cause either an acute infection followed by the clearance of the virus, or establish a persistent infection (192, 193). The disease outcome is determined by the IFN response at the early stage of the infection (192-194). Additional evidence suggests that mammarenaviruses such as LASV, MORV, and GAIV have adapted to rodent species in order to optimize the success of transmission, thus resulting in non-pathogenic outcomes in rodent reservoirs (72).

### 1.6.2 Mammarenavirus disease symptoms in humans

In humans, LCMV infection is most often asymptomatic; however, in some cases infection leads to aseptic meningitis, meningoencephalitis or encephalitis (195, 196) (Table 7). LCMV infections have been characterized as bi-phasic (197, 198). The initial symptoms include fever, headache, malaise, myalgia, anorexia, nausea, and vomiting. The second phase of symptoms is accompanied by temporal recovery and followed by central nervous system (CNS) deviations (199). The second-phase symptoms can be expressed by aseptic meningitis with raised sensitivity to light, headache, fever, and vomiting (199-201). Symptoms may persist for up to several months from the initial infection (202-204). The majority of adults and children infected with LCMV proceed to full recovery from the infection. Distinguishably, transplacental human fetal infections are often accompanied by severe consequences (205). Vertically transmitted infections frequently have severe consequences such as impairment and abnormal development of the brain functions (206, 207). In many embryonic infection cases, LCMV has been reported to induce the development of microcephaly (21, 198, 204, 208-210).

The most commonly proposed entry scenario for human infection is entry into the lungs through the inhalation of the aerosolized form of the virus (211). From the lungs, the virus travels through the blood stream, and ultimately reaches the meninges, choroid plexus and ventricular ependymal linings (212). LCMV infection may lead to secondary complications, such as myocarditis, pneumonitis parotitis, pharyngitis, orchitis, and dermatitis (213). CNS complications can also cause encephalitis, hydrocephalus, transverse myelitis, Guillain-Barre syndrome, and severe cases can lead to fatal outcomes (202).

Similarly to LCMV infections, the majority of cases of VHF are asymptomatic, although the percentage of severe symptoms is much higher in VHF mammarenavirus infections. LASV infections can induce severe symptoms in as many as 20% of infected cases (214) (Table 7). Symptoms expressed from VHF mammarenaviruses include fever, dry cough, chest and abdominal pain, headache, and myalgia (214). Complications are commonly supported with the

following symptoms: hemorrhage in multiple organs, respiratory difficulties, face and neck edema, pleural and pericardial effusion, and encephalopathy (214). Similarly to LCMV infection during pregnancy, complications for the fetus are far more severe and with higher mortality rates (215). In early stages of the infection, LASV targets human macrophages and dendritic cells (DC), which play a role in the immune response (216, 217). By infecting immune defense cells, LASV suppresses the human host's ability to stimulate T-cells to fight the infection (217). The impairment of the immune response leads to increased production of infectious LASV particles (217). Infection of the liver leads to elevated levels of transaminases and to hepatocellular necrosis (218). Progressive infection of hepatocytes and exacerbation of liver tissue has been a characteristic occurrence in infected patients, being the most contributing pathology in approximately 80% of mortality cases (219). As a result of VHF infection, death results from hypotensive, hypovolemic, and hypoxic shock in patients with severe complications (218). Surviving patients' symptoms start to disappear 10 to 15 days after the initiation of the disease (218).

**Table 7**. Summary of mammarenaviruses pathogenic to humans and officially registered cases. Adapted from Ly H 2017 (220)

| **Virus** | **Lineage (clade)** | **Reservoir** | **Geographical presence** | **Disease** | **Incidence rate and mortality** |
|---|---|---|---|---|---|
| LCMV | OW | *Mus Musculus* | Globally | Aseptic meningitis, encepaha-litis | Over 5% of people show evidence of prior exposure, mortality less than 1% |
| LASV | OW | *Mastomys spp* | West Africa | Hemorrh-agic fever | Up to 500 000 infections annually, mortality of approx. 5000 annually |
| LUJV | OW | N/A | South Africa | Hemorrh-agic fever | 5 identified cases, 4/5 were fatal |
| JUNV | NW (B) | *Akodon azare, Calomys laucha, Calomys musculinus* | Argentina | Hemorrh-agic fever | 300-1000 cases annually before the Candid#1 vaccination program. Post vaccination |

| | | | | | |
|---|---|---|---|---|---|
| | | | | | program cases: 30-50. Mortality rate 15-30% |
| MACV | NW (B) | *Calomys callosus* | Bolivia | Hemorrh-agic fever | 1962-1964: approx. 1000 cases reported; 1990s: 19 cases reported; 2007-2008: approx. 200 cases reported, Average mortality rate 20% |
| SBAV | NW (B) | N/A | Brazil | Hemorrh-agic fever | 1 reported case, fatal |
| GTOV | NW (B) | *Zygodontomys brevicauda* | Venezuela | Hemorrh-agic fever | 618 cases, 23% mortality rate |
| CHAPV | NW (B) | N/A | Bolivia | Hemorrh-agic fever | 1 reported case, fatal |
| WWAV (29) | NW (A) | *Neotoma albigula* | USA | Hemorrh-agic fever | 3 reported cases, all fatal |

## 1.7 Mammarenavirus global infections

Mammarenaviruses have caused epidemics in South America and West Africa, where the infections originated from contacting with specific reptarenavirus reservoir rodent species (10, 221). While mammarenaviruses often cause asymptomatic and persistent infections in rodents (9, 222, 223), some cause infections leading to severe and fatal outcomes in humans (224, 225). The impact on human health of mammarenaviruses can be significant in endemic regions, where mammarenavirus infections have reached a mortality rate of 50%, as recorded, for example, during West African epidemics (226) (Table 7). Human infections occur through contact with rodent excreta (urine, feces, saliva); through direct contact with infectious material if the skin is abraded, or through mucosal exposure to aerosols (9, 195, 222, 227). Since the reservoir host of LCMV is the common house mouse, *Mus musculus,* which is present in almost all geographical regions, sporadic infections are caused globally at very low frequencies in humans (228, 229). Within the rodent reservoir species, mammarenaviruses can be transmitted vertically and horizontally (230) (Figure 7). In humans, primary infection can occur after contact with the rodent that possesses the virus, allowing later possibility for vertical and horizontal virus transmission (197).

Due to the threat to human health from highly pathogenic mammarenaviruses such as LASV, JUNV, Guanarito virus (GTOV), Chapare virus (CHAPV), and Lujo virus (LUJV), the listed mammarenaviruses are classified as Class A pathogens, capable of causing fatal VHF, and are regarded as potential bioterrorism threat (231, 232). According to the Centers for Disease Control and Prevention (CDC), Class A pathogens are high-priority agents capable of posing a risk to public health, and have serious effects on national security. Highly pathogenic agents that can be easily transmitted and cause fatal disease are handled in high-level bio-safety level (BSL-3 and BSL-4) laboratories facilities (231, 232). With advances in research on mammarenaviruses, vaccines such as Candid#1 (233, 234) and antiviral compounds such as ribavirin (235) have been developed and successfully applied to control certain mammarenavirus diseases in endemic regions (235, 236). Notably ribavirin has been applied successfully in the treatment of LASV infections (235), while Candid#1 vaccination has decreased the incidence rate of infections caused by JUNV (236).

## 1.8 Reptarenavirus and hartmanivirus hosts and infections

In constrictor snakes, reptarenaviruses have been linked to BIBD, which has a direct impact on the health of captive constrictor snake populations. BIBD may lead to the eradication of entire snake collections possessed by a snake breeder or zoo. Moreover, BIBD has not been characterized in the literature in snakes from the wild. BIBD has been characterized in captive constrictor snakes since the 1970s, however identification of reptarenaviruses as the potential etiological disease agent was established only in 2012 (11, 13, 237). The work involving experimental infections of constrictor snakes with reptarenaviruses presented in this thesis was conducted to provide evidence for a virus-disease relationship. The research carried out in the snake reservoir suggests potential horizontal transmission of the virus within a captive population of constrictor snakes (238). In addition to horizontal transmission, reptarenaviruses can also be transmitted vertically (239) (Figure 7). Hartmaniviruses appear to circulate as co-infections with reptarenaviruses in constrictor snakes, and can also be vertically transmitted (74, 76). Horizontal transmission of hartmaniviruses within infected snake population has not been studied.

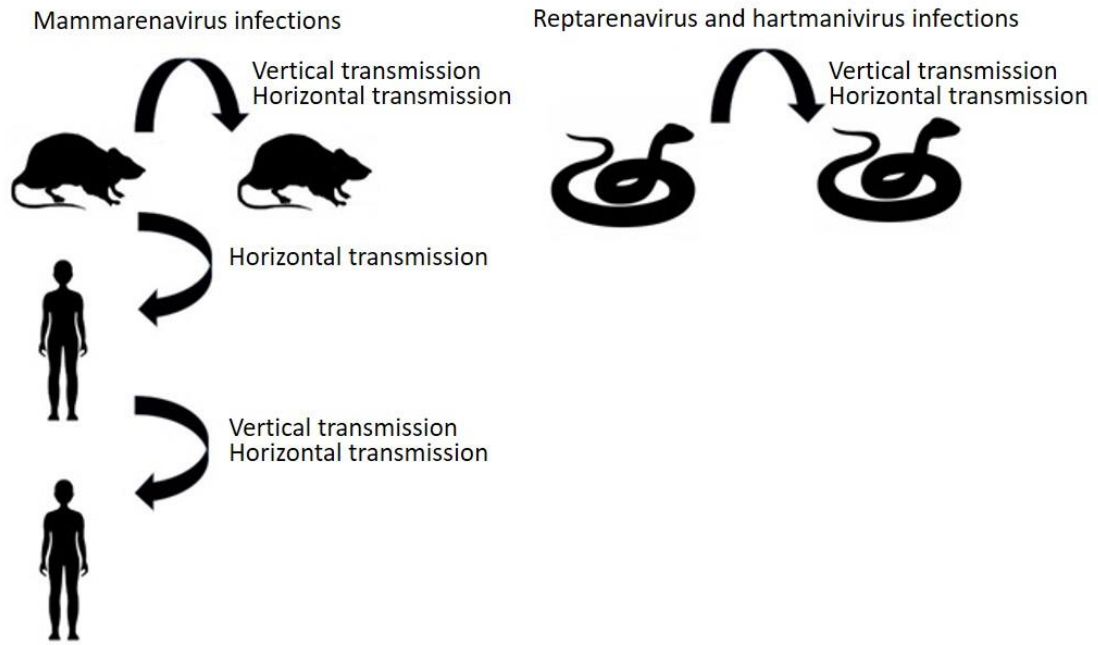



**Figure 7**. Illustration of arenavirus transmission in mammalian and reptilian species. Mammarenavirus infections are acquired from specific rodent species reservoirs, where the infection can be transmitted within rodent species or to a dead-end human host (9, 195, 222, 227). Human-to-human transmission has also been recorded for mammarenaviruses (9, 195, 222, 227). Reptarenaviruses and hartmaniviruses can be transmitted within constrictor snake species, although the original reservoir has not been established (76, 239).

### 1.8.1 Boid Inclusion Body Disease (BIBD)

Clinical signs of BIBD are generally associated with central nervous system complications, such as head tremor, opisthotonus, intermittent regurgitation, and eventual anorexia (237). "Stargazing'" is one of the signs occasionally present in affected snakes, characterized by the snake lifting its head along with the first third of its body and leaning backward for an extended amount of time (237). Snakes affected by BIBD can acquire secondary infections by bacteria, fungi, or protozoa, which may lead to severe complications with fatal outcomes. Secondary complications after infection have been characterized as lymphomas, encephalitis, pneumonia, hepatitis, enteritis, or osteomyelitis (240). BIBD's characteristic feature is the presence of IB within various tissues of the affected snake, where IB are characterized as eosinophilic to amphophilic, amorphous, intracytoplasmic inclusions (11, 13, 238, 241, 242).

Inoculation of reptarenaviruses varies in affected species. In boas, disease can vary from the asymptomatic and have persistent viremia for an extended amount of time (242). In pythons, viral infection may lead to rapid progression of BIBD signs, which are followed by secondary infections leading to death or inevitable

euthanasia of the affected snakes (11, 13). Similarly to mammarenaviruses, reptarenaviruses can be transmitted vertically and can cause BIBD in offspring populations (239). BIBD's geographical distribution has not been restricted, since confirmed BIBD cases have been documented in Europe, America, Australia, and Asia (11-13, 67, 69-71, 73, 75, 237, 243).

## 1.9 Immune response in humans

T-cell-mediated responses play a vital role in recovery from LASV infection (244). For example, survival of infected macaques shows considerable activated levels of T-cells, whereas infections with fatal outcomes have delayed T-cell activation (244) (Figure 8). Studies of LCMV have revealed the role of major histocompatibility complex (MHC) in cytotoxic T lymphocyte (CTL) responses against LCMV (245-247). In parallel analyses, human infections appear not to be dependent on the level of antibody titers (such as IgG and IgM), allowing the exacerbation of LASV infection (248). In addition, analysis of human serum samples from LASV-infected individuals indicate a correlation between survival and low levels of interleukins (IL) (IL-6, IL-8, and IL-10), blood urea nitrogen (BUN), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and liver enzyme aspartate aminotransferase (AST) (249).

MOPV is genetically closely related to LASV. Although it is not pathogenic to humans, it can provide protection against LASV infection in nonhuman primates (250). Infections mediated by MOPV also fail to activate DC, but macrophages become activated and produce IFN in response to the infection (251). NW infections caused by JUNV have demonstrated elevated levels of IL-6, IL-10, tumor necrosis factor alpha (TNFα), and IFNα (252-254). Although the exact role of cytokines in the pathogenesis of VHF by NW mammarenaviruses is not fully understood, the proposed theory links the delayed high level of cytokine production to the severity of the disease caused by VHF mammarenaviruses (255, 256) (Figure 8).

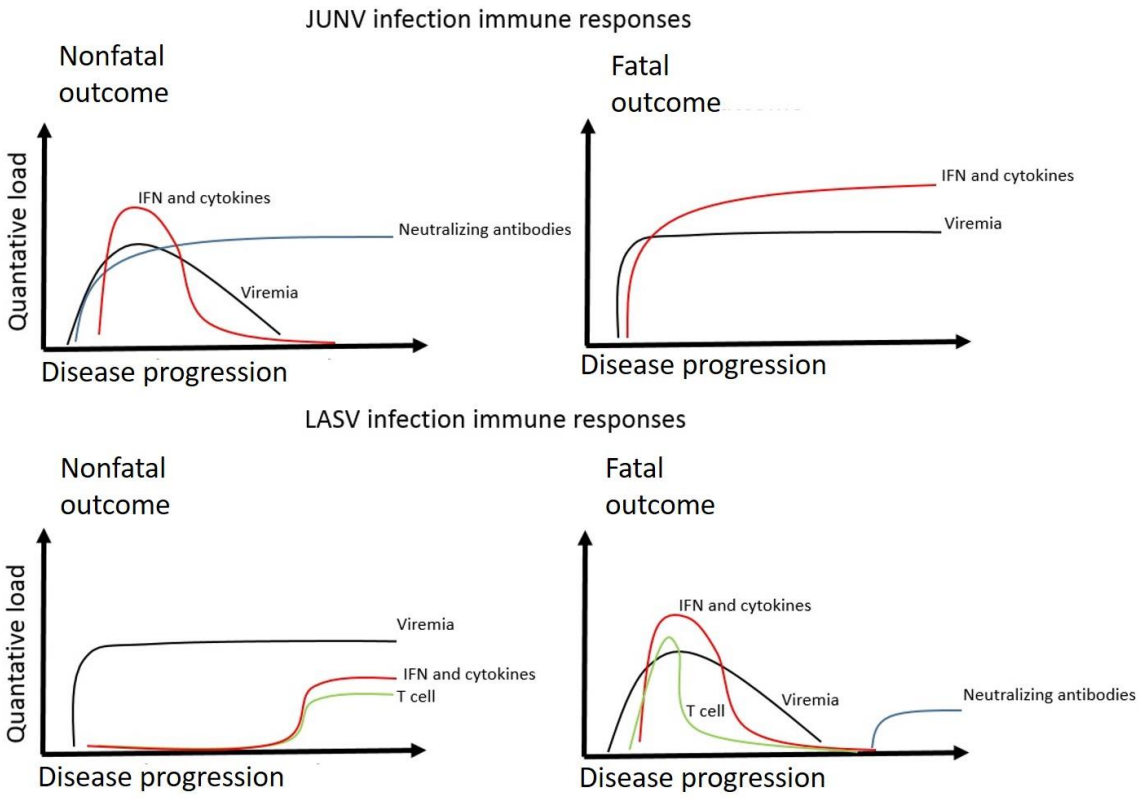



**Figure 8**. Schematic diagrams of immune response in LASV and JUNV infections in fatal and non-fatal outcomes. Adapted from (257) Mantlo E et al 2019.

## 1.10 Immune response in snakes

In vertebrates, immunoglobulins (Igs) that target viral antigens play the major role in the functionality of humoral immune response. In mammals, there are five Ig classes with different heavy chain classes, defined as α, δ, ε, γ and μ, and these classes give rise to IgG, IgA, IgE, IgD, and IgM, respectively (258). Heavy chain classes are paired with κ or λ light chains (258). In contrast, in snakes only four heavy chain Ig classes (IgD, IgM, IgYa and IgYb) have been described (259). Notably, snake Igs genes differ from Igs in mammalian species, due to diversification approximately 300 million years ago from ancestral animal species such as *Rhynchocephalia* (260, 261). Reptiles, such as snakes, are characterized as ectothermic animals, where the temperature directly affects the immune response (262), thus the humoral response is slower than in mammalian species (261). Antibody production in mammalian species usually reaches the maximum level at two weeks post-antigen encounter from the primary infection, while in reptiles it can take up to several months (263-266). In addition, the decline in post-production antibody levels in mammals usually occurs within weeks after the peak levels, while in reptiles peak titers can persist for up to 34

weeks (267). Another significant difference between mammalian and reptilian humoral immunity is the lack of increase in antibody titers upon a second encounter with the antigen in reptiles (261). The effects of reptarenavirus NP suppression of the innate immune responses have not been studied in infected snakes (261). A negative correlation between BIBD and the presence of antibodies against reptarenaviral NP has been detected in snakes infected with reptarenaviruses (268). In the same study, levels of reptarenavirus antibodies were lower in BIBD-positive snakes (268).

## 1.11 Diagnostics and treatment of arenavirus infections

### 1.11.1 Diagnosis, treatment and prevention of human infection

Several diagnostic methods have been used to identify mammarenavirus infection in humans. For serological assay, virus-specific IgM and IgG antibodies have been used in enzyme-linked immunosorbent (ELISA) and immunofluorescence assays (IFA) (269, 270). The application of serological assay is focused primarily on identifying VHF viruses. Rodent species are also tested in suspected cases of mammarenavirus transmission or for preventive measures by reverse transcription polymerase chain reaction (RT-PCR) and serologically, allowing the public to be informed of the presence of mammarenavirus infection in the area (271). Viral RNA in human samples is used to detect viral genetic material by RT-PCR in suspected human cases that have come into contact with the rodent and are experiencing mammarenavirus infection-associated symptoms (272-274). A neutralization assay can be applied to detect neutralizing antibodies, which typically have a higher detection rate for several years, although their generation upon infection is not immediate (275). Application of neutralization assay is performed to determine the efficiency of deployed vaccination of selected population, to ensure the presence of neutralizing antibodies against the virus.

Due to neurotropism of LCMV, isolation of the LCMV for an RT-PCR test from the cerebrospinal fluid is performed during suspected acute human infection (198). LASV infections have been successfully treated with ribavirin, which acts as a nucleoside analog interfering with RNA synthesis (235). In addition, vaccine candidates have been developed to combat LASV infection in endemic regions (276-279). In South America, application of the Candid #1 vaccine against JUNV has sharply reduced the number of mammarenavirus infections (233, 234).

### 1.11.2 BIBD diagnosis, treatment and prevention

Diagnosis of BIBD is based on observation of the characteristic signs of the disease, RT-PCR, and post-mortem histological analysis (68). Observational analysis of the suspected BIBD cases could initiate necessary quarantine actions. However, the progression of the disease can also be conducted asymptomatically, and not present clear BIBD-associated signs (70, 242). BIBD-associated signs can vary amongst constrictor snake species, and the presence with the effect of BIBD on non-constrictor snake species has not been established. Due to the large genetic diversity of reptarenaviruses (73, 74), the development of an RT-PCR with broad utility remains a challenge. Detection of numerous IB in the blood smear of snakes has been used as an *ante-mortem* standard for the diagnosis of BIBD for several decades since the identification of the disease in constrictor snakes (68). Currently, there are no treatments against BIBD. Preventive practice is based on the euthanasia of suspected or confirmed BIBD cases, with action preventing the spread of the disease to other snakes in captivity/an animal reservoir.

# 2 AIMS OF THE THESIS

- To identify the spectrum of reptarenavirus tissue tropism and to determine the replication potential of viruses in different cell lines

- To induce BIBD in snakes by experimental infection of reptarenaviruses

- To develop reagents for the detection of reptarenavirus-specific antibodies

- To compare the antibody repertoire in the sera of the snakes naturally and experimentally against reptarenavirus and to identify neutralizing antibodies in the sera

# 3 MATERIALS AND METHODS

Detailed protocols are provided in the respective articles/manuscripts.

## 3.1 Cell lines (I, II, III, IV)

Mammalian, reptilian and insect cell lines were used for the expression of virus proteins, virus propagation and infections.

African green monkey kidney (Vero E6, ATCC), human lung carcinoma (A549, ATCC), human embryonic kidney (HEK293FT, Thermo Fisher Scientific), human neuroblastoma (SK-N-SH, ATCC), Chinese hamster ovary (CHO wt, ATCC), and baby hamster kidney (BHK-21, ATCC) were maintained at standard growth and incubation conditions (5% $CO_2$, 37 °C) in a medium supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 IU/ml penicillin, and 100 µg/ml streptomycin, minimal essential medium (MEM) for Vero E6 and A549, and Dulbecco's Modified Eagle Medium (DMEM) for the other mammalian cell lines.

*Boa constrictor* kidney (I/1Ki) was cultured and maintained in MEM with 10% FBS, 2 mM L-glutamine, 100 IU/ml penicillin, and 100 µg/ml streptomycin. *B. constrictor* lung (V/5Lu), *B. constrictor* heart (V/2Hz), *B. constrictor* brain (V/2Br), and *Morelia viridis* liver (VII/2Liv) cells were maintained in DMEM using the same supplements as for I/1Ki (13, 280, 281). All reptile cell lines were maintained at 5% $CO_2$ and 30 °C, with the only exception of the incubation conditions for I/1Ki in for the study (I) where the medium was supplemented with 25 mM HEPES and the incubator had 0% $CO_2$ Collagen-coated bottles were used as described prior to plating the VII/2Liv and V/1Liv (281).

Tick embryo-derived cell lines, *Ixodes ricinus* IRE/CTVM19 (282, 283), *Rhipicephalus* (*Boophilus*) *microplus* BME/CTVM2 (284), and *Rhipicephalus appendiculatus* cell lines RAE/CTVM1 (284) were maintained in 2 ml L-15 (Leibovitz) medium (Sigma-Aldrich) supplemented with 20% FBS, 10% TPB, 2 mM L-glutamine, 100 IU/ml penicillin, and 100 µg/ml streptomycin in sealed flat-sided culture tubes (Nunc) at 30 °C.

## 3.2 Cloning, expression of glycoproteins (III, IV)

Mammalian expression plasmids, pCAGGS-HA and pCAGGS-FLAG (Figure 9), were used for the expression of viral GPC. The GPC sequences of UHV-1, UHV-2, UGV-1, HISV, ABV-1, ABV-2, TSMV-2, and S-5 arenaviruses were obtained

initially via sequencing at the University of Helsinki (74). The GPC sequences of GGV-1, CASV-1, LCMV and JUNV were obtained based on the sequence accession number provided in the table (Table 8). Sequences of reptarenaviruses and hartmaniviruses were cloned into pCAGGS plasmids, and mammarenavirus sequences were obtained from the Dr Luis Martinez-Sobrido (University of Rochester School of Medicine and Dentistry. Department of Microbiology and Immunology) and Dr Juan Carlos de la Torre (Scripps Institute, USA). Verification of cloning was performed by restriction digestion and Sanger sequencing (DNA Sequencing and Genomics Laboratory, Institute of Biotechnology, University of Helsinki).

**Table 8.** Arenavirus GPC sequences used in the experiments.

| **Virus GPC** | **Source of sequence (accession number)** |
|---|---|
| UHV-1 | KR870011.1 |
| UHV-2 | KR870016.1 |
| UGV-1 | NC_039005.1 |
| HISV-1 | NC_043444.1 |
| GGV-1 | NC_018483.1 |
| CASV-1 | JQ717262.1 |
| ABV-1 | KR870010.1 |
| ABV-2 | KR870018.1 |
| TSMV-2 | KX527575 |
| S-5 | KX527579.1 |
| LCMV | AY847350.1 |
| JUNV | NC_005081.1 |

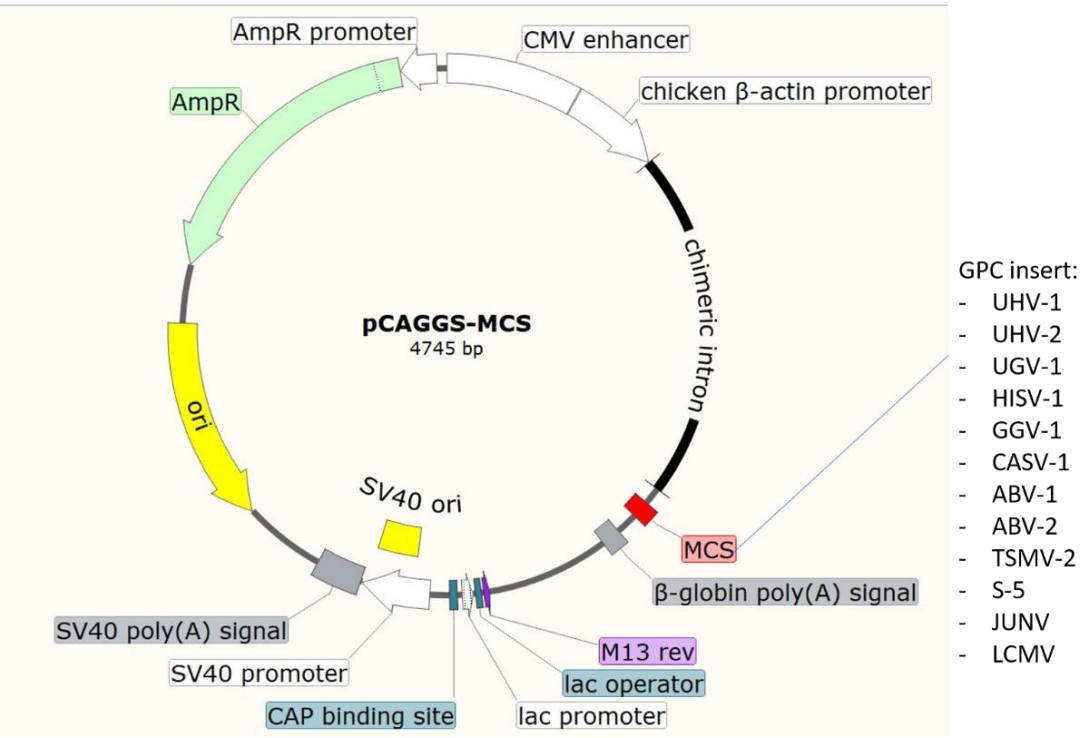



**Figure 9.** Schematic representation of cloning of arenavirus GPC sequence into pCAGGS vector.

HEK293FT cell line was used for the expression of arenavirus GP. HEK293FT was performed using FuGENE HD (Promega) transfections in a 6-well plate format. Prior to the transfection, cells were allowed to reach ~80% confluency, followed by the replacement of the medium containing 5% FBS and without antibiotics on the day of transfection. The reagent to DNA ratio for the transfection was 4:1 per well. The final DNA amount for the transfection of I/1Ki cells was at 2 500 ng per well, in a 6-well plate format. At 6-8 h post-transfection, the medium was changed. Cells were incubated for 48 h, followed by collection for either protein expression analysis or further pseudotyping infection.

## 3.3 Pseudotyping of recombinant vesicular stomatitis virus (III, IV)

A recombinant vesicular stomatitis virus (rVSVΔG-eGFP) was used to bear arenaviral GP. rVSVΔG-eGFP is constituted from all of its native proteins, but lacked its own glycoprotein G which was replaced with the eGFP signal sequence. The assembled rVSVΔG-eGFP virus was kindly provided by colleagues from the University of Helsinki, Dr Lev Levanov and Rommel Paneth Iheozor-Ejiofor (University of Helsinki, Medicum, Department of Virology). rVSVΔG-eGFP was

initially pseudotyped with its own G protein, conducted through the transfection of HEK293FT cells. Cells were transfected initially with pVSV-G plasmid and infected with rVSVΔG-eGFP to generate rVSV-G-eGFP stock (285). The generated virus was used to infect transfected HEK293FT cells that were expressing arenavirus GP, generating rVSV—GeGFP pseudotyped by arenaviral GP.

## 3.4 Reptarenavirus purification

Reptarenavirus supernatants propagated in the I/1Ki cells were collected and purified by density gradient ultracentrifugation using a 30% sucrose cushion in TEN buffer (50 mM Tris, 1 mM EDTA, 150 mM NaCl), loaded under the supernatant with a sterile needle, followed by ultracentrifugation for 2 h at 27,000 rpm and 5 °C, with a SW41 rotor and stored at −70 °C (supplemented with bovine serum albumin [BSA]) until further application. Reptarenaviruses adapted for the Vero E6 were additionally passaged three times through Vero E6 infection prior to the application of the adapted virus in infection experiments.

## 3.5 Pseudotyped virus purification

Rescued pseudotyped virus supernatants were filtered through 0.45 μm filter (Millipore), and pelleted by ultracentrifugation (Beckman coulter SW-55 rotor, 50,000 x g, 4 °C, 1 h) using a 0.5 ml 20% (w/v) sucrose cushion. The pelleted viruses were resuspended in phosphate-buffered saline (PBS) by pipetting, and the aliquots were stored at 4 °C or at -80 °C for further application.

## 3.6 Cloning, expression, and purification of recombinant UHV-1 NP protein (temperature paper and serological tools, I, II)

To clone UHV-NP recombinant variants, a template constructed from the pGEM-T vector (Promega) with a previously partial cloned S segment of UHV-1 (13). Three variants of UHV-NP were used in PCR cloning: NP (1 to 582 amino acids long, full length; rNP), N-terminal (1 to 339 amino acids long; rNP-N), and C-terminal (346 to 582 amino acids long; rNP-C).

PCR amplification of the fragments was achieved using Phusion high-fidelity DNA polymerase (Thermo Scientific) with the following primers: for NP, 5′-GGTACCATGGCTGCACTACAAAGAGC-3′ and 5′-CTCGAGGACCTCCACAGGCC-3′; for N-terminal NP, 5′-GGTACCATGGCTGCACTACAAAGAGC-3′ and 5′-CTCGAGCCTTCTCAAACGGAATACCG-3′; and for C-terminal NP, 5′-

CATATGAGGTTATACCCGGACTTGATGGA-3′ and 5′-CTCGAGGACCTCCACAGGCC-3′.

Recombinant proteins were produced using the baculovirus system (I). The recombinant proteins were obtained via purification procedures from the cells infected with recombinant baculoviruses.

## 3.7 RNA extraction, RT-PCR and qPCR (I, IV)

Viral RNAs were isolated from the corresponding reptarenavirus (UHV-1 and UGV-1) infected cells (I).Isolation was conducted using an RNeasy minikit (Qiagen) following manufacturer's guidelines. Viral RNAs from the experimentally infected snakes were collected in TriPure isolation reagents (Roche), and processed according to the manufacturer's protocol.

The isolated RNA was transcribed to cDNA using RevertAid premium reverse transcriptase (Thermo Scientific) following the manufacturer's protocol with the use of random hexamers. Stratagene MX3500P was used for the run of qPCR with the use of the Maxima SYBR green quantitative PCR (qPCR) master mix (Thermo Scientific). The target for the qPCR amplification was the UHV ZP region in the L segment with the use of 5′-CATATGAGCGAATCAACCGCAATAGGTC-3′ for the forward direction and 5′-CTCGAGTGGTTCGGGGAGG-3′ for the reverse direction.

Isolated RNAs from experimentally and naturally infected snakes (IV) were analyzed using one step TaqMan assay (Thermo Scientific) with the following primers and probes targeting the S segment, with sequence details indicated in table 9, and as was performed by K Windbichler et al in 2019 (239). PCR reactions for infected cell samples (I) were conducted using the Phusion Flash High-Fidelity PCR Master Mix (Thermo Scientific) with the primers indicated in Table 10.

**Table 9.** Primers and probes used for the amplification of extracted and reversely transcribed viral RNA from the snake tissue samples.

| Virus | Probe | Forward primer | Reverse primer |
|---|---|---|---|
| UGV-1 (IV) | 6-Fam-CTCGACAAGCGTGGGCGGAGG-BHQ-1 | CAAGAAAAACCACACTGCACA | AACCTGTTGTGTTCAGTAGT |
| UHV-1 (IV) | 6-Fam-TCCTCTGCCGCAAAAGACTATGTCACAG-BHQ-1 | ACAAACTGAATAAGACTGCTGCATT | AGGGCTATACACACATAGTTGGATG |
| ABV-1 (IV) | 6-Fam-CATGAATTCTTCATCGACATCAGAAACCG-BHQ-1 | CCGTACTGCACAACTGATGATG | AGCAACACAGGAGTAACCTGTCAC |

**Table 10.** Primers used for the amplification of extracted and reversely transcribed viral RNA from the snake tissue samples.

| Virus | Abbreviation | Forward primer | Reverse primer |
|---|---|---|---|
| University of Helsinki virus-1 | UHV-1 | TTTGTCGTCTGCCTTCAC | GCTTTGTTGACTATACAGAAGG |
| Aurora borealis virus-1 | ABV-1 | TCAAGTCCGGGTATAACCTAG | GAATTCAAGATAAAGATTGTCATAGATG |
| University of Giessen virus-1 | UGV-1 | ATAAGGTCAGGGTATAACTTGG | GAACTTGGCATAAAAATACAAATAAATG |
| S5-like virus (IV) | S5-like | GTCAGGATAGAGTCTGGGAGCAT | TGAACATTCAGAGGGAATTTGGCATC |
| Tavallinen suomalainen mies virus-2 (IV) | TSMV-2 | CAAGTCTGGATAAAGTCTTGGTGCAT | GTAATTGATGACGACAATAGGGTCGA |

## 3.8 Sequencing and DNA analysis (I, III, IV)

The PCR products were analyzed using standard agarose gel electrophoresis visualized by GelRed Nucleic Acid Stain (Biotium). Nucleic acid bands were gel-purified using the QIAquick gel extraction kit (Qiagen) following the manufacturer's instructions, eluted in Milli-Q water, and Sanger sequenced at the Sequencing Core Facility at Haartman Institute, University of Helsinki, Finland.

## 3.9 Protein works

The viral proteins were separated by SDS-PAGE under reducing or non-reducing conditions. For immunoblotting, the proteins were separated in SDS-PAGE and transferred onto nitrocellulose (Whatman) by wet blotting. Antigens, as indicated in the Table 4, rNP, NP-N and rNP-C were applied in SDS-PAGE, followed by immunoblotting with primary antibodies, then the application of secondary antibodies (Table 10). Visual results were obtained using the Odyssey Infrared Imaging System (LI-COR Biosciences).

**Table 11**. Western Blot analysis of viral proteins and correspondingly applied antibodies.

| Sample (antigen) | Primary antibody | Secondary antibody | Study |
|---|---|---|---|
| UHV-1 rNP, NP-N and rNP-C | Snake sera<br>Anti-snake anti-IgM or -IgY (applied to bind with snake sera IgM and IgY) | IR Dye 800 CW- labeled donkey-anti rabbit | II |
| Cell lysates | Rabbit anti-UHV rNP polyclonal | IR Dye 800 CW- labeled donkey-anti rabbit | I |
| Cell lysate and pseudotyped viruses | Mouse anti-HA-tag [clone 16B12, BioSite] and mouse anti-VSV-M [clone 23H12, KeraFast] | donkey anti-mouse AlexaFluor800 | III |

## 3.10 Indirect Immunofluorescence Assay (I, II)

Pre-grown and infected cells with reptarenavirus on diagnostic 10-well slides or in culture vessels were detached by pipetting (for tick cells) or trypsinized (for mammalian and reptilian cells), washed and diluted with PBS, and placed on slides for drying. Cells were fixed in 100% acetone followed by incubation with primary anti-ZP (II), anti-NP and anti-NP-C antibodies and staining with Alexa Fluor 488-labeled or Alexa Fluor 555-labeled goat anti-rabbit secondary antibodies (Invitrogen) for visual detection via fluorescence microscopy (I and II). Snake serum was applied as a primary antibody to bind with viral antigen in infected cells, followed by binding with anti-IgM and anti-IgY, with final binding with AF488 goat anti-rabbit (Molecular Probes) (II).

## 3.11 Histology and immunohistochemistry (IHC) (I, IV)

Cultured cells for immunohistochemistry (IHC) and histology were detached via trypsinization and centrifugation pelleting, followed by fixation in 2.5% paraformaldehyde (PFA) prepared in 0.2 M PBS for 24 h at 5 °C routinely embedded in paraffin wax. Sections in the size range of 3-5 µm were stained with hematoxylin-eosin (HE) generating samples for the examination for the presence of IB. For IHC, after the rescue of antigen with citrate buffer (pH 6.0) in a microwave oven, sections were incubated with affinity-purified rabbit anti-UHV-NP primary antibody (0.25 µg/ml in PBS), and stained with HRP-labeled goat anti-rabbit secondary antibodies (UltraVision anti-rabbit HRP detection system; Thermo Scientific). HRP was visualized using diaminobenzidine tetrahydrochloride (DAB) and hematoxylin counterstaining (13).

IHC and histological analyses were performed on cell pellets obtained from two culture flasks. The proportion of cells with one or multiple intracytoplasmic IB was subjected to grading of the diameter of IB in micrometers. Based on the staining intensity of NP, which is associated with the formation of IB, the IHC reaction was graded on a scale of 0.5 to 3. Scaling corresponded to the intensity of IB within positive cells, where faint intensity corresponded to (0.5), weak (1), weak moderate (1.5), moderate (2), moderate to strong (2.5), or strong (3).

Samples of extracted *B. constrictor* and *P. regious* brain, lung, liver, kidney, pancreas, spleen, small intestine, and heart tissues were fixed in paraformaldehyde (4% in PBS), prepared for hematoxylin-eosin (HE) and immunohistochemical staining, and stained as described (13).

## 3.12 Phylogeny (III)

Arenavirus GPC amino acid sequences were obtained from GenBank. Amino acid sequences were aligned by the INS-i algorithm embedded in MAFFT version 7 (286). To infer the phylogenetic tree, the Bayesian method with the Blosum model of amino acid substitution implemented in MrBayes v3.1.2 (287) was used. MrBayes was run for a million generations and sampled every 5000 generations, with a final standard deviation of 0.005 between 2 runs.

## 3.13 Infection of animals (IV)

A total of 16 *Python regius* (*P. regius*) and 16 *Boa constrictor* (*B. constrictor*) were used in the experimental infections. Housing was conducted under stable temperature conditions (27-30 °C) with application of daylight of fixed 12 h of light timing at 60-80% humidity. Three different experimental infections were conducted throughout the study on the selected group of snakes.

**The first** experimental infection involved eight *P. regius*. Three snakes from the set were infected with UHV preparation (containing UHV-1 and ABV-1), three with UGV-1, and two were used as non-infected controls. The inoculation of the virus amount and the route of infection was performed as follows: One received 5000 fluorescent focus forming units (fffus) intracoelamically, the second received 50 000 fffus intracoelamically, and the third received 50 000 fffus tracheally (the volume of inoculum was 500 ml in PBS).

**The second** experimental infection included 8 *P. regius*. Infection with the following viruses and quantity of snakes was performed in the following design: 2 snakes received UGV-1, and 4 received UHV preparation, and 2 received an equivalent amount of PBS. At 6 weeks post-inoculation, 2 *B. constrictors* were

added to the experiment. One *B. constrictor* was co-housed with UHV preparation-infected *P. regius*, and the other with UGV-1-infected *P. regius*.

**The third** experimental infection involved 14 *B. constrictor*. Three of the snakes were immunized with purified and inactivated UHV by the addition of Triton X-100 (with a final concentration of 0.2% final v/v). One *B. constrictor* was administered recombinant UHV-NP (described above). On day 0, the snakes were administered subcutaneously (multiple locations) 50 000 fffus of inactivated UHV or recombinant UHV-NP emulsified in Freund's incomplete adjuvant (Sigma Aldrich). Immunization boosters were administered on days 14 and 28 after the initial challenge. Post-immunization 8 *B. constrictors*, also including the vaccinated snakes, received via trachea 250 000 fffus of UHV, 2 *B. constrictors* received 250 000 fffus of UGV-1, and 2 *B. constrictors* received 125,000 fffus of both UHV and UGV-1. On day 30 post-inoculation, 2 of the vaccinated snakes were administered 250 000 fffus of UGV-1, and the snakes initially inoculated with UGV-1 were placed into boxes with UHV-inoculated snakes for co-housing.

All infected animals were monitored daily for the presence of signs associated with BIBD, and fed at one- to three-week intervals. The post-mortem procedure was conducted using sedation of the snake with $CO_2$, followed by decapitation and extraction of organs with Trizol (Life Technologies, for RT-PCR) or paraformaldehyde (4% solution in PBS, for histology and immunohistology). Fresh tissue was stored unfixed at -70 °C for virus isolation and further analyses.

## 3.14 Infection of cells (I)

Prior to the infection of I/1ki, Vero E6, A549, and BHK-21 were cultured in minimal essential medium (MEM) (Sigma-Aldrich) supplemented with 10% fetal bovine serum (FBS), 25 mM HEPES, 2 mM l-glutamine, 100 IU/ml penicillin, and 100 µg/ml streptomycin at 7.2-7.3 pH. For infection with UHV-1 and UGV-1, the medium was changed to minimal essential medium (MEM) (Sigma-Aldrich) supplemented with 10% fetal bovine serum (FBS), 25 mM HEPES, 2 mM l-glutamine, 100 IU/ml penicillin, and 100 µg/ml streptomycin. Infection of tick cells was performed by the addition of UHV-1 to the culture medium, allowing incubation for 14 days at 30 °C. The effect of the temperature switch was assayed in the following manner: infected and non-infected cells kept at either 30 °C or 37 °C for 4 to 5 days after virus inoculation were divided into two groups; half of the cells were transferred to the opposite temperature while the other half remained in the same temperature, i.e., cells grown at 30 °C were maintained at 37 °C and vice versa, or remained at a constant temperature. Cell samples were collected at 1- and 2-day intervals for western blot analysis and qPCR, Samples for histology and IHC were collected 4 days after the temperature swap.

## 3.15 Infection with pseudotyped viruses (III, IV)

Mammalian and reptilian cells grown in a 96-well plate were inoculated with pseudotyped viruses at a dilution yielding 50-100 fffus per well (based on the initial titration results) for 24 h. After the incubation period, virus-containing media was removed and cells were washed with PBS and fixed with 4% PFA. The fixed cells were stained for nuclei using Hoechst 33342 (Thermo Fischer Scientific), followed by washing with PBS and addition of PBS to cover the layer of the cells. The analysis of fluorescence for the detection of a GFP signal was conducted using PerkinElmer Opera Phenix High Content Screening System (Institute for Molecular Medicine Finland, University of Helsinki).

## 3.16 Neutralization assay (IV)

The extracted sera from the experimentally infected snakes were mixed with pseudotyped viruses in MEM without supplements at 37 °C for 1 h. I/1ki cells were grown to 80% confluence, the medium was removed and the virus-serum complex (dilution range of serum 1:50 to 1:6400) was inoculated with cells for at 30 °C for 1-2 h. After incubation, the medium containing the virus-serum complex was replaced with a fresh virus-free medium with all supplements as described previously, allowing cells to be incubated for 22-24 h. Neutralization was determined using fluorescence microscopy for the detection of a GFP signal.

# 4. RESULTS AND DISCUSSION

## 4.1 Replication of UHV-1 and UGV-1 is dependent on lower than mammalian body temperatures (I)

BIBD is characterized by the presence of cytoplasmic intracytoplasmic IB in infected snake tissue (11, 13, 237, 238). Reptarenaviruses induce IB formation, which mainly comprises reptarenaviral NP (13, 241), and thus the presence of IB within cells would indicate viral replication. The body temperature of snakes, in contrast to mammals, alternates between lower and higher temperature variations on daily and seasonal bases (288). Isolation of reptarenaviruses from the boid species and the ability of reptarenaviruses to infect boid cells had been previously established (13). Infection experiments were conducted in non-boid cell species, including mammalian and arthropod cells. The goal was to determine whether reptarenaviruses can infect and replicate in non-boid species.

Infection experiments on boid and non-boid cell types using two different incubation temperature conditions were performed to determine the role of temperature on the replication of reptarenaviruses. Snakes have alternating body temperatures and range from 25 to 30, and maintain body temperature closer to the surrounding enviroment °C (289). The 30 °C temperature was selected to mimic equator *B. constrictor* natural habitant area body temperature conditions. Thus, two 30 °C and 37 °C temperature conditions were compared for the infectivity and replication of reptarenaviruses in the tested cells. The infection experiments on Vero E6 was conducted using Vero E6-adapted UHV described in an earlier study (13). Viral growth was monitored by immunoblotting with anti-NP antiserum. Cells infected and incubated at 30 °C already showed accumulation of viral NP at two days post-infection (d.p.i.). Furthermore, NP expression was detected in I/1Ki and Vero E6 cells at 4, 6, and 8 d.p.i. In contrast, NP expression was not seen in either of the cell lines at 37 °C (Figure 10), suggesting inhibition or severely impaired replication.

Reptarenavirus infection leads to the formation of IB within infected I/1Ki cells (13). IB formation was evaluated in infected boid (I/1ki), arthropod (IRE/CTVM19, BME/CTVM2, and RAE/CTVM1), and mammalian (Vero E6, A549, and BHK-21) cells using IHC and transmission electron microscopy (TEM). The intensity of the formed IB was graded on the intensity within positive cells and the proportion of positive cells, where the scale from 0.5 (faint) to 3 (strong) corresponded to the overall staining intensity. UHV and Vero E6-adapted UHV I/1Ki infected with UHV and incubated at 30 °C demonstrated the presence of IB, with up to 40% of cells presenting moderate NP expression. In parallel, in I/1Ki

infected with Vero E6-adapted UHV, the formation of IB was as high as 80% of positive cells with moderate NP expression intensity. However, the rise from 30 °C to 37 °C, while keeping the infection conditions unaltered, reduced IB formation in I/1Ki cells, where IB was barely detectable and faint expression of NP was detected. The infection of Vero E6 with UHV (non-Vero E6-adapted) and incubation at 30 °C, resulted in lower IB formation, reaching only 20% of IB-positive cells and with weak expression of NP. Infection and incubation experiments on Vero E6 performed at 37 °C resulted in even lower IB formation. Thus, Vero E6 was permissive for reptarenavirus, however it allowed lower replication efficiency as indicated by the IB within the cells. Cells were further investigated initially grown and infected under 30 °C conditions, and the follow-up effect of temperature elevation to 37 °C. UHV-infected cells were passaged at 15 and 12 d.p.i. for I/1Ki and Vero E6, respectively, followed by incubation in 6-well plates at 30 °C or 37 °C. Western blot analysis was conducted at 1, 3, 5, and 7 days post incubation. Analysis of the time points showed a decrease of NP in cells. Thus, the temperature rise had a direct negative impact on the replication process of reptarenavirus (Figure 10).

The amount of viral RNA was compared after incubation at both 30 °C and 37 °C, to assess whether the decreased NP expression was due to diminished viral replication. The transfer of infected cells from 30 °C to 37 °C resulted in the reduction of viral replication, as demonstrated by qPCR. In contrast, when cells were transferred from 37 °C to 30 °C, viral replication was re-initiated. Transfer of cells from 30 °C to 37 °C caused a decrease in the amount of viral RNA, surprisingly; however, the amount of viral RNA started to increase after 4 days at 37 °C. An increase of viral RNA after 4 days at 37 °C is suggestive of an adaptation to higher temperature by the reptarenavirus, however more detailed studies on the adaptation of reptarenaviruses to higher temperatures is required to establish adaptation. However, the amount of viral RNA remained at significantly lower levels when compared to control cells incubated constantly at 30 °C. Evidently, the transfer from 30 °C to 37 °C, with a modest increase in the viral RNA levels, does not produce the expression of NP in cells grown at 37 °C. Lack of viral NP at 37 °C could be linked to the inability of viral RdRp to operate efficiently at 37 °C. Similar characteristics, dependent equally on the same temperature conditions as UHV, were demonstrated in the experiments involving UGV-1 (referred to in the original publication as T10404).

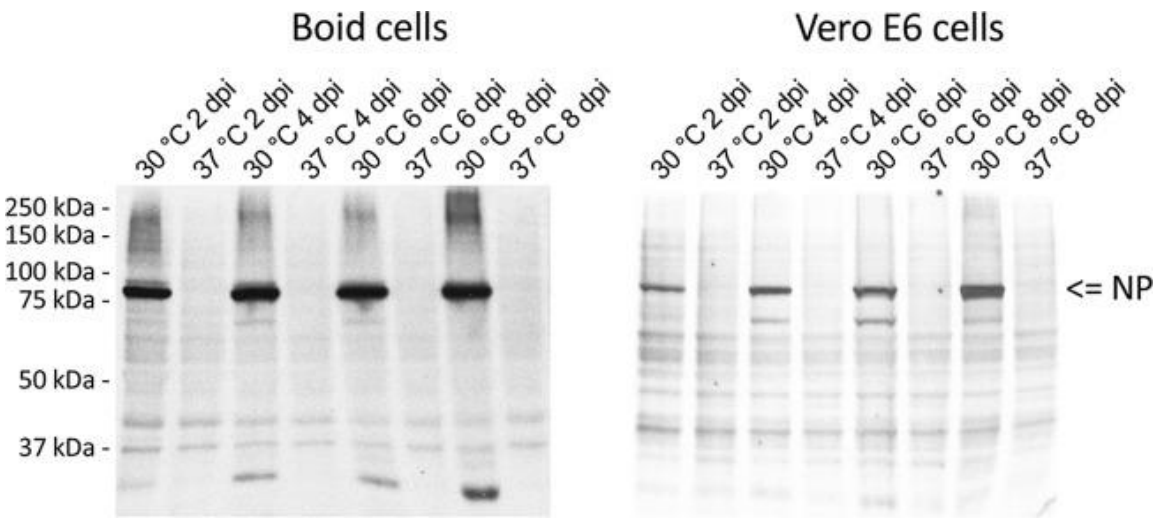



**Figure 10.** SDS-PAGE analysis of viral NP in reptilian and mammalian cells. Expression of NP in reptilian (I/1Ki) and mammalian (Vero E6) cells using comparative culturing and incubating temperatures. Infected cell suspension was collected at different time points post-infection (2, 4, 6, and 8 days post-infection). Cell incubation temperatures were set at 30 °C and 37 °C for both cell lines.

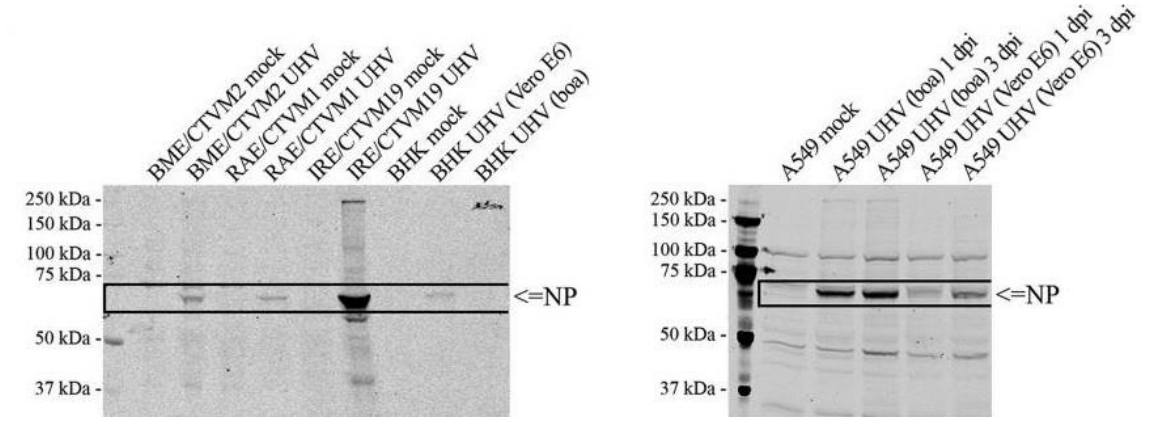



**Figure 11**. Immunoblot of tick (BME/CTVM2, RAE/CTVM1, IRE/CTVM19), rodent (BHK-21), and human (A549) cell lines infected with UHV. Arrow indicating target protein, NP, in the immunoblot using anti-rNP polyclonal antibodies.

Arthropod cell lines were tested as possible vectors for reptarenavirus transmission. Snakes with BIBD have been accompanied with mite infestations (68), which could be involved as carriers for reptarenavirus. To determine whether reptarenaviruses can replicate in arthropod cells, tick cell lines were employed, since mite cell lines were unavailable. Reptarenaviruses were able to infect the selected tick cell lines, and also depending on 30 °C, allowing viral propagation. Arenaviruses such as TCRV and LCMV have been shown to have the ability to infect tick cell lines (290, 291), indicating that tick cell lines can be permissive for arenaviruses. Ticks can in theory act solely as a preservative

transmitter of reptarenavirus from an infected snake to an uninfected, thus causing efficient virus transmission. This theory would suggest that snake blood would contain reptarenaviruses, which have been detected in snake blood smears in BIBD-positive snakes (13, 237). However, an experimental infection of ticks following by the housing of infected ticks with snakes is necessary to test this theory. Arthropod species may have played a role in the evolution of arenaviruses, leading to the adaptation to replicating at lower than mammalian body temperatures. Since arthropod cell lines are generally cultured at a 27-30 °C temperature range, snake cell lines are also cultured at the same temperature range.

A model human cell line A549 was used for infection experiments with reptarenaviruses. The A549 cell line was selected for its known IFN-generation competency. Mammarenaviruses possess suppressive properties against IFN responses (111, 129, 192); however, it is not known whether reptarenaviruses possess similar IFN-suppressing properties. Detection of NP and viral RNA after infection in this experiment suggests that reptarenaviruses are able to infect and replicate in A549. Infectivity of A549 by reptarenaviruses can also in theory be caused via TfR1 pathway, since A549 express TfR1 (292). However, Vero E6-adapted UHV caused a considerably lower level of infection than the infection mediated by the original UHV isolate in A549. The decreased infection efficiency is possibly linked to Vero E6 ability to secrete IFN-λ, which has been demonstrated with NW infection by hantaviruses (293). BHK-21 cells were used to test whether reptarenaviruses can infect rodent cells. Summarizing the cell line infection studies through the IFA and immunoblot analysis, the generated results have indicated that UHV is permeable in all tested cell lines: Mammalian (Vero, Vero E6, A549, and BHK-21) and arthropod [tick cell lines RAE/CTVM1 from *R. appendiculatus*, IRE/CTVM19 from *I. ricinus*, and BME/CTVM2 from *R.* (*B.*) *microplus*]. Notably, the original UHV isolate contained two viruses, UHV-1 and ABV-1. Thus, it is possible that only one of the viruses was able to infect the cell line.

Based on the results, reptarenaviruses infect and replicate in non-boid cells, although requiring lower temperature conditions for successful viral replication (Figure 11). Based on the infection of non-boid cell types, parasite arthropods may theoretically serve as a vector for the transmission of the virus, although testing of this hypothesis requires *in vivo* studies. Isolation of reptarenaviruses from many wild arthropod species provides strong evidence for such species serving as vectors for reptarenaviruses. Strict dependency on temperature for efficient viral replication is possibly linked to the properties of viral polymerase. Further experiments using recombinant viruses, for instance LCMV-containing reptarenaviruses RdRp, could test the adaptability of viral polymerase under different temperature conditions.

## 4.2 Reptarenavirus glycoprotein expression in mammalian cells (II)

After determining the ability of reptarenaviruses to infect non-boid cell types, the expression of viral GPC in mammalian cells was tested. Viral GPC expression was conducted in HEK293FT cells. A panel of 9 reptarenavirus GPCs was selected, while the GPCs of a hartmanivirus and two mammarenaviruses served as controls. The results showed that the GPCs of all arenaviruses tested can be expressed in mammalian cells. Expression of GPC was determined via the immunoblotting method, with the HA and FLAG to the C-terminus of GPC. Due to a lack of specific anti-GPC antibodies for each species, all GPC species were universally blotted against HA and FLAG tags.

The migration pattern of biotinylated cell surface proteins and whole cell lysates of cells expressing GPCs were compared to determine the trafficking of viral proteins to the plasma membrane. According to the immunoblot analysis of the biotinylated proteins, whole cell lysates and biotinylated proteins had highly similar migration patterns, which suggests efficient transport of viral proteins to the plasma membrane. The expression dynamics were the same for all arenaviral GPCs used in the experiments in HEK293FT cells. The processing of all arenaviral GPCs in HEK293FT occurred with considerably low efficiency. According to the migration patterns of the proteins, reptarenaviruses displayed in immunoblot bands in the size range of 10-37 kDa, with varying intensity. Bands migrating in the range 25-37 kDa most likely represent GP2, and additionally lower molecular weight bands in the range 10-15 kDa most likely represented degraded fragments of GP2 or products from alternative translation. Based on the evidence of GPC processing in mammalian cells, the ability to produce processed GPC subunits and allow trafficking of proteins to the plasma membrane would suggest homologue cellular protease activity in mammalian and reptilian cells. In parallel, expression of GPC was conducted in I/1Ki cells, where all GPC were expressed, including the control mammarenaviruses and hartmanivirus. A study describing the expression of reptarenavirus GPC indicated that GP alone may be preserved separately independent of other arenavirus proteins' properties of expression at 37 °C, since mammalian cells were cultured constantly at 37 °C. However, lower expression efficiency would indicate a dependency on a lower temperature which would be closer to the range of cell snake culturing conditions. Arenavirus GPC expression in boid and mammalian cell types also suggested the same intracellular conditions and presence of necessary factors for expression along with polyprotein processing and trafficking to the cell membrane surface. Based on the obtained results, transfected cells expressing arenavirus GPC were further applied to generate pseudotyping of rVSV.

## 4.3 Tissue and reptarenavirus species tropism using pseudotyped recombinant vesicular stomatitis virus (II)

Recombinant replication deficient vesicular stomatitis virus system (rVSVΔG-eGFP), has been used as an efficient and safe tool in studies of other non-VSV species' protein interactions (294). In this system, the structure of the virus lacks native GP, which is replaced by the GP of alternative virus species, thus making it a single cycle infection virus. Visualization of eGFP within cells allows determination of the efficiency of infectivity of a particular GP, whereupon a successful infection GFP signal is generated within the target cell. In this study, rVSVΔG-eGFP system was used for pseudotyping with a selection of reptarenavirus, hartmanivirus, and mammarenavirus GPs. The system was further used in infection experiments on mammalian and reptilian cell types. The aim of the experiment was to establish or block of entry of different reptarenaviruses GPs into reptilian and mammalian cell types. Mammarenavirus and hartmanivirus pseudotypes were used as controls.

Reptarenavirus species differ genetically, and may have differences in GP interactions with target cell surface receptors. Reptarenaviruses have demonstrated the ability to infect and replicate in mammalian, arthropod, and boid cell types, as was demonstrated earlier in the study. However, only UHV-1, ABV-1, and UGV-1 were used in the study and a limited selection of cell species. Here the infection efficiency was determined by the ability of virus to enter target cell via the attachment of arenaviral GP to the target receptor with the internalization eGFP signal into the cell. The efficiency was determined on the intensity and the quantity of generated GFP signal within infected cell by the Opera Phenix High Content Screening System (as described in Methods) detecting eGFP-generated fluorescence. The spectrum of the reptilian and mammalian cell type diversity was expanded, as indicated in Methods. Nine reptarenaviral GPC species were included in the experiments. In addition, two mammarenavirus controls for known cell tropism in mammalian hosts, were included: and one hartmanivirus as a distant arenavirus control representative. The selected control mammarenaviruses were LCMV and JUNV, for which A-DG and TfR1 serve as the respective cell surface receptors, where known GP1 and GP2 characteristics have been identified (155, 168).

Immunoblots of transfected HEK293FT cells, followed up by pseudotyping by rVSVΔG-eGFP, contained the incorporation of reptarenavirus and hartmanivirus GPC into the rVSV vector platform. Infectivity of the pseudotyped viruses was supported by the titration of viruses. Negative and positive control pseudotyped viruses were applied in titration, with rVSV-G and r-VSV-G0, pseudotyped with own GP insert and no incorporation of GP, respectively. In rVSV-G, the virus is pseudotyped with its own G protein where the infection caused by its pseudotyped virus yields the highest infectivity and rVSV-G0 yielding the lowest or no

infectivity due to absence of the incorporated GP. According to the results, reptarenavirus GPs enabled entry into the cultured mammalian and reptilian cells. The highest entry efficiency in mammalian cells was recorded in BHK-21, HEK293FT, and CHO wt for all arenaviruses used in the study. Relatively lower entry efficiency was recorded in Vero E6 and A549, when the results were analyzed based on the infectivity of mammalian cell lines. Entry of the viruses was present for most of the viruses, with similar efficiency in mammalian and reptilian cell lines, CASV-1 being the only distinguishing and the least permissive virus. Nevertheless, CASV-1 did demonstrate entry into HEK293FT, and lower entry efficiency for A549, I/1ki, and V/2Hz.

Distinguishable entry amongst reptarenaviruses was present for GGV-1, relatively higher for SK-N-SH than in CHO wt, while in all other reptarenaviruses, excluding CASV-1, entry into SK-N-SH was lower than in CHO wt. GGV-1 was isolated previously from brain tissue (11, 242), which may resemble higher neurotropism in mammalian species. Amongst the closely related reptarenaviruses ABV-1 and ABV-2, ABV-2 had almost twofold higher entry efficiency into BHK-21. The demonstration of entry efficiency between reptarenavirus species shows that GPs have considerable differences in structure that impact entry efficiency. Interestingly, equally high entry efficiency was demonstrated for all viruses in the V/2Hz constrictor snake heart cell line (13, 240). In the conducted experiments, the lung and brain cell lines of *B. constrictor* showed surprisingly low permeability for reptarenaviruses. IB has been identified along with reptarenaviruses in lung and brain tissue in analyzed snakes with BIBD (13, 237, 242); however the entry of reptarenaviruses remained at very low levels in the conducted experiments.

Mammarenaviruses showed the ability to infect cells with low permeability where an alternative entry route with the use of unidentified receptors or co-receptors has been suggested (295). In the conducted study, natural entry occurred with a possibility to use TfR1 or A-DG and unknown endocytic pathways. Mammarenaviruses have demonstrated even higher entry efficiency into V/2Hz than the vast majority of reptarenaviruses. The ability of mammarenaviruses to infect reptilian cell types could indicate an evolutionary relation to reptarenavirus GP. However, viral entry efficiency only indicated the potential of viral entry into particular target cells and does not indicate an ability to replicate or to produce progeny virions from the infected cells. Python liver, VII/1Liv, was not permissive for any arenavirus used in the study, where the infectivity levels were as low as rVSV-G0 levels, thus indicating a lack of permissiveness for arenaviruses. The python liver cell line, based on its apparent naturally non-permissive properties for arenaviruses, could be utilized as a platform for research on specific cell surface receptor and virus-specific GP interactions. Knowing that non-permissive cell lines would not naturally allow the entry of arenaviruses, the expression of known mammarenavirus cell surface receptors, such as TfR1, NRP-2, and A-DG,

would in theory allow testing on whether reptarenaviral entry depends on the same or orthologs of such receptors.

The arenaviruses used in the study showed wide tropism amongst mammalian and reptilian cell types. The ability of arenaviruses to infect different cell type could maximize the likelihood of recombinations to occur in the wild. However, the use of pseudotyped viruses does not demonstrate a natural infection process, due to the artificial expression of GP on the surface of non-native carrying virus. Pseudotyped viruses used in the study have demonstrated only the ability of selected arenaviral GP to attach and allow the entry into the target cell. Replication processes, with essential maturation and budding stages of progeny virions should involve in the future studies the use of wild type viruses, possessing all viral proteins and unmodified genome. The role of other than GP arenaviral protein should also be taken into consideration in future studies. In addition, purification of pseudotyped viruses could lead to the decrease of viral titers. Reptarenaviruses did demonstrate the ability to infect mammalian cell lines at different efficiencies and mammarenaviruses were able to enter reptilian cells. The ability to replicate in corresponding arenavirus-permeable cell lines and identification of receptor usage would constitute the infection potential of viruses in specific tissues and animals possessing such cell types.

## 4.4 Generation of anti-boa IgM and IgY and their application/use in serodiagnostics (III)

Four classes of immunoglobulins (IgM, IgD, and two classes of IgY) play a role in the humoral immunity of snakes (259). Previous reports characterizing UHV-1 have provided information about anti-reptarenavirus antibodies in snakes with BIBD (13). However, a lack of specific reagents recognizing reptarenavirus-specific antibodies hampers the classification of antibody response. Notably, due to a lack of reptarenavirus-specific reagents, serodiagnostics also remain less specific for the detection of BIBD etiological agents. Snakes possess IgY, IgD and IgM class antibodies, that are generated upon encounter with an antigen (259). To identify the presence and diversity of antibodies in snakes infected with reptarenaviruses, reagents were generated to detect snake-specific IgM and IgY antibodies. The generation of anti-boa antibodies would allow the detection of the presence of reptarenavirus-specific antibodies. A serum from a reptarenavirus-infected snake would be incubated with a reptarenavirus antigen. Thus, the introduction of the reptarenavirus antigen to the serum from a reptarenavirus-infected snake would create an antibody-antigen-specific complex. The complex was further incubated with anti-IgM and anti-IgY antibodies that were generated and purified from rabbit. Upon generation of anti-IgY and anti-IgM antibodies

bound to the complex, the final addition of IRDye 800CW-labeled donkey anti-rabbit antibodies would allow visual detection of an infrared signal.

The generation of anti-IgM and anti-IgY reagents was performed from sera initially isolated from a pool of two boa constrictor snakes and concentrated via PEG precipitation. The separation of IgY and IgM was performed by gel filtration and size-exclusion chromatography, resulting in two fractions representing IgM and IgY. The resulting fractions were submitted further to mass spectrometry, yielding three peptides that matched *Python bivitattus* IgY, and a single peptide matching *Orthriophis taeniurus* IgD for IgM. Notably, mass spectrometry analysis could not confirm the exact relation of the results to boa constrictor, since the database on boa constrictor Ig was absent at the point when the analyses were conducted. Nevertheless, the closest indication of peptide matchings suggested that the IgY fraction was related to boa constrictor. Evidence from the protein analysis via SDS-PAGE would suggest that IgD matchings are actually IgM-based. Since the source of Ig was the serum, the presence of IgD would not be expected since it has been shown to be mostly membrane-bound in mammals (296, 297), although there is no evidence for IgD being bound to the membrane in reptiles, particularly in snakes.

The IgM and IgY fractions were used to immunize rabbits to obtain boa-specific secondary antisera, thus generating antibodies able to recognize boa IgM and IgY antibodies. The antisera were tested by immunoblotting with the protein pellets containing both IgY and IgM. Immunoblot analysis of antisera showed almost identical protein bands, suggesting cross-reactivity between the two Igs. To separate the IgY and IgM, an affinity chromatography purification process was enabled. IgY and IgM fractions were coupled separately to CnBr-activated Sepharose (GE Healthcare) to generate affinity columns. The initial passing of anti-IgY antiserum through the IgM-coupled column resulted in the removal of cross-reactive antibodies. The final IgY fraction purification from the bound protein was used with a protein G column. Similarly, an anti-IgM antiserum purification process was conducted. The resulting purification products of IgM and IgM were used as antigens against immunized sera, and showed only minimal cross-reactivity. To use the generated secondary antibodies with enhanced effect, the Igs fractions were coupled with horseradish peroxidase (HRP), also analyzing IgM and IgY as test antigens. Thus, both reagents resulted in considerably good performance in chemiluminescence (ECL) detection (268). The validity of the reagents was later subjected to testing with reptarenavirus antigens, with the aim to detect reptarenavirus-specific IgM and IgY antibodies in the serum.

The application of anti-snake IgY and IgM in western blot has shown to be functional, when they were used to detect reptarenavirus-specific antibodies from infected snake serum (Figure 12). Viral NP was selected as an antigen to test the generated reagents. Nine different snake sera were incubated with rNP antigen,

resulting in the detection of IgM and IgY in one snake serum, and another IgY solely in another snake. Overall, out of nine tested snake sera, only two possessed reptarenavirus NP-specific antibodies. Infection of the Vero E6 with UHV-adapted virus confirmed the presence of viral proteins via immunofluorescence analysis. Serum lacking antibodies against NP was additionally confirmed in the IFA analysis of infected cells by UHV-1, and followed up by staining with IgM and IgY negative serum from snake #6. Positive serum for IgY and IgM deriving from snakes #7 and #9 showed positive fluorescence, confirming the presence of rNP-recognizing antibodies. Experimentally infected snake sera were screened for the presence of anti-reptarenavirus antibodies, similarly using rNP as an antigen in the blotting. As a result of experimental infection, the majority of sera had IgY present, yet most of the snakes lacked IgM (Figure 12). A pool of experimentally infected snakes after serum screening showed that only two out of thirteen possessed both IgM and IgY antibodies, and eleven possessed only IgY class antibodies against rNP (Figure 12).

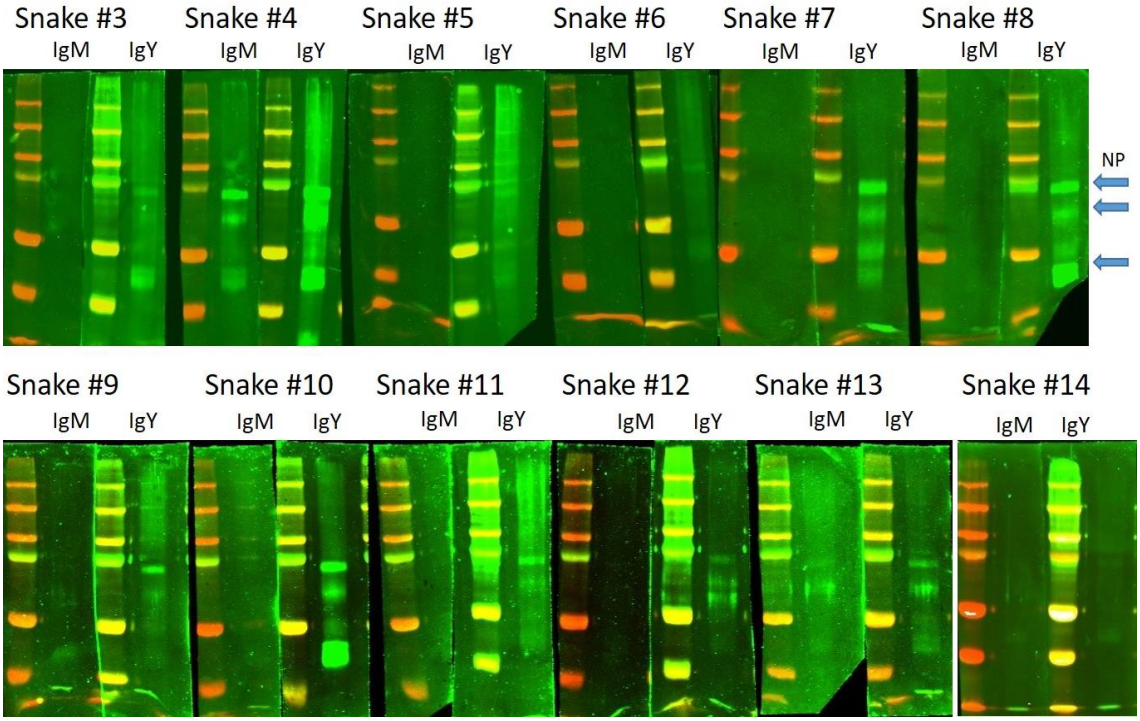



Figure 12. Screening of sera from experimentally infected *Boa constrictor* snakes with reptarenaviruses, by immunoblotting. Screening was conducted using rNP reptarenavirus as an antigen, indicated by an arrow. Multiple arrows indicate NP fractions.

According to the conducted experiments, the generated reagents have been tested and validated using immunoblotting and immunofluorescence staining. Application of the developed reagent has allowed detection of the immune response in reptarenavirus-infected snakes and can be utilized in the future for serological diagnostic purposes.

## 4.5 Experimentally and naturally infected snakes with reptarenavirus generate neutralizing antibodies (IV)

Previous reports have suggested the presence of anti-reptarenavirus antibodies in the serum from BIBD-positive snakes, conducted via indirect ELISA assay (13). In another report, serum analysis of reptarenavirus-infected snakes living in the wild indicated the presence of anti-NP antibodies (268). Experimental infection with reptarenaviruses was conducted in order to induce BIBD and detect immune response against reptarenaviruses. Notably, snakes experimentally infected with reptarenaviruses were monitored for the presence of virus-induced disease signs. UHV-1, ABV-1, and UGV-1 reptarenaviruses were used in the experimental infections. However, clear and progressive BIBD-associated signs were not observed, particularly absence of IB was evident in post mortem tissue analysis. Neurological signs were detected in some snakes, which could be linked to virus infection. Experimental infection of python and boa constrictor snake species was conducted, with the goal to determine the presence of neutralizing antibodies against reptarenaviruses. Serum extracted from experimentally infected snakes was subjected to ELISA analysis to verify the presence or absence of IgY and IgM antibodies. Python sera presented low values in the ELISA analysis for both IgM and IgY antibodies against NP, that was used an antigen. Absence of antibodies would indicate a lack of cross-reactivity of antibodies in the sera. In contrast, both experimentally and naturally infected *B. constrictor* snakes showed consistent and much higher values for IgY class antibodies in the ELISA analysis; however, IgM class antibodies were considerably lower. Detection of IgY with higher values would also be associated with the later generation and longer half-life of the antibodies in contrast to IgM antibodies. Post-mortem extracted sera from experimentally infected snakes were subjected to testing with pseudotyped rVSV with respective reptarenavirus GP species that were used in the infection experiments with the whole viruses. Serial serum dilution was tested with constant viral load, where the neutralization titer was determined. Neutralizing antibodies against all reptarenaviruses with various degrees of neutralization were detected (Figure 13). Cross-reactivity of antibodies against other reptarenaviruses was detected in most cases, suggesting similarities between reptarenavirus GP confirmation and the target epitopes.

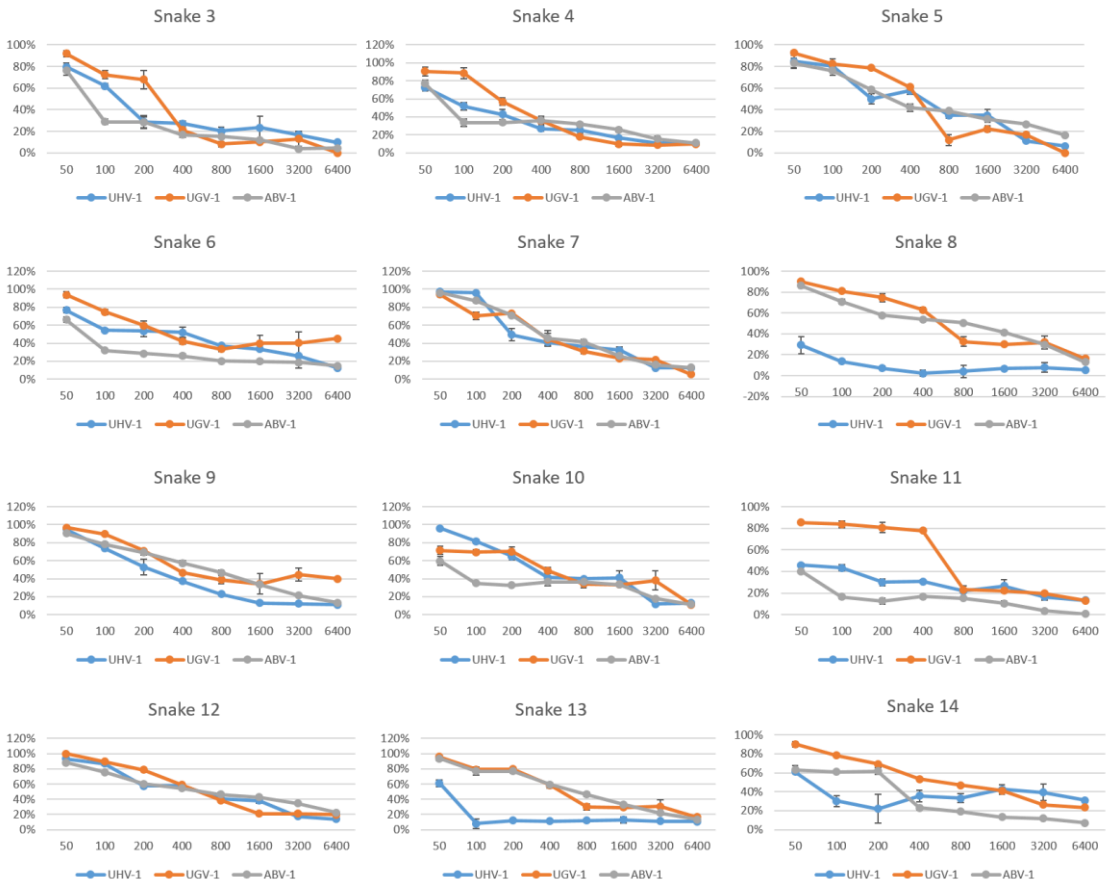
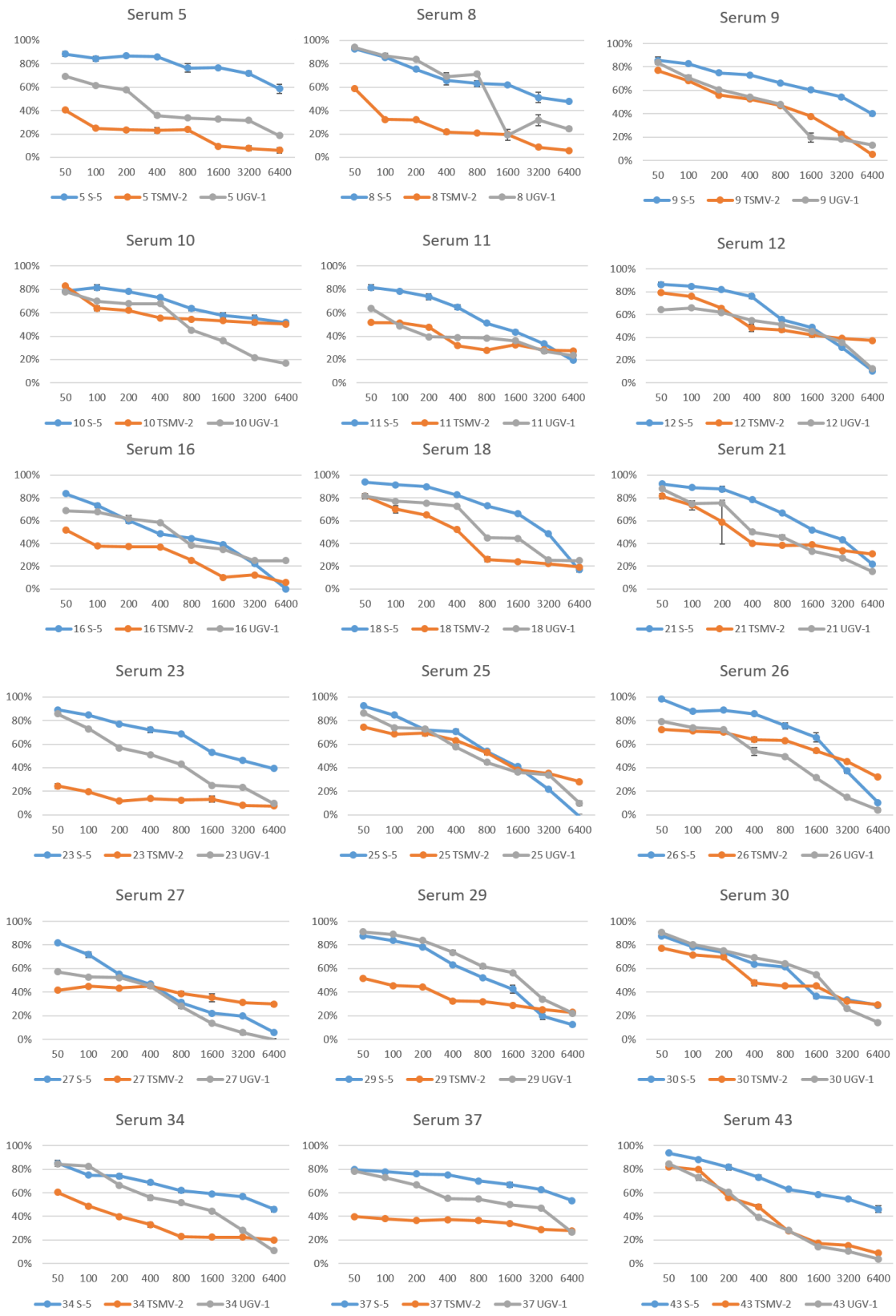



**Figure 13**. Neutralization assay of sera from experimentally infected constrictor snakes with reptarenaviruses. The pseudotyped viruses represented GP UHV-1, UGV-1, and ABV-1. Y axis represents the percentage of neutralization, X axis represents the dilution of the serum.


Serum 5
100%
80%
60%
40%
20%
0%
50 100 200 400 800 1600 3200 6400
5 S-5
5 TSMV-2
5 UGV-1
Serum 8
8 S-5
8 TSMV-2
8 UGV-1
Serum 9
9 S-5
9 TSMV-2
9 UGV-1
Serum 10
10 S-5
10 TSMV-2
10 UGV-1
Serum 11
11 S-5
11 TSMV-2
11 UGV-1
Serum 12
12 S-5
12 TSMV-2
12 UGV-1
Serum 16
16 S-5
16 TSMV-2
16 UGV-1
Serum 18
18 S-5
18 TSMV-2
18 UGV-1
Serum 21
21 S-5
21 TSMV-2
21 UGV-1
Serum 23
23 S-5
23 TSMV-2
23 UGV-1
Serum 25
25 S-5
25 TSMV-2
25 UGV-1
Serum 26
26 S-5
26 TSMV-2
26 UGV-1
Serum 27
27 S-5
27 TSMV-2
27 UGV-1
Serum 29
29 S-5
29 TSMV-2
29 UGV-1
Serum 30
30 S-5
30 TSMV-2
30 UGV-1
Serum 34
34 S-5
34 TSMV-2
34 UGV-1
Serum 37
37 S-5
37 TSMV-2
37 UGV-1
Serum 43
43 S-5
43 TSMV-2
43 UGV-1

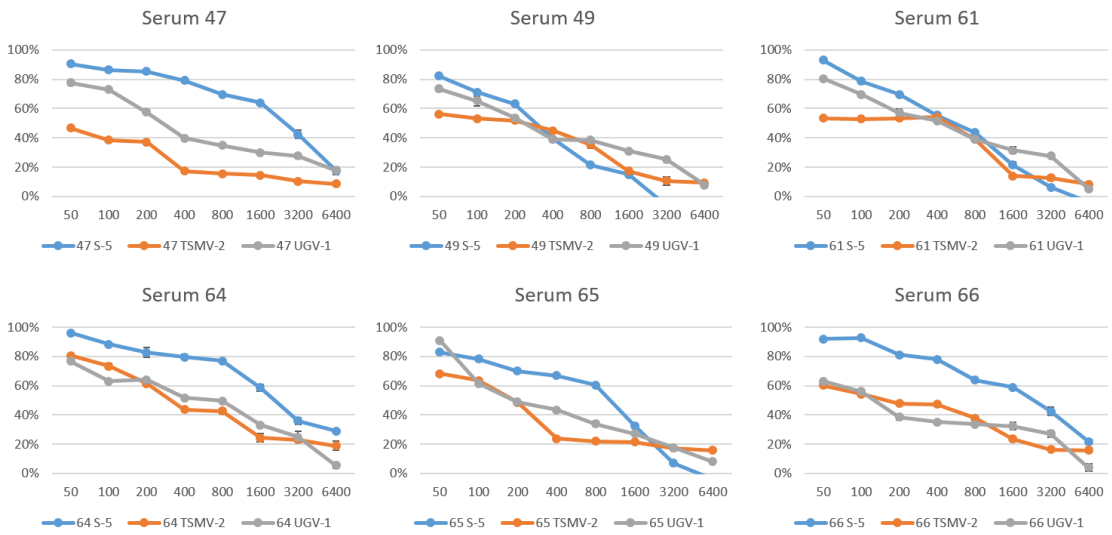



**Figure 14**. Neutralization assay of sera from natural infections with reptarenaviruses. The pseudotyped viruses represented a GP of S-5 like, TSMV-2 and UGV-1. Y axis represents the percentage of neutralization, X axis represents the dilution of the serum.

Neutralization assay was set up to further determine the presence or absence of neutralizing antibodies in naturally infected snakes with reptarenavirus. A total of 25 sera from naturally infected constrictor snakes were screened for reptarenaviruses. Based on the RT-PCR results of infected snakes, the following viruses were present: TSMV-2, UGV-1, and S-5 (268). Based on the viruses identified in the snakes, the rVSV pseudotyped with the corresponding reptarenavirus GP was applied in the neutralization assay. According to the neutralization assay results, the majority of naturally infected snakes were able to generate neutralizing antibodies against viral GP with relatively high titers of neutralization (Figure 14). The experiments demonstrated that reptarenaviruses can induce the production of neutralizing antibodies in boa constrictor snakes, although progressive disease signs were absent. The application of a wider selection of reptarenaviruses in experimental infection would help to identify the causative agents of BIBD. While it is evident that reptarenaviruses infect snakes, the identification of a BIBD-inducing virus or virus combination still requires establishment. In addition, the role of other reptarenavirus proteins and co-infections by reptarenaviruses would be required in future testing to better understand the pathogenesis of reptarenaviruses in snakes.

# 5. CONCLUDING REMARKS

BIBD has been strongly linked to reptarenavirus infection in captive constrictor snakes (11-13). Here, studies were conducted to examine the temperature dependency of reptarenavirus infection and the ability of reptarenaviruses to replicate in various host cells, including non-boid cells. The results from the conducted studies showed that reptarenaviruses can infect the cells of different species including rodent, human, arthropod, and snake cells. In addition to inducing IB in reptilian cells, reptarenaviruses have demonstrated the ability to induce the formation of IB within mammalian cells. However, viral replication is restricted to lower temperatures characteristic of reptilian species, which can be associated with the adaptation of reptarenavirus RdRp to operate optimally at reptilian body temperature.

Experimental infections were conducted in constrictor snakes using reptarenaviruses. The experimental infections of captive snakes did not yield a consistent presence of IB, even though the RT-PCR results were positive for the virus. In contrast, in naturally infected snake tissue samples, histological analysis allowed the detection of IB in some of the infected snakes. Lack of IB formation in experimentally infected snakes could be linked to a strong immune response against NP, main component associated with the formation of IB. However, snakes may still experience BIBD signs, such as neurological signs and yet not develop IB as was discovered in earlier studies (237, 238, 242). Experimental infection in conducted study of snakes with one or multiple reptarenaviruses did not induce clear and progressive BIBD signs in infected snakes, such as progressive head tremors, so-called ‘’stargazing” or also known as opisthotonus, regurgitation, lack of appetite, and eventual anorexia (240). While the conditions that were used in the experiments for snake housing ranged from 27-29 °C, the snake owners use heat lamps to regulate temperature in snake terrariums. This creates heat gradients, allowing snakes to naturally regulate their body temperature. The unregulated heat conditions in the experimental setting may have affected the progression of the infection and have had a negative impact on the progression of BIBD. Interestingly, it was noted that snakes naturally infected with reptarenaviruses have weak anti-NP antibody levels with high neutralizing titers. In contrast, experimentally infected snakes had inverse values for anti-NP antibodies. A similar study by Stenglein et al in 2017 (242) successfully established the formation of IB by the reptarenavirus. However, they used reptarenavirus GGV-1, which was not used in this study. Previous studies have also established the formation of IB in snakes (68, 237, 238), but at the time those studies were conducted, reptarenaviruses’ existence was not known. Thus, viral

diversity amongst reptarenaviruses can vary in pathogenicity and causing of BIBD.

Pseudotyped viruses were used in the tissue tropism experiments, where the panel of diverse mammalian and reptilian cells were tested. Reptarenavirus GPs were able to enter not only reptilian cell lines, but also mammalian cell lines, regardless of the incubation temperatures during infection. Viral entry was recorded with various efficiencies, and even for some reptilian cell lines, such as python liver cells, cells seemed to be impermeable to all arenaviruses used in the experiments. Mammarenavirus entry was not restricted to mammalian cell lines, and was also evident in reptilian cell lines. The dependency of viral entry on the known mammarenavirus receptors A-DG, TfR1, or NRP-2 requires further investigations for reptarenaviruses. However, the ability of LCMV and JUNV to infect reptilian cell lines would suggest a role of A-DG and/or TfR1 in reptilian cell line infections. Temperature dependency plays a crucial role in the replication of reptarenaviruses in mammalian cells; however, wild-type viruses with expanded diversity reptilian cell line panel require future testing.

Mammarenavirus infections in humans induce strong humoral immune response, resulting in the generation of IgM and IgG class antibodies (257, 298-301). Here, reagents were developed to recognize anti-boa IgM and IgY, and applied as serological tools to detect boa IgM and IgY bound to reptarenavirus antigen. The developed reagents were tested in immunoblotting with viral antigens, allowing the detection of the presence or absence of the immune response in reptarenavirus-infected snakes. Experimentally infected snakes showed for the most part an absence of IgM antibodies against reptarenavirus NP, although IgY class antibodies were detected. With available serum from experimentally infected snakes and with the pseudotyped rVSV system, the presence of neutralizing antibodies in snakes infected with reptarenaviruses was tested. Reptarenavirus infection appear to stimulate the consistent generation of neutralizing antibodies in experimentally infected snakes. However, detailed analysis of viral antibody epitopes requires further investigation.

To summarize, it is quite possible that extended reptarenavirus infection leads to BIBD. The conducted studies were able to show that the spectrum of selected mammalian and reptilian target cells was tested with reptarenaviruses. The association of viral replication dependency on restricted temperature conditions could further determine the role of viral polymerase properties under various temperatures. Identification of antibodies against reptarenaviruses can be used to determine the level of protection in animals that have been priorly infected with reptarenavirus. While there are currently no licensed treatments against reptarenavirus infection, this study has provided novel information on reptarenavirus infections in mammalian and reptilian cells. Future research is aimed at identifying specific antiviral targets.

# 6. ACKNOWLEDGEMENTS

This given thesis work was conducted initially at the Department of Virology, Medicum, University of Helsinki, and final stages were completed at the Department of Veterinary Medicine, University of Helsinki. I want to thank the Head of the Virology department, Professor Kalle Saksela, for providing an outstanding facility and professional atmosphere for conducting virology research. I want to thank Docent Maija Vihinen-Ranta and Adjunct Professor Petri Susi for their critical review, essential comments, and discussion input on my thesis.

I would like to sincerely express my gratitude to my supervisors Professor Olli Vapalahti and Docent Jussi Hepojoki for their invaluable advice, support, and patience during my PhD studies. Your extended experience, knowledge, and expertise in science have provided me with inspiration to excel in virology research. You will always be my role models in science. I would also like to thank our Swiss collaborators; Dr Udo Hetzel and Professor Anja Kipar for indispensable animal work and irreplaceable teamwork. I want to express appreciation to Leonora Szirovicza for all the help in laboratory work and for helping our projects to move forward.

I want to express my exclusive appreciation to the best team of technicians; Sanna, Mira, Johanna, and Irina. Your help with cell cultures, upkeep of the laboratory reagents created a professional and wonderful working atmosphere in Meilahti. You make the dream team of technicians to have!

I want to thank viral zoonosis researchers, Tomas, Tarja, Lev, Rommel, Suvi, Teemu, Lauri, Alex, Angelina and Kirsi for providing guidance and support during my PhD research. The viral zoonosis research group has been very supportive inside and outside the research. Enjoyed every single day working with all of you! I have also enjoyed all the fun outside the laboratory life, such as floor ball games and viral zoonosis Olympics.

I want to thank Professor Volodymir Ivanitsa and Docent Tatyana Gudzenko, my previous supervisors at the Odessa I. I. Mechnikov National University for providing me with academic and practical undergraduate training in microbiology.

My gratitude goes to my wrestling teammates Jouni, Rolle, Jonas, Jere K, Jere H, Jukka, Jarmo, Henri, Matias, Niko K, Niko R, Ville, Jani, Juha and Timo. Many of you have helped me to overcome through tough times before and during my PhD studies. Your support on the mat, on the field, and outside my research has

been irreplaceable. Additional appreciation goes to AC Karhuryhmä, providing me with discipline and commitment for working hard.

I want to express my warmest appreciation to the ‘’Ronians’’ - international friends all over the world with true friendship and support. Jay Whiten, you are the link to the ‘’Ronians’’, I am truly grateful for bringing such diversity together. For all of you Ronians, I truly value your friendship.

Anna P, my dear friend, thank you for all your support and belief in hard work.

I want to thank my parents and brother for supporting me from the very first morphogenesis stages. I want to thank my girlfriend Roosa and her family for supporting me through all these years of PhD studies and all the guidance in Finland.

# 7. REFERENCES


1. **Armstrong C, Lillie RD.** 1934. Experimental Lymphocytic Choriomeningitis of Monkeys and Mice Produced by a Virus Encountered in Studies of the 1933 St. Louis Encephalitis Epidemic. Public Health Reports (1896-1970) **49:**1019-1027.
2. **Parodi AS, Greenway DJ, Rugiero HR, Frigerio M, De La Barrera JM, Mettler N, Garzon F, Boxaca M, Guerrero L, Nota N.** 1958. [Concerning the epidemic outbreak in Junin]. Dia Med **30:**2300-2301.
3. **Downs WG, Anderson CR, Spence L, Aitken TH, Greenhall AH.** 1963. Tacaribe virus, a new agent isolated from Artibeus bats and mosquitoes in Trinidad, West Indies. Am J Trop Med Hyg **12:**640-646.
4. **Johnson KM, Mackenzie RB, Webb PA, Kuns ML.** 1965. Chronic infection of rodents by Machupo virus. Science **150:**1618-1619.
5. **Buckley SM, Casals J, Downs WG.** 1970. Isolation and antigenic characterization of Lassa virus. Nature **227:**174.
6. **Fenner F.** 1976. Classification and nomenclature of viruses. Second report of the International Committee on Taxonomy of Viruses. Intervirology **7:**1-115.
7. **Rodas JD, Salvato MS.** 2006. Tales of mice and men: Natural History of Arenaviruses %J Revista Colombiana de Ciencias Pecuarias. **19:**382-400.
8. **Maes P, Alkhovsky SV, Bao Y, Beer M, Birkhead M, Briese T, Buchmeier MJ, Calisher CH, Charrel RN, Choi IR, Clegg CS, de la Torre JC, Delwart E, DeRisi JL, Di Bello PL, Di Serio F, Digiaro M, Dolja VV, Drosten C, Druciarek TZ, Du J, Ebihara H, Elbeaino T, Gergerich RC, Gillis AN, Gonzalez JJ, Haenni AL, Hepojoki J, Hetzel U, Ho T, Hong N, Jain RK, Jansen van Vuren P, Jin Q, Jonson MG, Junglen S, Keller KE, Kemp A, Kipar A, Kondov NO, Koonin EV, Kormelink R, Korzyukov Y, Krupovic M, Lambert AJ, Laney AG, LeBreton M, Lukashevich IS, Marklewitz M, Markotter W, Martelli GP, Martin RR, Mielke-Ehret N, Muhlbach HP, Navarro B, Ng TFF, Nunes MRT, Palacios G, Paweska JT, Peters CJ, Plyusnin A, Radoshitzky SR, Romanowski V, Salmenpera P, Salvato MS, Sanfacon H, Sasaya T, Schmaljohn C, Schneider BS, Shirako Y, Siddell S, Sironen TA, Stenglein MD, Storm N, Sudini H, Tesh RB, Tzanetakis IE, Uppala M, Vapalahti O, Vasilakis N, Walker PJ, Wang G, Wang L, Wang Y, Wei T, Wiley MR, Wolf YI, Wolfe ND, Wu Z, Xu W, Yang L, Yang Z, Yeh SD, Zhang YZ, Zheng Y, Zhou X, Zhu C, Zirkel F, Kuhn JH.** 2018. Taxonomy of the family Arenaviridae and the order Bunyavirales: update 2018. Arch Virol **163:**2295-2310.
9. **Peralta LA, Laguens RP, Cossio PM, Sabattini MS, Maiztegui JI, Arana RM.** 1979. Presence of viral particles in the salivary gland of Calomys musculinus infected with Junin virus by a natural route. Intervirology **11:**111-116.
10. **Enria DA, Pinheiro F.** 2000. Rodent-borne emerging viral zoonosis. Hemorrhagic fevers and hantavirus infections in South America. Infect Dis Clin North Am **14:**167-184, x.
11. **Stenglein MD, Sanders C, Kistler AL, Ruby JG, Franco JY, Reavill DR, Dunker F, Derisi JL.** 2012. Identification, characterization, and in vitro culture of highly divergent arenaviruses from boa constrictors and annulated tree boas: candidate etiological agents for snake inclusion body disease. MBio **3:**e00180-00112.

12. **Bodewes R, Kik MJ, Raj VS, Schapendonk CM, Haagmans BL, Smits SL, Osterhaus AD.** 2013. Detection of novel divergent arenaviruses in boid snakes with inclusion body disease in The Netherlands. J Gen Virol **94:**1206-1210.
13. **Hetzel U, Sironen T, Laurinmaki P, Liljeroos L, Patjas A, Henttonen H, Vaheri A, Artelt A, Kipar A, Butcher SJ, Vapalahti O, Hepojoki J.** 2013. Isolation, identification, and characterization of novel arenaviruses, the etiological agents of boid inclusion body disease. J Virol **87:**10918-10935.
14. **Zhang YZ, Wu WC, Shi M, Holmes EC.** 2018. The diversity, evolution and origins of vertebrate RNA viruses. Curr Opin Virol **31:**9-16.
15. **Hetzel U, Sironen T, Laurinmaki P, Liljeroos L, Patjas A, Henttonen H, Vaheri A, Artelt A, Kipar A, Butcher SJ, Vapalahti O, Hepojoki J.** 2014. Reply to "Updated phylogenetic analysis of arenaviruses detected in boid snakes". J Virol **88:**1401.
16. **Abudurexiti A, Adkins S, Alioto D, Alkhovsky SV, Avsic-Zupanc T, Ballinger MJ, Bente DA, Beer M, Bergeron E, Blair CD, Briese T, Buchmeier MJ, Burt FJ, Calisher CH, Chang C, Charrel RN, Choi IR, Clegg JCS, de la Torre JC, de Lamballerie X, Deng F, Di Serio F, Digiaro M, Drebot MA, Duan X, Ebihara H, Elbeaino T, Ergunay K, Fulhorst CF, Garrison AR, Gao GF, Gonzalez JJ, Groschup MH, Gunther S, Haenni AL, Hall RA, Hepojoki J, Hewson R, Hu Z, Hughes HR, Jonson MG, Junglen S, Klempa B, Klingstrom J, Kou C, Laenen L, Lambert AJ, Langevin SA, Liu D, Lukashevich IS, Luo T, Lu C, Maes P, de Souza WM, Marklewitz M, Martelli GP, Matsuno K, Mielke-Ehret N, Minutolo M, Mirazimi A, Moming A, Muhlbach HP, Naidu R, Navarro B, Nunes MRT, Palacios G, Papa A, Pauvolid-Correa A, Paweska JT, Qiao J, Radoshitzky SR, Resende RO, Romanowski V, Sall AA, Salvato MS, Sasaya T, Shen S, Shi X, Shirako Y, Simmonds P, Sironi M, Song JW, Spengler JR, Stenglein MD, Su Z, Sun S, Tang S, Turina M, Wang B, Wang C, Wang H, Wang J, Wei T, Whitfield AE, Zerbini FM, Zhang J, Zhang L, Zhang Y, Zhang YZ, Zhang Y, Zhou X, Zhu L, Kuhn JH.** 2019. Taxonomy of the order Bunyavirales: update 2019. Arch Virol **164:**1949-1965.
17. **Charrel RN, de Lamballerie X, Emonet S.** 2008. Phylogeny of the genus Arenavirus. Curr Opin Microbiol **11:**362-368.
18. **Salazar-Bravo J, Ruedas LA, Yates TL.** 2002. Mammalian reservoirs of arenaviruses. Curr Top Microbiol Immunol **262:**25-63.
19. **Charrel RN, de Lamballerie X.** 2010. Zoonotic aspects of arenavirus infections. Vet Microbiol **140:**213-220.
20. **Li K, Lin XD, Wang W, Shi M, Guo WP, Zhang XH, Xing JG, He JR, Wang K, Li MH, Cao JH, Jiang ML, Holmes EC, Zhang YZ.** 2015. Isolation and characterization of a novel arenavirus harbored by Rodents and Shrews in Zhejiang province, China. Virology **476:**37-42.
21. **Palacios G, Druce J, Du L, Tran T, Birch C, Briese T, Conlan S, Quan PL, Hui J, Marshall J, Simons JF, Egholm M, Paddock CD, Shieh WJ, Goldsmith CS, Zaki SR, Catton M, Lipkin WI.** 2008. A new arenavirus in a cluster of fatal transplant-associated diseases. N Engl J Med **358:**991-998.
22. **Bowen MD, Peters CJ, Nichol ST.** 1996. The phylogeny of New World (Tacaribe complex) arenaviruses. Virology **219:**285-290.
23. **Reignier T, Oldenburg J, Flanagan ML, Hamilton GA, Martin VK, Cannon PM.** 2008. Receptor use by the Whitewater Arroyo virus glycoprotein. Virology **371:**439-446.
24. **Flanagan ML, Oldenburg J, Reignier T, Holt N, Hamilton GA, Martin VK, Cannon PM.** 2008. New world clade B arenaviruses can use transferrin receptor 1 (TfR1)-dependent

and -independent entry pathways, and glycoproteins from human pathogenic strains are associated with the use of TfR1. J Virol **82:**938-948.

25. **Fernandes J, de Oliveira RC, Guterres A, de Carvalho Serra F, Bonvicino CR, D'Andrea PS, Cunha RV, Levis S, de Lemos ERS.** 2015. Co-circulation of Clade C New World Arenaviruses: New geographic distribution and host species. Infection, Genetics and Evolution **33:**242-245.
26. **Calisher CH, Tzianabos T, Lord RD, Coleman PH.** 1970. Tamiami virus, a new member of the TaCaribe group. Am J Trop Med Hyg **19:**520-526.
27. **Fulhorst CF, Bowen MD, Ksiazek TG, Rollin PE, Nichol ST, Kosoy MY, Peters CJ.** 1996. Isolation and characterization of Whitewater Arroyo virus, a novel North American arenavirus. Virology **224:**114-120.
28. **Fulhorst CF, Bennett SG, Milazzo ML, Murray HL, Jr., Webb JP, Jr., Cajimat MN, Bradley RD.** 2002. Bear Canyon virus: an arenavirus naturally associated with the California mouse (Peromyscus californicus). Emerg Infect Dis **8:**717-721.
29. **Enserink M.** 2000. Emerging diseases. New arenavirus blamed for recent deaths in California. Science **289:**842-843.
30. **Moncayo AC, Hice CL, Watts DM, Travassos de Rosa AP, Guzman H, Russell KL, Calampa C, Gozalo A, Popov VL, Weaver SC, Tesh RB.** 2001. Allpahuayo virus: a newly recognized arenavirus (arenaviridae) from arboreal rice rats (oecomys bicolor and oecomys paricola) in northeastern peru. Virology **284:**277-286.
31. **Mills JN, Ellis BA, McKee KT, Jr., Calderon GE, Maiztegui JI, Nelson GO, Ksiazek TG, Peters CJ, Childs JE.** 1992. A longitudinal study of Junin virus activity in the rodent reservoir of Argentine hemorrhagic fever. Am J Trop Med Hyg **47:**749-763.
32. **Lisieux T, Coimbra M, Nassar ES, Burattini MN, de Souza LT, Ferreira I, Rocco IM, da Rosa AP, Vasconcelos PF, Pinheiro FP, et al.** 1994. New arenavirus isolated in Brazil. Lancet **343:**391-392.
33. **Trapido H, Sanmartin C.** 1971. Pichinde virus, a new virus of the Tacaribe group from Colombia. Am J Trop Med Hyg **20:**631-641.
34. **Delgado S, Erickson BR, Agudo R, Blair PJ, Vallejo E, Albarino CG, Vargas J, Comer JA, Rollin PE, Ksiazek TG, Olson JG, Nichol ST.** 2008. Chapare virus, a newly discovered arenavirus isolated from a fatal hemorrhagic fever case in Bolivia. PLoS Pathog **4:**e1000047.
35. **Charrel RN, Feldmann H, Fulhorst CF, Khelifa R, de Chesse R, de Lamballerie X.** 2002. Phylogeny of New World arenaviruses based on the complete coding sequences of the small genomic segment identified an evolutionary lineage produced by intrasegmental recombination. Biochem Biophys Res Commun **296:**1118-1124.
36. **Pinheiro FP, Woodall JP, Travassosdarosa APA, Travossosdarosa JF.** 1977. Studies on Arenaviruses in Brazil. Medicina-Buenos Aire **37:**175-181.
37. **Gryseels S, Rieger T, Oestereich L, Cuypers B, Borremans B, Makundi R, Leirs H, Gunther S, Gouy de Bellocq J.** 2015. Gairo virus, a novel arenavirus of the widespread Mastomys natalensis: Genetically divergent, but ecologically similar to Lassa and Morogoro viruses. Virology **476:**249-256.
38. **Tesh RB, Jahrling PB, Salas R, Shope RE.** 1994. Description of Guanarito virus (Arenaviridae: Arenavirus), the etiologic agent of Venezuelan hemorrhagic fever. Am J Trop Med Hyg **50:**452-459.
39. **Swanepoel R, Leman PA, Shepherd AJ, Shepherd SP, Kiley MP, McCormick JB.** 1985. Identification of Ippy as a Lassa-fever-related virus. Lancet **1:**639.

40. **Buckley SM, Casals J.** 1970. Lassa fever, a new virus disease of man from West Africa. 3. Isolation and characterization of the virus. Am J Trop Med Hyg **19:**680-691.
41. **Webb PA, Justines G, Johnson KM.** 1975. Infection of wild and laboratory animals with Machupo and Latino viruses. Bull World Health Organ **52:**493-499.
42. **Blasdell KR, Duong V, Eloit M, Chretien F, Ly S, Hul V, Deubel V, Morand S, Buchy P.** 2016. Evidence of human infection by a new mammarenavirus endemic to Southeastern Asia. Elife **5**.
43. **Briese T, Paweska JT, McMullan LK, Hutchison SK, Street C, Palacios G, Khristova ML, Weyer J, Swanepoel R, Egholm M, Nichol ST, Lipkin WI.** 2009. Genetic detection and characterization of Lujo virus, a new hemorrhagic fever-associated arenavirus from southern Africa. PLoS Pathog **5:**e1000455.
44. **Ishii A, Thomas Y, Moonga L, Nakamura I, Ohnuma A, Hang'ombe BM, Takada A, Mweene AS, Sawa H.** 2012. Molecular surveillance and phylogenetic analysis of Old World arenaviruses in Zambia. J Gen Virol **93:**2247-2251.
45. **Traub E.** 1935. A Filterable Virus Recovered from White Mice. Science **81:**298-299.
46. **Rivers TM, Scott TF.** 1936. Meningitis in Man Caused by a Filterable Virus : Ii. Identification of the Etiological Agent. J Exp Med **63:**415-432.
47. **Johnson KM, Kuns ML, Mackenzie RB, Webb PA, Yunker CE.** 1966. Isolation of Machupo virus from wild rodent Calomys callosus. Am J Trop Med Hyg **15:**103-106.
48. **Witkowski PT, Kallies R, Hoveka J, Auste B, Ithete NL, Soltys K, Szemes T, Drosten C, Preiser W, Klempa B, Mfune JK, Kruger DH.** 2015. Novel Arenavirus Isolates from Namaqua Rock Mice, Namibia, Southern Africa. Emerg Infect Dis **21:**1213-1216.
49. **Palacios G, Savji N, Hui J, Travassos da Rosa A, Popov V, Briese T, Tesh R, Lipkin WI.** 2010. Genomic and phylogenetic characterization of Merino Walk virus, a novel arenavirus isolated in South Africa. J Gen Virol **91:**1315-1324.
50. **Gonzalez JP, McCormick JB, Saluzzo JF, Herve JP, Georges AJ, Johnson KM.** 1983. An arenavirus isolated from wild-caught rodents (Pramys species) in the Central African Republic. Intervirology **19:**105-112.
51. **Wulff H, McIntosh BM, Hamner DB, Johnson KM.** 1977. Isolation of an arenavirus closely related to Lassa virus from Mastomys natalensis in south-east Africa. Bull World Health Organ **55:**441-444.
52. **Gunther S, Hoofd G, Charrel R, Roser C, Becker-Ziaja B, Lloyd G, Sabuni C, Verhagen R, van der Groen G, Kennis J, Katakweba A, Machang'u R, Makundi R, Leirs H.** 2009. Mopeia virus-related arenavirus in natal multimammate mice, Morogoro, Tanzania. Emerg Infect Dis **15:**2008-2012.
53. **Mills JN, Barrera Oro JG, Bressler DS, Childs JE, Tesh RB, Smith JF, Enria DA, Geisbert TW, McKee KT, Jr., Bowen MD, Peters CJ, Jahrling PB.** 1996. Characterization of Oliveros virus, a new member of the Tacaribe complex (Arenaviridae: Arenavirus). Am J Trop Med Hyg **54:**399-404.
54. **Webb PA, Johnson KM, Hibbs JB, Kuns ML.** 1970. Parana, a new Tacaribe complex virus from Paraguay. Arch Gesamte Virusforsch **32:**379-388.
55. **Fulhorst CE, Bowen MD, Salas RA, de Manzione NM, Duno G, Utrera A, Ksiazek TG, Peters CJ, Nichol ST, De Miller E, Tovar D, Ramos B, Vasquez C, Tesh RB.** 1997. Isolation and characterization of pirital virus, a newly discovered South American arenavirus. Am J Trop Med Hyg **56:**548-553.
56. **Bisordi I, Levis S, Maeda AY, Suzuki A, Nagasse-Sugahara TK, de Souza RP, Pereira LE, Garcia JB, Cerroni Mde P, de AeSF, dos Santos CL, da Fonseca BA.** 2015. Pinhal Virus, a

New Arenavirus Isolated from Calomys tener in Brazil. Vector Borne Zoonotic Dis **15:**694-700.

57. **Pinheiro FP, Shope RE, de Andrade AHP, Bensabath G, Cacios GV, Casals J.** 1966. Amapari, a New Virus of the Tacaribe Group from Rodents and Mites of Amapa Territory, Brazil. **122:**531-535.
58. **Milazzo ML, Cajimat MN, Haynie ML, Abbott KD, Bradley RD, Fulhorst CF.** 2008. Diversity among tacaribe serocomplex viruses (family Arenaviridae) naturally associated with the white-throated woodrat (Neotoma albigula) in the southwestern United States. Vector Borne Zoonotic Dis **8:**523-540.
59. **Cajimat MN, Milazzo ML, Bradley RD, Fulhorst CF.** 2007. Catarina virus, an arenaviral species principally associated with Neotoma micropus (southern plains woodrat) in Texas. Am J Trop Med Hyg **77:**732-736.
60. **Cajimat MN, Milazzo ML, Borchert JN, Abbott KD, Bradley RD, Fulhorst CF.** 2008. Diversity among Tacaribe serocomplex viruses (family Arenaviridae) naturally associated with the Mexican woodrat (Neotoma mexicana). Virus Res **133:**211-217.
61. **Coulibaly-N'Golo D, Allali B, Kouassi SK, Fichet-Calvet E, Becker-Ziaja B, Rieger T, Olschlager S, Dosso H, Denys C, Ter Meulen J, Akoua-Koffi C, Gunther S.** 2011. Novel arenavirus sequences in Hylomyscus sp. and Mus (Nannomys) setulosus from Cote d'Ivoire: implications for evolution of arenaviruses in Africa. PLoS One **6:**e20893.
62. **Kronmann KC, Nimo-Paintsil S, Guirguis F, Kronmann LC, Bonney K, Obiri-Danso K, Ampofo W, Fichet-Calvet E.** 2013. Two novel arenaviruses detected in pygmy mice, Ghana. Emerg Infect Dis **19:**1832-1835.
63. **Lecompte E, ter Meulen J, Emonet S, Daffis S, Charrel RN.** 2007. Genetic identification of Kodoko virus, a novel arenavirus of the African pigmy mouse (Mus Nannomys minutoides) in West Africa. Virology **364:**178-183.
64. **Inizan CC, Cajimat MN, Milazzo ML, Barragan-Gomez A, Bradley RD, Fulhorst CF.** 2010. Genetic evidence for a tacaribe serocomplex virus, Mexico. Emerg Infect Dis **16:**1007-1010.
65. **Cajimat MN, Milazzo ML, Bradley RD, Fulhorst CF.** 2012. Ocozocoautla de espinosa virus and hemorrhagic fever, Mexico. Emerg Infect Dis **18:**401-405.
66. **Fernandes J, Guterres A, de Oliveira RC, Jardim R, Davila AMR, Hewson R, de Lemos ERS.** 2019. Apore virus, a novel mammarenavirus (Bunyavirales: Arenaviridae) related to highly pathogenic virus from South America. Mem Inst Oswaldo Cruz **114:**e180586.
67. **Carlisle-Nowak MS, Sullivan N, Carrigan M, Knight C, Ryan C, Jacobson ER.** 1998. Inclusion body disease in two captive Australian pythons (Morelia spilota variegata and Morelia spilota spilota). Aust Vet J **76:**98-100.
68. **Chang L-W, Jacobson ER.** 2010. Inclusion Body Disease, A Worldwide Infectious Disease of Boid Snakes: A Review. Journal of Exotic Pet Medicine **19:**216-225.
69. **Aqrawi T, Stohr AC, Knauf-Witzens T, Krengel A, Heckers KO, Marschang RE.** 2015. Identification of snake arenaviruses in live boas and pythons in a zoo in Germany. Tierarztl Prax Ausg K Kleintiere Heimtiere **43:**239-247.
70. **Chang L, Fu D, Stenglein MD, Hernandez JA, DeRisi JL, Jacobson ER.** 2016. Detection and prevalence of boid inclusion body disease in collections of boas and pythons using immunological assays. Vet J **218:**13-18.
71. **Hyndman TH, Marschang RE, Bruce M, Clark P, Vitali SD.** 2019. Reptarenaviruses in apparently healthy snakes in an Australian zoological collection. Aust Vet J **97:**93-102.

72. **Argenta FF, Hepojoki J, Smura T, Szirovicza L, Hammerschmitt ME, Driemeier D, Kipar A, Hetzel U.** 2020. Identification of Reptarenaviruses, Hartmaniviruses, and a Novel Chuvirus in Captive Native Brazilian Boa Constrictors with Boid Inclusion Body Disease. J Virol **94**.
73. **Stenglein MD, Jacobson ER, Chang LW, Sanders C, Hawkins MG, Guzman DS, Drazenovich T, Dunker F, Kamaka EK, Fisher D, Reavill DR, Meola LF, Levens G, DeRisi JL.** 2015. Widespread recombination, reassortment, and transmission of unbalanced compound viral genotypes in natural arenavirus infections. PLoS Pathog **11:**e1004900.
74. **Hepojoki J, Salmenpera P, Sironen T, Hetzel U, Korzyukov Y, Kipar A, Vapalahti O.** 2015. Arenavirus Coinfections Are Common in Snakes with Boid Inclusion Body Disease. J Virol **89:**8657-8660.
75. **Abba Y, Hassim H, Hamzah H, Ibrahim OE, Ilyasu Y, Bande F, Mohd Lila MA, Noordin MM.** 2016. In vitro isolation and molecular identification of reptarenavirus in Malaysia. Virus Genes **52:**640-650.
76. **Hepojoki J, Hepojoki S, Smura T, Szirovicza L, Dervas E, Prahauser B, Nufer L, Schraner EM, Vapalahti O, Kipar A, Hetzel U.** 2018. Characterization of Haartman Institute snake virus-1 (HISV-1) and HISV-like viruses-The representatives of genus Hartmanivirus, family Arenaviridae. PLoS Pathog **14:**e1007415.
77. **Young PR, Chanas AC, Lee SR, Gould EA, Howard CR.** 1987. Localization of an arenavirus protein in the nuclei of infected cells. J Gen Virol **68 ( Pt 9):**2465-2470.
78. **Neuman BW, Adair BD, Burns JW, Milligan RA, Buchmeier MJ, Yeager M.** 2005. Complementarity in the supramolecular design of arenaviruses and retroviruses revealed by electron cryomicroscopy and image analysis. J Virol **79:**3822-3830.
79. **Schlie K, Maisa A, Freiberg F, Groseth A, Strecker T, Garten W.** 2010. Viral protein determinants of Lassa virus entry and release from polarized epithelial cells. J Virol **84:**3178-3188.
80. **Schlie K, Maisa A, Lennartz F, Stroher U, Garten W, Strecker T.** 2010. Characterization of Lassa virus glycoprotein oligomerization and influence of cholesterol on virus replication. J Virol **84:**983-992.
81. **Salas R, de Manzione N, Tesh RB, Rico-Hesse R, Shope RE, Betancourt A, Godoy O, Bruzual R, Pacheco ME, Ramos B, et al.** 1991. Venezuelan haemorrhagic fever. Lancet **338:**1033-1036.
82. **Rawls WE, Buchmeier M.** 1975. Arenaviruses: purification and physicochemical nature. Bull World Health Organ **52:**393-401.
83. **Pfau CJ.** 1965. Biophysical and Biochemical Characterization of Lymphocytic Choriomeningitis Virus. 2. Partial Purification by Differential Centrifugation and Fluorocarbon Techniques. Acta Pathol Microbiol Scand **63:**198-205.
84. **Podoplekina LE, Chyorny NB, Fyodorov Yu V.** 1986. Sensitivity of lymphocytic choriomeningitis and Tacaribe viruses to several physical factors. Virologie **37:**37-42.
85. **Mitchell SW, McCormick JB.** 1984. Physicochemical inactivation of Lassa, Ebola, and Marburg viruses and effect on clinical laboratory analyses. J Clin Microbiol **20:**486-489.
86. **Sagripanti JL, Lytle CD.** 2011. Sensitivity to ultraviolet radiation of Lassa, vaccinia, and Ebola viruses dried on surfaces. Arch Virol **156:**489-494.
87. **Elliott LH, McCormick JB, Johnson KM.** 1982. Inactivation of Lassa, Marburg, and Ebola viruses by gamma irradiation. J Clin Microbiol **16:**704-708.
88. **Sepulveda CS, Garcia CC, Damonte EB.** 2010. Inhibition of arenavirus infection by thiuram and aromatic disulfides. Antiviral Res **87:**329-337.

89. **Li S, Sun Z, Pryce R, Parsy ML, Fehling SK, Schlie K, Siebert CA, Garten W, Bowden TA, Strecker T, Huiskonen JT.** 2016. Acidic pH-Induced Conformations and LAMP1 Binding of the Lassa Virus Glycoprotein Spike. PLoS Pathog **12:**e1005418.
90. **Salvato MS, Shimomaye EM.** 1989. The completed sequence of lymphocytic choriomeningitis virus reveals a unique RNA structure and a gene for a zinc finger protein. Virology **173:**1-10.
91. **Auperin DD, Romanowski V, Galinski M, Bishop DH.** 1984. Sequencing studies of pichinde arenavirus S RNA indicate a novel coding strategy, an ambisense viral S RNA. J Virol **52:**897-904.
92. **Salvato M, Shimomaye E, Southern P, Oldstone MB.** 1988. Virus-lymphocyte interactions. IV. Molecular characterization of LCMV Armstrong (CTL+) small genomic segment and that of its variant, Clone 13 (CTL-). Virology **164:**517-522.
93. **Beyer WR, Popplau D, Garten W, von Laer D, Lenz O.** 2003. Endoproteolytic processing of the lymphocytic choriomeningitis virus glycoprotein by the subtilase SKI-1/S1P. J Virol **77:**2866-2872.
94. **Hallam SJ, Koma T, Maruyama J, Paessler S.** 2018. Review of Mammarenavirus Biology and Replication. Front Microbiol **9:**1751.
95. **Albarino CG, Bird BH, Chakrabarti AK, Dodd KA, Erickson BR, Nichol ST.** 2011. Efficient rescue of recombinant Lassa virus reveals the influence of S segment noncoding regions on virus replication and virulence. J Virol **85:**4020-4024.
96. **Auperin DD, Compans RW, Bishop DH.** 1982. Nucleotide sequence conservation at the 3' termini of the virion RNA species of New World and Old World arenaviruses. Virology **121:**200-203.
97. **Howard CR, Young PR.** 1984. Variation among New and Old World arenaviruses. Trans R Soc Trop Med Hyg **78:**299-306.
98. **Salvato M, Shimomaye E, Oldstone MB.** 1989. The primary structure of the lymphocytic choriomeningitis virus L gene encodes a putative RNA polymerase. Virology **169:**377-384.
99. **Pinschewer DD, Perez M, de la Torre JC.** 2005. Dual role of the lymphocytic choriomeningitis virus intergenic region in transcription termination and virus propagation. J Virol **79:**4519-4526.
100. **Archer AM, Rico-Hesse R.** 2002. High genetic divergence and recombination in Arenaviruses from the Americas. Virology **304:**274-281.
101. **Jay MT, Glaser C, Fulhorst CF.** 2005. The arenaviruses. J Am Vet Med Assoc **227:**904-915.
102. **Emonet S, Lemasson JJ, Gonzalez JP, de Lamballerie X, Charrel RN.** 2006. Phylogeny and evolution of old world arenaviruses. Virology **350:**251-257.
103. **Pinschewer DD, Perez M, de la Torre JC.** 2003. Role of the virus nucleoprotein in the regulation of lymphocytic choriomeningitis virus transcription and RNA replication. J Virol **77:**3882-3887.
104. **Qi X, Lan S, Wang W, Schelde LM, Dong H, Wallat GD, Ly H, Liang Y, Dong C.** 2010. Cap binding and immune evasion revealed by Lassa nucleoprotein structure. Nature **468:**779-783.
105. **Buchmeier MJ, Elder JH, Oldstone MB.** 1978. Protein structure of lymphocytic choriomeningitis virus: identification of the virus structural and cell associated polypeptides. Virology **89:**133-145.

106. **Hastie KM, Kimberlin CR, Zandonatti MA, MacRae IJ, Saphire EO.** 2011. Structure of the Lassa virus nucleoprotein reveals a dsRNA-specific 3' to 5' exonuclease activity essential for immune suppression. Proc Natl Acad Sci U S A **108:**2396-2401.
107. **Pythoud C, Rodrigo WW, Pasqual G, Rothenberger S, Martinez-Sobrido L, de la Torre JC, Kunz S.** 2012. Arenavirus nucleoprotein targets interferon regulatory factor-activating kinase IKKepsilon. J Virol **86:**7728-7738.
108. **Zhang Y, Li L, Liu X, Dong S, Wang W, Huo T, Guo Y, Rao Z, Yang C.** 2013. Crystal structure of Junin virus nucleoprotein. J Gen Virol **94:**2175-2183.
109. **Iwasaki M, Ngo N, Cubitt B, de la Torre JC.** 2015. Efficient Interaction between Arenavirus Nucleoprotein (NP) and RNA-Dependent RNA Polymerase (L) Is Mediated by the Virus Nucleocapsid (NP-RNA) Template. J Virol **89:**5734-5738.
110. **Lee KJ, Novella IS, Teng MN, Oldstone MB, de La Torre JC.** 2000. NP and L proteins of lymphocytic choriomeningitis virus (LCMV) are sufficient for efficient transcription and replication of LCMV genomic RNA analogs. J Virol **74:**3470-3477.
111. **Martinez-Sobrido L, Zuniga EI, Rosario D, Garcia-Sastre A, de la Torre JC.** 2006. Inhibition of the type I interferon response by the nucleoprotein of the prototypic arenavirus lymphocytic choriomeningitis virus. J Virol **80:**9192-9199.
112. **West BR, Hastie KM, Saphire EO.** 2014. Structure of the LCMV nucleoprotein provides a template for understanding arenavirus replication and immunosuppression. Acta crystallographica. Section D, Biological crystallography **70:**1764-1769.
113. **Jiang X, Huang Q, Wang W, Dong H, Ly H, Liang Y, Dong C.** 2013. Structures of arenaviral nucleoproteins with triphosphate dsRNA reveal a unique mechanism of immune suppression. J Biol Chem **288:**16949-16959.
114. **Jacobs BL, Langland JO.** 1996. When two strands are better than one: the mediators and modulators of the cellular responses to double-stranded RNA. Virology **219:**339-349.
115. **Rodrigo WW, Ortiz-Riano E, Pythoud C, Kunz S, de la Torre JC, Martinez-Sobrido L.** 2012. Arenavirus nucleoproteins prevent activation of nuclear factor kappa B. J Virol **86:**8185-8197.
116. **Volpon L, Osborne MJ, Borden KL.** 2008. NMR assignment of the arenaviral protein Z from Lassa fever virus. Biomol NMR Assign **2:**81-84.
117. **Volpon L, Osborne MJ, Capul AA, de la Torre JC, Borden KL.** 2010. Structural characterization of the Z RING-eIF4E complex reveals a distinct mode of control for eIF4E. Proc Natl Acad Sci U S A **107:**5441-5446.
118. **Wang J, Danzy S, Kumar N, Ly H, Liang Y.** 2012. Biological roles and functional mechanisms of arenavirus Z protein in viral replication. J Virol **86:**9794-9801.
119. **Fehling SK, Lennartz F, Strecker T.** 2012. Multifunctional nature of the arenavirus RING finger protein Z. Viruses **4:**2973-3011.
120. **Salvato MS, Schweighofer KJ, Burns J, Shimomaye EM.** 1992. Biochemical and immunological evidence that the 11 kDa zinc-binding protein of lymphocytic choriomeningitis virus is a structural component of the virus. Virus Res **22:**185-198.
121. **Perez M, Craven RC, de la Torre JC.** 2003. The small RING finger protein Z drives arenavirus budding: implications for antiviral strategies. Proc Natl Acad Sci U S A **100:**12978-12983.
122. **Strecker T, Eichler R, Meulen J, Weissenhorn W, Dieter Klenk H, Garten W, Lenz O.** 2003. Lassa virus Z protein is a matrix protein and sufficient for the release of virus-like particles [corrected]. J Virol **77:**10700-10705.

123. **Gibadulinova A, Zelnik V, Reiserova L, Zavodska E, Zatovicova M, Ciampor F, Pastorekova S, Pastorek J.** 1998. Sequence and characterisation of the Z gene encoding ring finger protein of the lymphocytic choriomeningitis virus MX strain. Acta Virol **42:**369-374.
124. **Kranzusch PJ, Whelan SP.** 2011. Arenavirus Z protein controls viral RNA synthesis by locking a polymerase-promoter complex. Proc Natl Acad Sci U S A **108:**19743-19748.
125. **Regad T, Chelbi-Alix MK.** 2001. Role and fate of PML nuclear bodies in response to interferon and viral infections. Oncogene **20:**7274-7286.
126. **Regad T, Saib A, Lallemand-Breitenbach V, Pandolfi PP, de The H, Chelbi-Alix MK.** 2001. PML mediates the interferon-induced antiviral state against a complex retrovirus via its association with the viral transactivator. EMBO J **20:**3495-3505.
127. **Borden KL, Campbelldwyer EJ, Carlile GW, Djavani M, Salvato MS.** 1998. Two RING finger proteins, the oncoprotein PML and the arenavirus Z protein, colocalize with the nuclear fraction of the ribosomal P proteins. J Virol **72:**3819-3826.
128. **Djavani M, Topisirovic I, Zapata JC, Sadowska M, Yang Y, Rodas J, Lukashevich IS, Bogue CW, Pauza CD, Borden KL, Salvato MS.** 2005. The proline-rich homeodomain (PRH/HEX) protein is down-regulated in liver during infection with lymphocytic choriomeningitis virus. J Virol **79:**2461-2473.
129. **Fan L, Briese T, Lipkin WI.** 2010. Z proteins of New World arenaviruses bind RIG-I and interfere with type I interferon induction. J Virol **84:**1785-1791.
130. **Morin B, Coutard B, Lelke M, Ferron F, Kerber R, Jamal S, Frangeul A, Baronti C, Charrel R, de Lamballerie X, Vonrhein C, Lescar J, Bricogne G, Gunther S, Canard B.** 2010. The N-terminal domain of the arenavirus L protein is an RNA endonuclease essential in mRNA transcription. PLoS Pathog **6:**e1001038.
131. **Pyle JD, Whelan SPJ.** 2019. RNA ligands activate the Machupo virus polymerase and guide promoter usage. Proc Natl Acad Sci U S A **116:**10518-10524.
132. **Kranzusch PJ, Schenk AD, Rahmeh AA, Radoshitzky SR, Bavari S, Walz T, Whelan SP.** 2010. Assembly of a functional Machupo virus polymerase complex. Proc Natl Acad Sci U S A **107:**20069-20074.
133. **Lan S, McLay L, Aronson J, Ly H, Liang Y.** 2008. Genome comparison of virulent and avirulent strains of the Pichinde arenavirus. Arch Virol **153:**1241-1250.
134. **Poch O, Blumberg BM, Bougueleret L, Tordo N.** 1990. Sequence comparison of five polymerases (L proteins) of unsegmented negative-strand RNA viruses: theoretical assignment of functional domains. J Gen Virol **71 ( Pt 5):**1153-1162.
135. **Chandrika R, Horikami SM, Smallwood S, Moyer SA.** 1995. Mutations in conserved domain I of the Sendai virus L polymerase protein uncouple transcription and replication. Virology **213:**352-363.
136. **Jin H, Elliott RM.** 1992. Mutagenesis of the L protein encoded by Bunyamwera virus and production of monospecific antibodies. J Gen Virol **73 ( Pt 9):**2235-2244.
137. **Sleat DE, Banerjee AK.** 1993. Transcriptional activity and mutational analysis of recombinant vesicular stomatitis virus RNA polymerase. J Virol **67:**1334-1339.
138. **Wang X, Gillam S.** 2001. Mutations in the GDD motif of rubella virus putative RNA-dependent RNA polymerase affect virus replication. Virology **285:**322-331.
139. **Smith AE, Helenius A.** 2004. How viruses enter animal cells. Science **304:**237-242.
140. **Marsh M, Helenius A.** 2006. Virus entry: open sesame. Cell **124:**729-740.
141. **Yamauchi Y, Helenius A.** 2013. Virus entry at a glance. J Cell Sci **126:**1289-1295.

142. **Helenius A.** 2018. Virus Entry: Looking Back and Moving Forward. J Mol Biol **430:**1853-1862.
143. **Rojek JM, Kunz S.** 2008. Cell entry by human pathogenic arenaviruses. Cell Microbiol **10:**828-835.
144. **de la Torre JC.** 2009. Molecular and cell biology of the prototypic arenavirus LCMV: implications for understanding and combating hemorrhagic fever arenaviruses. Ann N Y Acad Sci **1171 Suppl 1:**E57-64.
145. **Lenz O, ter Meulen J, Klenk HD, Seidah NG, Garten W.** 2001. The Lassa virus glycoprotein precursor GP-C is proteolytically processed by subtilase SKI-1/S1P. Proc Natl Acad Sci U S A **98:**12701-12705.
146. **Rojek JM, Lee AM, Nguyen N, Spiropoulou CF, Kunz S.** 2008. Site 1 protease is required for proteolytic processing of the glycoproteins of the South American hemorrhagic fever viruses Junin, Machupo, and Guanarito. J Virol **82:**6045-6051.
147. **Burri DJ, Pasqual G, Rochat C, Seidah NG, Pasquato A, Kunz S.** 2012. Molecular characterization of the processing of arenavirus envelope glycoprotein precursors by subtilisin kexin isozyme-1/site-1 protease. J Virol **86:**4935-4946.
148. **Burri DJ, Pasquato A, da Palma JR, Igonet S, Oldstone MB, Kunz S.** 2013. The role of proteolytic processing and the stable signal peptide in expression of the Old World arenavirus envelope glycoprotein ectodomain. Virology **436:**127-133.
149. **Burri DJ, da Palma JR, Kunz S, Pasquato A.** 2012. Envelope glycoprotein of arenaviruses. Viruses **4:**2162-2181.
150. **Kunz S, Edelmann KH, de la Torre JC, Gorney R, Oldstone MB.** 2003. Mechanisms for lymphocytic choriomeningitis virus glycoprotein cleavage, transport, and incorporation into virions. Virology **314:**168-178.
151. **von Heijne G.** 1985. Signal sequences. The limits of variation. J Mol Biol **184:**99-105.
152. **Froeschke M, Basler M, Groettrup M, Dobberstein B.** 2003. Long-lived signal peptide of lymphocytic choriomeningitis virus glycoprotein pGP-C. J Biol Chem **278:**41914-41920.
153. **Garry CE, Garry RF.** 2019. Proteomics Computational Analyses Suggest that the Antennavirus Glycoprotein Complex Includes a Class I Viral Fusion Protein (alpha-Penetrene) with an Internal Zinc-Binding Domain and a Stable Signal Peptide. Viruses **11**.
154. **Parekh BS, Buchmeier MJ.** 1986. Proteins of lymphocytic choriomeningitis virus: antigenic topography of the viral glycoproteins. Virology **153:**168-178.
155. **Radoshitzky SR, Abraham J, Spiropoulou CF, Kuhn JH, Nguyen D, Li W, Nagel J, Schmidt PJ, Nunberg JH, Andrews NC, Farzan M, Choe H.** 2007. Transferrin receptor 1 is a cellular receptor for New World haemorrhagic fever arenaviruses. Nature **446:**92-96.
156. **Radoshitzky SR, Kuhn JH, Spiropoulou CF, Albarino CG, Nguyen DP, Salazar-Bravo J, Dorfman T, Lee AS, Wang E, Ross SR, Choe H, Farzan M.** 2008. Receptor determinants of zoonotic transmission of New World hemorrhagic fever arenaviruses. Proc Natl Acad Sci U S A **105:**2664-2669.
157. **York J, Agnihothram SS, Romanowski V, Nunberg JH.** 2005. Genetic analysis of heptad-repeat regions in the G2 fusion subunit of the Junin arenavirus envelope glycoprotein. Virology **343:**267-274.
158. **Di Simone C, Zandonatti MA, Buchmeier MJ.** 1994. Acidic pH triggers LCMV membrane fusion activity and conformational change in the glycoprotein spike. Virology **198:**455-465.

159. **Di Simone C, Buchmeier MJ.** 1995. Kinetics and pH dependence of acid-induced structural changes in the lymphocytic choriomeningitis virus glycoprotein complex. Virology **209:**3-9.
160. **Castilla V, Mersich SE.** 1996. Low-pH-induced fusion of Vero cells infected with Junin virus. Arch Virol **141:**1307-1317.
161. **Eichler R, Lenz O, Strecker T, Eickmann M, Klenk HD, Garten W.** 2003. Identification of Lassa virus glycoprotein signal peptide as a trans-acting maturation factor. EMBO Rep **4:**1084-1088.
162. **York J, Nunberg JH.** 2006. Role of the stable signal peptide of Junin arenavirus envelope glycoprotein in pH-dependent membrane fusion. J Virol **80:**7775-7780.
163. **York J, Nunberg JH.** 2007. Distinct requirements for signal peptidase processing and function in the stable signal peptide subunit of the Junin virus envelope glycoprotein. Virology **359:**72-81.
164. **York J, Nunberg JH.** 2009. Intersubunit interactions modulate pH-induced activation of membrane fusion by the Junin virus envelope glycoprotein GPC. J Virol **83:**4121-4126.
165. **Bonhomme CJ, Capul AA, Lauron EJ, Bederka LH, Knopp KA, Buchmeier MJ.** 2011. Glycosylation modulates arenavirus glycoprotein expression and function. Virology **409:**223-233.
166. **Perez M, Greenwald DL, de la Torre JC.** 2004. Myristoylation of the RING finger Z protein is essential for arenavirus budding. J Virol **78:**11443-11448.
167. **Bederka LH, Bonhomme CJ, Ling EL, Buchmeier MJ.** 2014. Arenavirus stable signal peptide is the keystone subunit for glycoprotein complex organization. mBio **5:**e02063.
168. **Cao W, Henry MD, Borrow P, Yamada H, Elder JH, Ravkov EV, Nichol ST, Compans RW, Campbell KP, Oldstone MB.** 1998. Identification of alpha-dystroglycan as a receptor for lymphocytic choriomeningitis virus and Lassa fever virus. Science **282:**2079-2081.
169. **Raaben M, Jae LT, Herbert AS, Kuehne AI, Stubbs SH, Chou YY, Blomen VA, Kirchhausen T, Dye JM, Brummelkamp TR, Whelan SP.** 2017. NRP2 and CD63 Are Host Factors for Lujo Virus Cell Entry. Cell Host Microbe **22:**688-696 e685.
170. **Spiropoulou CF, Kunz S, Rollin PE, Campbell KP, Oldstone MB.** 2002. New World arenavirus clade C, but not clade A and B viruses, utilizes alpha-dystroglycan as its major receptor. J Virol **76:**5140-5146.
171. **Kunz S, Borrow P, Oldstone MB.** 2002. Receptor structure, binding, and cell entry of arenaviruses. Curr Top Microbiol Immunol **262:**111-137.
172. **Pasqual G, Rojek JM, Masin M, Chatton JY, Kunz S.** 2011. Old world arenaviruses enter the host cell via the multivesicular body and depend on the endosomal sorting complex required for transport. PLoS Pathog **7:**e1002232.
173. **Eschli B, Quirin K, Wepf A, Weber J, Zinkernagel R, Hengartner H.** 2006. Identification of an N-terminal trimeric coiled-coil core within arenavirus glycoprotein 2 permits assignment to class I viral fusion proteins. J Virol **80:**5897-5907.
174. **Henne WM, Buchkovich NJ, Emr SD.** 2011. The ESCRT pathway. Developmental cell **21:**77-91.
175. **Quirin K, Eschli B, Scheu I, Poort L, Kartenbeck J, Helenius A.** 2008. Lymphocytic choriomeningitis virus uses a novel endocytic pathway for infectious entry via late endosomes. Virology **378:**21-33.
176. **Urata S, Yasuda J.** 2012. Molecular mechanism of arenavirus assembly and budding. Viruses **4:**2049-2079.

177. **Lopez N, Jacamo R, Franze-Fernandez MT.** 2001. Transcription and RNA replication of tacaribe virus genome and antigenome analogs require N and L proteins: Z protein is an inhibitor of these processes. J Virol **75:**12241-12251.
178. **Garcin D, Kolakofsky D.** 1990. A novel mechanism for the initiation of Tacaribe arenavirus genome replication. J Virol **64:**6196-6203.
179. **Iapalucci S, Lopez N, Franze-Fernandez MT.** 1991. The 3' end termini of the Tacaribe arenavirus subgenomic RNAs. Virology **182:**269-278.
180. **Decroly E, Ferron F, Lescar J, Canard B.** 2011. Conventional and unconventional mechanisms for capping viral mRNA. Nat Rev Microbiol **10:**51-65.
181. **Reich S, Guilligay D, Pflug A, Malet H, Berger I, Crepin T, Hart D, Lunardi T, Nanao M, Ruigrok RW, Cusack S.** 2014. Structural insight into cap-snatching and RNA synthesis by influenza polymerase. Nature **516:**361-366.
182. **Raju R, Raju L, Hacker D, Garcin D, Compans R, Kolakofsky D.** 1990. Nontemplated bases at the 5' ends of Tacaribe virus mRNAs. Virology **174:**53-59.
183. **Polyak SJ, Zheng S, Harnish DG.** 1995. 5' termini of Pichinde arenavirus S RNAs and mRNAs contain nontemplated nucleotides. J Virol **69:**3211-3215.
184. **Casabona JC, Levingston Macleod JM, Loureiro ME, Gomez GA, Lopez N.** 2009. The RING domain and the L79 residue of Z protein are involved in both the rescue of nucleocapsids and the incorporation of glycoproteins into infectious chimeric arenavirus-like particles. J Virol **83:**7029-7039.
185. **Shtanko O, Imai M, Goto H, Lukashevich IS, Neumann G, Watanabe T, Kawaoka Y.** 2010. A role for the C terminus of Mopeia virus nucleoprotein in its incorporation into Z protein-induced virus-like particles. J Virol **84:**5415-5422.
186. **Capul AA, Perez M, Burke E, Kunz S, Buchmeier MJ, de la Torre JC.** 2007. Arenavirus Z-glycoprotein association requires Z myristoylation but not functional RING or late domains. J Virol **81:**9451-9460.
187. **Urata S, Weyer J, Storm N, Miyazaki Y, van Vuren PJ, Paweska JT, Yasuda J.** 2015. Analysis of Assembly and Budding of Lujo Virus. J Virol **90:**3257-3261.
188. **Gorelick RJ, Henderson LE, Hanser JP, Rein A.** 1988. Point mutants of Moloney murine leukemia virus that fail to package viral RNA: evidence for specific RNA recognition by a "zinc finger-like" protein sequence. Proc Natl Acad Sci U S A **85:**8420-8424.
189. **Agnihothram SS, York J, Trahey M, Nunberg JH.** 2007. Bitopic membrane topology of the stable signal peptide in the tripartite Junin virus GP-C envelope glycoprotein complex. J Virol **81:**4331-4337.
190. **Votteler J, Sundquist WI.** 2013. Virus budding and the ESCRT pathway. Cell Host Microbe **14:**232-241.
191. **Fazakerley JK, Southern P, Bloom F, Buchmeier MJ.** 1991. High resolution in situ hybridization to determine the cellular distribution of lymphocytic choriomeningitis virus RNA in the tissues of persistently infected mice: relevance to arenavirus disease and mechanisms of viral persistence. J Gen Virol **72 ( Pt 7):**1611-1625.
192. **Borrow P, Martinez-Sobrido L, de la Torre JC.** 2010. Inhibition of the type I interferon antiviral response during arenavirus infection. Viruses **2:**2443-2480.
193. **Merigan TC, Oldstone MB, Welsh RM.** 1977. Interferon production during lymphocytic choriomeningitis virus infection of nude and normal mice. Nature **268:**67-68.
194. **Wilson EB, Yamada DH, Elsaesser H, Herskovitz J, Deng J, Cheng G, Aronow BJ, Karp CL, Brooks DG.** 2013. Blockade of chronic type I interferon signaling to control persistent LCMV infection. Science **340:**202-207.

195. **Lehmann-Grube F.** 1984. Portraits of viruses: arenaviruses. Intervirology **22:**121-145.
196. **Hoey J.** 2005. Lymphocytic choriomeningitis virus. CMAJ **173:**1033.
197. **Bonthius DJ.** 2009. Lymphocytic choriomeningitis virus: a prenatal and postnatal threat. Adv Pediatr **56:**75-86.
198. **Bonthius DJ.** 2012. Lymphocytic choriomeningitis virus: an underrecognized cause of neurologic disease in the fetus, child, and adult. Semin Pediatr Neurol **19:**89-95.
199. **Asnis DS, Muana O, Kim DG, Garcia M, Rollin PE, Slavinski S.** 2010. Lymphocytic choriomeningitis virus meningitis, New York, NY, USA, 2009. Emerg Infect Dis **16:**328-330.
200. **Barton LL, Hyndman NJ.** 2000. Lymphocytic choriomeningitis virus: reemerging central nervous system pathogen. Pediatrics **105:**E35.
201. **Bonthius DJ, Karacay B.** 2002. Meningitis and encephalitis in children. An update. Neurol Clin **20:**1013-1038, vi-vii.
202. **Meyer HM, Jr., Johnson RT, Crawford IP, Dascomb HE, Rogers NG.** 1960. Central nervous system syndromes of "vital" etiology. A study of 713 cases. Am J Med **29:**334-347.
203. **Stone GS, Glover M, Jilg N, Sfeir MM.** 2019. Case 40-2019: A 26-Year-Old Returning Traveler with Headache. N Engl J Med **381:**2553-2560.
204. **Delaine M, Weingertner AS, Nougairede A, Lepiller Q, Fafi-Kremer S, Favre R, Charrel R.** 2017. Microcephaly Caused by Lymphocytic Choriomeningitis Virus. Emerg Infect Dis **23:**1548-1550.
205. **Barton LL, Mets MB, Beauchamp CL.** 2002. Lymphocytic choriomeningitis virus: emerging fetal teratogen. Am J Obstet Gynecol **187:**1715-1716.
206. **Barton LL, Peters CJ, Ksiazek TG.** 1995. Lymphocytic choriomeningitis virus: an unrecognized teratogenic pathogen. Emerg Infect Dis **1:**152-153.
207. **Wright R, Johnson D, Neumann M, Ksiazek TG, Rollin P, Keech RV, Bonthius DJ, Hitchon P, Grose CF, Bell WE, Bale JF, Jr.** 1997. Congenital lymphocytic choriomeningitis virus syndrome: a disease that mimics congenital toxoplasmosis or Cytomegalovirus infection. Pediatrics **100:**E9.
208. **Peters CJ.** 2006. Lymphocytic choriomeningitis virus--an old enemy up to new tricks. N Engl J Med **354:**2208-2211.
209. **Fischer SA, Graham MB, Kuehnert MJ, Kotton CN, Srinivasan A, Marty FM, Comer JA, Guarner J, Paddock CD, DeMeo DL, Shieh WJ, Erickson BR, Bandy U, DeMaria A, Jr., Davis JP, Delmonico FL, Pavlin B, Likos A, Vincent MJ, Sealy TK, Goldsmith CS, Jernigan DB, Rollin PE, Packard MM, Patel M, Rowland C, Helfand RF, Nichol ST, Fishman JA, Ksiazek T, Zaki SR, Team LiTRI.** 2006. Transmission of lymphocytic choriomeningitis virus by organ transplantation. N Engl J Med **354:**2235-2249.
210. **Perez-Ruiz M, Navarro-Mari JM, Sanchez-Seco MP, Gegundez MI, Palacios G, Savji N, Lipkin WI, Fedele G, de Ory-Manchon F.** 2012. Lymphocytic choriomeningitis virus-associated meningitis, southern Spain. Emerg Infect Dis **18:**855-858.
211. **Danes L, Benda R, Fuchsova M.** 1963. [Experimental Inhalation Infection of Monkeys of the Macacus Cynomolgus and Macacus Rhesus Species with the Virus of Lymphocytic Choriomeningitis (We)]. Bratisl Lek Listy **2:**71-79.
212. **Roebroek RM, Postma BH, Dijkstra UJ.** 1994. Aseptic meningitis caused by the lymphocytic choriomeningitis virus. Clin Neurol Neurosurg **96:**178-180.
213. **Lewis JM, Utz JP.** 1961. Orchitis, parotitis and meningoencephalitis due to lymphocytic-choriomeningitis virus. N Engl J Med **265:**776-780.

214. **Richmond JK, Baglole DJ.** 2003. Lassa fever: epidemiology, clinical features, and social consequences. BMJ **327:**1271-1275.
215. **Cobo F.** 2016. Viruses Causing Hemorrhagic Fever. Safety Laboratory Procedures. Open Virol J **10:**1-9.
216. **Lukashevich IS, Maryankova R, Vladyko AS, Nashkevich N, Koleda S, Djavani M, Horejsh D, Voitenok NN, Salvato MS.** 1999. Lassa and Mopeia virus replication in human monocytes/macrophages and in endothelial cells: different effects on IL-8 and TNF-alpha gene expression. J Med Virol **59:**552-560.
217. **Baize S, Kaplon J, Faure C, Pannetier D, Georges-Courbot MC, Deubel V.** 2004. Lassa virus infection of human dendritic cells and macrophages is productive but fails to activate cells. J Immunol **172:**2861-2869.
218. **Edington GM, White HA.** 1972. The pathology of Lassa fever. Trans R Soc Trop Med Hyg **66:**381-389.
219. **McCormick JB, Fisher-Hoch SP.** 2002. Lassa fever. Curr Top Microbiol Immunol **262:**75-109.
220. **Ly H.** 2017. Differential Immune Responses to New World and Old World Mammalian Arenaviruses. Int J Mol Sci **18**.
221. **Peters CJ.** 2002. Human infection with arenaviruses in the Americas. Curr Top Microbiol Immunol **262:**65-74.
222. **Justines G, Johnson KM.** 1968. Use of oral swabs for detection of Machupo-virus infection in rodents. Am J Trop Med Hyg **17:**788-790.
223. **Skinner HH, Knight EH, Grove R.** 1977. Murine lymphocytic choriomeningitis: the history of a natural cross-infection from wild to laboratory mice. Lab Anim **11:**219-222.
224. **Gunther S, Lenz O.** 2004. Lassa virus. Crit Rev Clin Lab Sci **41:**339-390.
225. **Gomez RM, Jaquenod de Giusti C, Sanchez Vallduvi MM, Frik J, Ferrer MF, Schattner M.** 2011. Junin virus. A XXI century update. Microbes Infect **13:**303-311.
226. **Fisher-Hoch SP, Tomori O, Nasidi A, Perez-Oronoz GI, Fakile Y, Hutwagner L, McCormick JB.** 1995. Review of cases of nosocomial Lassa fever in Nigeria: the high price of poor medical practice. BMJ **311:**857-859.
227. **Salvato MS.** 1993. The Arenaviridae. Plenum Press, New York.
228. **Ledesma J, Fedele CG, Carro F, Lledo L, Sanchez-Seco MP, Tenorio A, Soriguer RC, Saz JV, Dominguez G, Rosas MF, Barandika JF, Gegundez MI.** 2009. Independent lineage of lymphocytic choriomeningitis virus in wood mice (Apodemus sylvaticus), Spain. Emerg Infect Dis **15:**1677-1680.
229. **Tagliapietra V, Rosa R, Hauffe HC, Laakkonen J, Voutilainen L, Vapalahti O, Vaheri A, Henttonen H, Rizzoli A.** 2009. Spatial and temporal dynamics of lymphocytic choriomeningitis virus in wild rodents, northern Italy. Emerg Infect Dis **15:**1019-1025.
230. **McLay L, Liang Y, Ly H.** 2014. Comparative analysis of disease pathogenesis and molecular mechanisms of New World and Old World arenavirus infections. J Gen Virol **95:**1-15.
231. **Franz DR, Jahrling PB, Friedlander AM, McClain DJ, Hoover DL, Bryne WR, Pavlin JA, Christopher GW, Eitzen EM, Jr.** 1997. Clinical recognition and management of patients exposed to biological warfare agents. JAMA **278:**399-411.
232. **Borio L, Inglesby T, Peters CJ, Schmaljohn AL, Hughes JM, Jahrling PB, Ksiazek T, Johnson KM, Meyerhoff A, O'Toole T, Ascher MS, Bartlett J, Breman JG, Eitzen EM, Jr., Hamburg M, Hauer J, Henderson DA, Johnson RT, Kwik G, Layton M, Lillibridge S, Nabel GJ, Osterholm MT, Perl TM, Russell P, Tonat K, Working Group on Civilian B.** 2002.

Hemorrhagic fever viruses as biological weapons: medical and public health management. JAMA **287:**2391-2405.

233. **Barrera Oro JG, McKee KT, Jr.** 1991. Toward a vaccine against Argentine hemorrhagic fever. Bull Pan Am Health Organ **25:**118-126.
234. **Ambrosio A, Saavedra M, Mariani M, Gamboa G, Maiza A.** 2011. Argentine hemorrhagic fever vaccines. Hum Vaccin **7:**694-700.
235. **McCormick JB, King IJ, Webb PA, Scribner CL, Craven RB, Johnson KM, Elliott LH, Belmont-Williams R.** 1986. Lassa fever. Effective therapy with ribavirin. N Engl J Med **314:**20-26.
236. **Enria DA, Ambrosio AM, Briggiler AM, Feuillade MR, Crivelli E, Study Group on Argentine Hemorrhagic Fever V.** 2010. [Candid#1 vaccine against Argentine hemorrhagic fever produced in Argentina. Immunogenicity and safety]. Medicina (B Aires) **70:**215-222.
237. **Schumacher J, Jacobson ER, Homer BL, Gaskin JM.** 1994. Inclusion Body Disease in Boid Snakes. Journal of Zoo and Wildlife Medicine **25:**511-524.
238. **Wozniak E, McBride J, DeNardo D, Tarara R, Wong V, Osburn B.** 2000. Isolation and characterization of an antigenically distinct 68-kd protein from nonviral intracytoplasmic inclusions in Boa constrictors chronically infected with the inclusion body disease virus (IBDV: Retroviridae). Vet Pathol **37:**449-459.
239. **Keller S, Hetzel U, Sironen T, Korzyukov Y, Vapalahti O, Kipar A, Hepojoki J.** 2017. Co-infecting Reptarenaviruses Can Be Vertically Transmitted in Boa Constrictor. PLoS Pathog **13:**e1006179.
240. **Schilliger L, Selleri P, Frye FL.** 2011. Lymphoblastic lymphoma and leukemic blood profile in a red-tail boa (Boa constrictor constrictor) with concurrent inclusion body disease. J Vet Diagn Invest **23:**159-162.
241. **Chang LW, Fu A, Wozniak E, Chow M, Duke DG, Green L, Kelley K, Hernandez JA, Jacobson ER.** 2013. Immunohistochemical detection of a unique protein within cells of snakes having inclusion body disease, a world-wide disease seen in members of the families Boidae and Pythonidae. PLoS One **8:**e82916.
242. **Stenglein MD, Sanchez-Migallon Guzman D, Garcia VE, Layton ML, Hoon-Hanks LL, Boback SM, Keel MK, Drazenovich T, Hawkins MG, DeRisi JL.** 2017. Differential Disease Susceptibilities in Experimentally Reptarenavirus-Infected Boa Constrictors and Ball Pythons. J Virol **91**.
243. **Vancraeynest D, Pasmans F, Martel A, Chiers K, Meulemans G, Mast J, Zwart P, Ducatelle R.** 2006. Inclusion body disease in snakes: a review and description of three cases in boa constrictors in Belgium. Vet Rec **158:**757-760.
244. **Baize S, Marianneau P, Loth P, Reynard S, Journeaux A, Chevallier M, Tordo N, Deubel V, Contamin H.** 2009. Early and strong immune responses are associated with control of viral replication and recovery in lassa virus-infected cynomolgus monkeys. J Virol **83:**5890-5903.
245. **Zinkernagel RM, Doherty PC.** 1974. Restriction of in vitro T cell-mediated cytotoxicity in lymphocytic choriomeningitis within a syngeneic or semiallogeneic system. Nature **248:**701-702.
246. **Zinkernagel RM, Doherty PC.** 1974. Immunological surveillance against altered self components by sensitised T lymphocytes in lymphocytic choriomeningitis. Nature **251:**547-548.

247. **Doherty PC, Zinkernagel RM.** 1975. H-2 compatibility is required for T-cell-mediated lysis of target cells infected with lymphocytic choriomeningitis virus. J Exp Med **141:**502-507.
248. **Johnson KM, McCormick JB, Webb PA, Smith ES, Elliott LH, King IJ.** 1987. Clinical virology of Lassa fever in hospitalized patients. J Infect Dis **155:**456-464.
249. **Branco LM, Grove JN, Boisen ML, Shaffer JG, Goba A, Fullah M, Momoh M, Grant DS, Garry RF.** 2011. Emerging trends in Lassa fever: redefining the role of immunoglobulin M and inflammation in diagnosing acute infection. Virol J **8:**478.
250. **Fisher-Hoch SP, Hutwagner L, Brown B, McCormick JB.** 2000. Effective vaccine for lassa fever. J Virol **74:**6777-6783.
251. **Pannetier D, Faure C, Georges-Courbot MC, Deubel V, Baize S.** 2004. Human macrophages, but not dendritic cells, are activated and produce alpha/beta interferons in response to Mopeia virus infection. J Virol **78:**10516-10524.
252. **Heller MV, Saavedra MC, Falcoff R, Maiztegui JI, Molinas FC.** 1992. Increased tumor necrosis factor-alpha levels in Argentine hemorrhagic fever. J Infect Dis **166:**1203-1204.
253. **Levis SC, Saavedra MC, Ceccoli C, Feuillade MR, Enria DA, Maiztegui JI, Falcoff R.** 1985. Correlation between endogenous interferon and the clinical evolution of patients with Argentine hemorrhagic fever. J Interferon Res **5:**383-389.
254. **Marta RF, Montero VS, Hack CE, Sturk A, Maiztegui JI, Molinas FC.** 1999. Proinflammatory cytokines and elastase-alpha-1-antitrypsin in Argentine hemorrhagic fever. Am J Trop Med Hyg **60:**85-89.
255. **Groseth A, Hoenen T, Weber M, Wolff S, Herwig A, Kaufmann A, Becker S.** 2011. Tacaribe virus but not junin virus infection induces cytokine release from primary human monocytes and macrophages. PLoS Negl Trop Dis **5:**e1137.
256. **Meyer B, Ly H.** 2016. Inhibition of Innate Immune Responses Is Key to Pathogenesis by Arenaviruses. J Virol **90:**3810-3818.
257. **Mantlo E, Paessler S, Huang C.** 2019. Differential Immune Responses to Hemorrhagic Fever-Causing Arenaviruses. Vaccines (Basel) **7**.
258. **Schroeder HW, Jr., Cavacini L.** 2010. Structure and function of immunoglobulins. The Journal of allergy and clinical immunology **125:**S41-52.
259. **Gambon-Deza F, Sanchez-Espinel C, Mirete-Bachiller S, Magadan-Mompo S.** 2012. Snakes antibodies. Dev Comp Immunol **38:**1-9.
260. **Litman GW, Anderson MK, Rast JP.** 1999. Evolution of antigen binding receptors. Annual review of immunology **17:**109-147.
261. **Zimmerman LM, Vogel LA, Bowden RM.** 2010. Understanding the vertebrate immune system: insights from the reptilian perspective. J Exp Biol **213:**661-671.
262. **Kluger MJ, Ringler DH, Anver MR.** 1975. Fever and survival. Science **188:**166-168.
263. **Grey HM.** 1963. Phylogeny of the Immune Response. Studies on Some Physical Chemical and Serologic Characteristics of Antibody Produced in the Turtle. J Immunol **91:**819-825.
264. **Marchalonis JJ, Ealey EH, Diener E.** 1969. Immune response of the Tuatara, Sphenodon punctatum. The Australian journal of experimental biology and medical science **47:**367-380.
265. **Ingram GA, Molyneux DH.** 1983. The humoral immune response of the spiny-tailed agamid lizard (Agama caudospinosum) to injection with Leishmania agamae promastigotes. Veterinary immunology and immunopathology **4:**479-491.

266. **Work TM, Balazs GH, Rameyer RA, Chang SP, Berestecky J.** 2000. Assessing humoral and cell-mediated immune response in Hawaiian green turtles, Chelonia mydas. Veterinary immunology and immunopathology **74:**179-194.
267. **Origgi FC, Klein PA, Mathes K, Blahak S, Marschang RE, Tucker SJ, Jacobson ER.** 2001. Enzyme-linked immunosorbent assay for detecting herpesvirus exposure in Mediterranean tortoises (spur-thighed tortoise [Testudo graeca] and Hermann's tortoise [Testudo hermanni]). J Clin Microbiol **39:**3156-3163.
268. **Windbichler K, Michalopoulou E, Palamides P, Pesch T, Jelinek C, Vapalahti O, Kipar A, Hetzel U, Hepojoki J.** 2019. Antibody response in snakes with boid inclusion body disease. PLoS One **14:**e0221863.
269. **Bausch DG, Rollin PE, Demby AH, Coulibaly M, Kanu J, Conteh AS, Wagoner KD, McMullan LK, Bowen MD, Peters CJ, Ksiazek TG.** 2000. Diagnosis and clinical virology of Lassa fever as evaluated by enzyme-linked immunosorbent assay, indirect fluorescent-antibody test, and virus isolation. J Clin Microbiol **38:**2670-2677.
270. **Emmerich P, Thome-Bolduan C, Drosten C, Gunther S, Ban E, Sawinsky I, Schmitz H.** 2006. Reverse ELISA for IgG and IgM antibodies to detect Lassa virus infections in Africa. J Clin Virol **37:**277-281.
271. **Blasdell KR, Becker SD, Hurst J, Begon M, Bennett M.** 2008. Host range and genetic diversity of arenaviruses in rodents, United Kingdom. Emerg Infect Dis **14:**1455-1458.
272. **Enders G, Varho-Gobel M, Lohler J, Terletskaia-Ladwig E, Eggers M.** 1999. Congenital lymphocytic choriomeningitis virus infection: an underdiagnosed disease. Pediatr Infect Dis J **18:**652-655.
273. **Cordey S, Sahli R, Moraz ML, Estrade C, Morandi L, Cherpillod P, Charrel RN, Kunz S, Kaiser L.** 2011. Analytical validation of a lymphocytic choriomeningitis virus real-time RT-PCR assay. J Virol Methods **177:**118-122.
274. **Lozano ME, Enria D, Maiztegui JI, Grau O, Romanowski V.** 1995. Rapid diagnosis of Argentine hemorrhagic fever by reverse transcriptase PCR-based assay. J Clin Microbiol **33:**1327-1332.
275. **Pfau CJ.** 1996. Arenaviruses. *In* th, Baron S (ed.), Medical Microbiology, Galveston (TX).
276. **Lukashevich IS, Pushko P.** 2016. Vaccine platforms to control Lassa fever. Expert Rev Vaccines **15:**1135-1150.
277. **Carrion R, Jr., Bredenbeek P, Jiang X, Tretyakova I, Pushko P, Lukashevich IS.** 2012. Vaccine Platforms to Control Arenaviral Hemorrhagic Fevers. J Vaccines Vaccin **3**.
278. **Enria DA, Barrera Oro JG.** 2002. Junin virus vaccines. Curr Top Microbiol Immunol **263:**239-261.
279. **Ortiz-Riano E, Cheng BY, Carlos de la Torre J, Martinez-Sobrido L.** 2013. Arenavirus reverse genetics for vaccine development. J Gen Virol **94:**1175-1188.
280. **Dervas E, Hepojoki J, Laimbacher A, Romero-Palomo F, Jelinek C, Keller S, Smura T, Hepojoki S, Kipar A, Hetzel U.** 2017. Nidovirus-Associated Proliferative Pneumonia in the Green Tree Python (Morelia viridis). J Virol **91**.
281. **Hetzel U, Szirovicza L, Smura T, Prahauser B, Vapalahti O, Kipar A, Hepojoki J.** 2019. Identification of a Novel Deltavirus in Boa Constrictors. MBio **10**.
282. **Bell-Sakyi L, Zweygarth E, Blouin EF, Gould EA, Jongejan F.** 2007. Tick cell lines: tools for tick and tick-borne disease research. Trends Parasitol **23:**450-457.
283. **Pedra JH, Narasimhan S, Rendic D, DePonte K, Bell-Sakyi L, Wilson IB, Fikrig E.** 2010. Fucosylation enhances colonization of ticks by Anaplasma phagocytophilum. Cell Microbiol **12:**1222-1234.

284. **Bell-Sakyi L.** 2004. Ehrlichia ruminantium grows in cell lines from four ixodid tick genera. J Comp Pathol **130:**285-293.
285. **Paneth Iheozor-Ejiofor R, Levanov L, Hepojoki J, Strandin T, Lundkvist A, Plyusnin A, Vapalahti O.** 2016. Vaccinia virus-free rescue of fluorescent replication-defective vesicular stomatitis virus and pseudotyping with Puumala virus glycoproteins for use in neutralization tests. J Gen Virol **97:**1052-1059.
286. **Katoh K, Standley DM.** 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol **30:**772-780.
287. **Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Hohna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP.** 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol **61:**539-542.
288. **Benedict FG, Fox EL.** 1931. Body Temperature and Heat Regulation of Large Snakes. Proceedings of the National Academy of Sciences **17:**584.
289. **Brattstrom BH.** 1965. Body Temperatures of Reptiles. The American Midland Naturalist **73:**376-422.
290. **Sayler KA, Barbet AF, Chamberlain C, Clapp WL, Alleman R, Loeb JC, Lednicky JA.** 2014. Isolation of Tacaribe virus, a Caribbean arenavirus, from host-seeking Amblyomma americanum ticks in Florida. PLoS One **9:**e115769.
291. **Rehacek J.** 1965. Cultivation of different viruses in tick tissue cultures. Acta Virol **9:**332-337.
292. **Whitney JF, Clark JM, Griffin TW, Gautam S, Leslie KO.** 1995. Transferrin receptor expression in nonsmall cell lung cancer. Histopathologic and clinical correlates. Cancer **76:**20-25.
293. **Prescott J, Hall P, Acuna-Retamar M, Ye C, Wathelet MG, Ebihara H, Feldmann H, Hjelle B.** 2010. New World hantaviruses activate IFNlambda production in type I IFN-deficient vero E6 cells. PLoS One **5:**e11159.
294. **Whitt MA.** 2010. Generation of VSV pseudotypes using recombinant DeltaG-VSV for studies on virus entry, identification of entry inhibitors, and immune responses to vaccines. J Virol Methods **169:**365-374.
295. **Grant A, Seregin A, Huang C, Kolokoltsova O, Brasier A, Peters C, Paessler S.** 2012. Junin virus pathogenesis and virus replication. Viruses **4:**2317-2339.
296. **Geisberger R, Lamers M, Achatz G.** 2006. The riddle of the dual expression of IgM and IgD. Immunology **118:**429-437.
297. **Yuan D.** 1984. Regulation of IgM and IgD synthesis in B lymphocytes. II. Translational and post-translational events. J Immunol **132:**1566-1570.
298. **Djavani M, Yin C, Lukashevich IS, Rodas J, Rai SK, Salvato MS.** 2001. Mucosal immunization with Salmonella typhimurium expressing Lassa virus nucleocapsid protein cross-protects mice from lethal challenge with lymphocytic choriomeningitis virus. Journal of human virology **4:**103-108.
299. **Geisbert TW, Jones S, Fritz EA, Shurtleff AC, Geisbert JB, Liebscher R, Grolla A, Stroher U, Fernando L, Daddario KM, Guttieri MC, Mothe BR, Larsen T, Hensley LE, Jahrling PB, Feldmann H.** 2005. Development of a new vaccine for the prevention of Lassa fever. PLoS medicine **2:**e183.
300. **Clark LE, Mahmutovic S, Raymond DD, Dilanyan T, Koma T, Manning JT, Shankar S, Levis SC, Briggiler AM, Enria DA, Wucherpfennig KW, Paessler S, Abraham J.** 2018. Vaccine-elicited receptor-binding site antibodies neutralize two New World hemorrhagic fever arenaviruses. Nature communications **9:**1884.

301. **Ter Meulen J, Koulemou K, Wittekindt T, Windisch K, Strigl S, Conde S, Schmitz H.** 1998. Detection of Lassa virus antinucleoprotein immunoglobulin G (IgG) and IgM antibodies by a simple recombinant immunoblot assay for field use. J Clin Microbiol **36:**3143-3148.

# 8. ORIGINAL PUBLICATIONS