



An updated review and current challenges of Guanarito virus infection, Venezuelan hemorrhagic fever

Carlos Ramiro Silva-Ramos¹ · Carolina Montoya-Ruiz² · Álvaro A. Faccini-Martínez^{3,4} · Juan David Rodas⁵

Received: 23 October 2021 / Accepted: 8 March 2022 / Published online: 17 May 2022
© The Author(s), under exclusive licence to Springer-Verlag GmbH Austria, part of Springer Nature 2022

Abstract

Guanarito virus (GTOV) is a member of the family *Arenaviridae* and has been designated a category A bioterrorism agent by the US Centers for Disease Control and Prevention. It is endemic to Venezuela's western region, and it is the etiological agent of "Venezuelan hemorrhagic fever" (VHF). Similar to other arenaviral hemorrhagic fevers, VHF is characterized by fever, mild hemorrhagic signs, nonspecific symptoms, thrombocytopenia, and leukopenia. Patients with severe disease usually develop signs of internal bleeding. Due to the absence of reference laboratories that can handle GTOV in endemic areas, diagnosis is primarily clinical and epidemiological. No antiviral therapies are available; thus, treatment includes only supportive analgesia and fluids. GTOV is transmitted by contact with the excreta of its rodent reservoir, *Zygodontomys brevicauda*. The main reasons for the emergence of the disease may be the increase in the human population, migration, and changes in land use patterns in rural areas. Social and environmental changes could make VHF an important cause of underdiagnosed acute febrile illnesses in regions near the endemic areas. Although there is evidence that GTOV circulates among rodents in different Venezuelan states, VHF cases have only been reported in the states of Portuguesa and Barinas. However, due to the increased frequency of invasions by humans into wildlife habitats, it is probable that VHF could become a public health problem in the nearby regions of Colombia and Brazil. The current Venezuelan political crisis is causing an increase in the migration of people and livestock, representing a risk for the redistribution and re-emergence of infectious diseases.

Introduction

Guanarito virus (GTOV) is a negative-strand RNA virus of the the genus *Mammarenavirus*, family *Arenaviridae*, order *Bunyavirales* [1]. This genus includes both Old and New

World arenaviruses. The New World arenaviruses to which GTOV belongs are also known as the Tacaribe complex of viruses and are grouped in four clades: A, B, C, and A/Rec (clade D) [1]. Human-pathogenic New World arenaviruses belong to clade B, which includes Junin virus (JUNV), the etiological agent of Argentine hemorrhagic fever; Machupo virus (MACV), the agent of Bolivian hemorrhagic fever; Sabia virus (SABV), the agent of Brazilian hemorrhagic fever; Chapare virus (CHAPV), the agent of Chapare hemorrhagic fever; and GTOV, the agent of Venezuelan hemorrhagic fever (VHF) [2, 3]. An outbreak of VHF was first described in the Venezuelan state of Portuguesa and its adjacent areas, and the lethality rate of confirmed cases was 60% (9/15) [4]. The first strain of GTOV was isolated from a fatal case in September 1989, and subsequent studies indicated that this virus was a novel member of the Tacaribe complex [4–6]. Pirital virus (PIRV), another arenavirus, discovered in 1997, is widely distributed in rural areas in which GTOV is also present [7, 8], but human cases due to PIRV have not yet been identified. The geographical focalicity of VHF may be due to a restricted distribution of the rodent reservoir host *Zygodontomys brevicauda* in the western area of Venezuela

Handling Editor: Hideki Ebihara.

✉ Carolina Montoya-Ruiz
cmontoyru@unal.edu.co; carolinamontoyaruiz@gmail.com

- ¹ Grupo de Enfermedades Infecciosas, Departamento de Microbiología, Facultad de Ciencias, Pontificia Universidad Javeriana, Bogotá, Colombia
- ² Facultad de Ciencias, Universidad Nacional de Colombia, Carrera 65, #59a, 110, Medellín, Antioquia, Colombia
- ³ Department of Pathology, University of Texas Medical Branch, Galveston, TX, USA
- ⁴ Comité de Medicina Tropical, Zoonosis y Medicina del Viajero, Asociación Colombiana de Infectología, Bogotá, Colombia
- ⁵ Grupo de Investigación en Ciencias Veterinarias Centauro, Universidad de Antioquia, Medellín, Colombia

[9]. Although the exact reason for the emergence of VHF is not known, it has been suggested that it can be partially explained by human migration and changes in land use patterns in uninhabited forest areas in the states of Portuguesa and Barinas. When forest areas were replaced by agricultural fields and pastures, grassland rodents such as *Z. brevicauda* thrived, which increased the probability of contact between susceptible people and GTOV-infected rodents [10]. However, due to recent social and environmental changes, including increasing human population, deforestation, and migration [11, 12], it is possible that GTOV is an important cause of acute undifferentiated febrile illness in nearby regions and that the number of cases has been underestimated.

Endemic area

The VHF endemic region includes an area of approximately 9000 km² in Venezuela, in the southern and southwestern regions of the state of Portuguesa and adjacent regions, mainly in the state of Barinas [13]. The climate in these regions is tropical, with a mean annual temperature of 28°C, an average precipitation of 1300 mm/year, and a seasonal rainfall that is heavier between May and November each year, with a dry period between December and April [13]. The endemic area of GTOV is shown in Fig. 1, and the regions where human cases of VHF and evidence of GTOV in rodents have been reported are indicated [4, 9, 13–15].

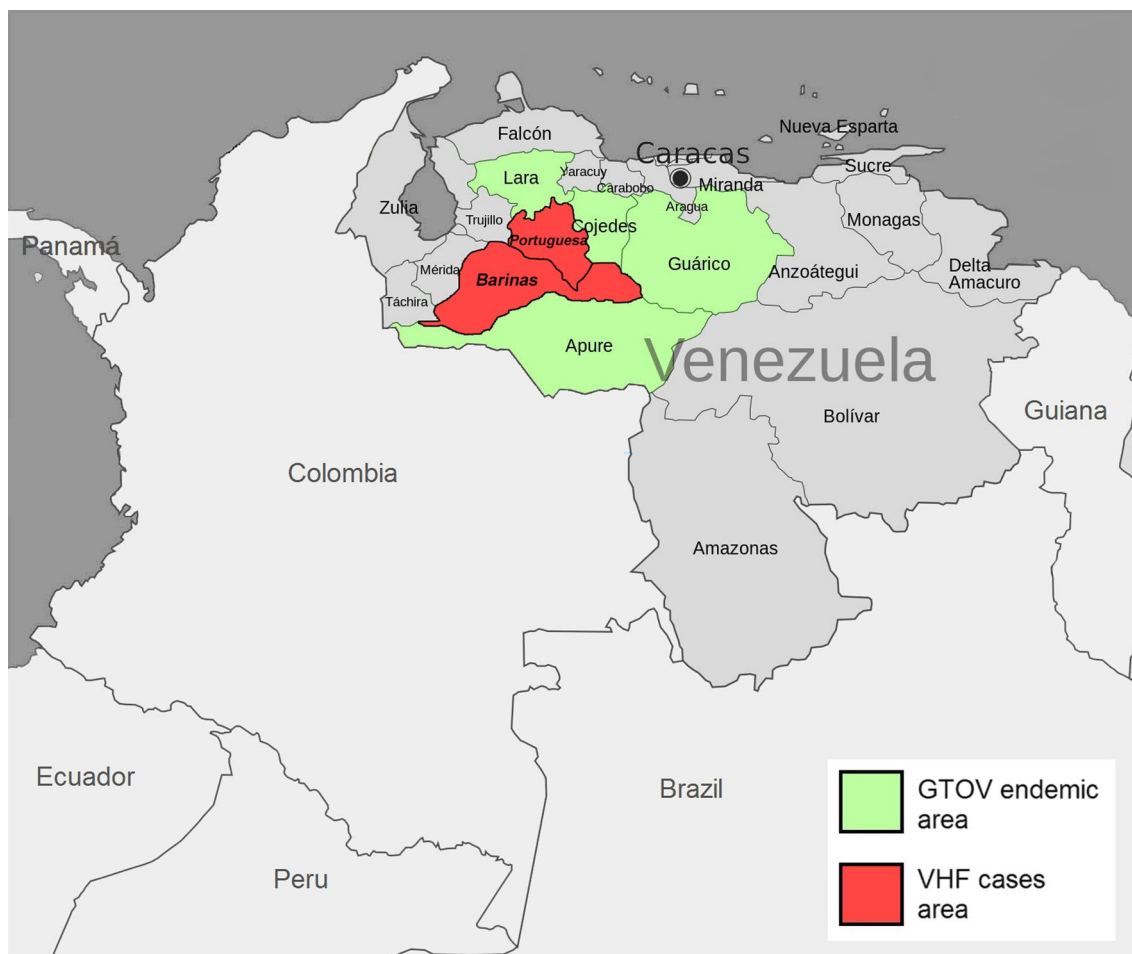


Fig. 1 Areas where Guanarito virus is endemic. Venezuelan states in green are considered endemic for Guanarito virus due to seropositivity and virus isolation from *Zygodontomys brevicauda* rodents [9, 14,

15]. Human VHF cases associated with Guanarito virus have only been identified in Portuguesa and Barinas states (in red) [4, 13, 14, 16]. GTOV, Guanarito virus; VHF, Venezuelan hemorrhagic fever

Reported cases and outbreaks

During September of 1989, an outbreak of severe hemorrhagic febrile illness occurred in the municipality of Guanarito, Portuguesa state, Venezuela, which is considered the first outbreak of GTOV infection reported in the scientific literature [4]. Although local physicians had reported similar clinical cases before the 1989 outbreak, no reports were found about previous cases [16]. Surveillance in the same municipality between 1990 and 1991 identified a total of 104 suspected cases with 26 deaths, which were reported to the Venezuelan Health Ministry [4]; 15 of these patients were admitted and treated at the Miguel Orea Hospital in Guanare, where they received a confirmed diagnosis of VHF [4].

During 1991, serum samples from family contacts of VHF-confirmed patients were collected to determine their exposure to GTOV. The study found that 10.5% had antibodies against GTOV, suggesting a possible mild form of VHF [4]. A year later, in 1992, another serological study was performed in the community of La Hoyada in the state of Portuguesa due to possible VHF cases that were occurring in this and other nearby communities; however, the study showed a low seroprevalence of GTOV antibodies (3.6%) among residents [16].

Since the discovery of GTOV as the etiological agent of VHF, a total of 165 probable cases have been reported (1989 to 1997), with only 40% confirmed by virus isolation and/or detection of seroconversion [13]. Although VHF cases were reported every month, more than half of these cases occurred during November, December, and January [13]. A continuous occurrence of cases was reported from September 1989 through August 1992; these numbers then continued to decrease until August 1996, when little disease activity was detected; however, the number of VHF cases then increased again until May 1997 [13]. The most recent data suggest that from September 1989 through December 2006, a total of 618 VHF cases were reported in the state of Portuguesa, with a case-fatality rate of 23.1% [15]. Due to the poor surveillance system and a lack of epidemiological studies in Venezuela, there are no updated data on the number of cases from 2006 to 2021, when a recent report described 36 confirmed cases [17].

Risk factors

Due to the rapid onset and high mortality rates associated with infection, the CDC has classified GTOV as a high-priority category A bioterrorism agent [18] that may only be manipulated in maximum-containment laboratories (biosafety level 4) [19].

All of the confirmed and probable cases of VHF have occurred in people residing in endemic areas, or in people who lived elsewhere but had recently travelled to the endemic region [13, 14]; thus, exposure to endemic areas may be an important risk factor for GTOV infection. Males of reproductive age represent the group with the highest risk due to occupational exposure to infected rodents such as *Z. brevicauda* (short-tailed cane mice), which inhabit wild and rural areas [13]. Records from 165 VHF cases from September 1989 to January 1997 show that most of the cases occurred between November and January, coinciding with the end of the rainy season and the highest level of agricultural activity in the region [13]. It is worth mentioning that, in 1998, almost 50% of the population in those rural areas was involved in agriculture and/or cattle raising as their principal work activity. However, during the planting and harvest seasons, many temporary agricultural workers from Venezuela and nearby Colombia go into VHF endemic areas for work [13], thus risking infection with GTOV. Despite the high transmissibility from wild rodents, cases of VHF by interhuman transmission may not be common, as only one probable secondary case has been reported in the scientific literature [13].

Natural reservoir

The first information about the natural reservoir host of GTOV was obtained in 1991 during a surveillance of rodents captured in dwellings of VHF cases in the municipality of Guanarito. GTOV was isolated from *Sigmodon hispidus*, and seropositivity was detected in *Oryzomys* spp. [4]. Almost a year later, field surveillance of the communities of La Hoyada, Pirital, La Arenosa, and Pirital (Portuguesa state) was performed to determine the prevalence of infection in wild rodents, and GTOV was isolated from 19 out of 40 *Sigmodon alstoni* (Alston's cotton rats) and 12 out of 106 *Z. brevicauda* samples [16]. Interestingly, *S. alstoni* developed a persistent seronegative infection and *Z. brevicauda* developed a seropositive infection [16]. These first results suggested that *S. alstoni* might be the principal reservoir host of GTOV in the wild environment [16]. During the same surveillance, seropositivity was also observed in 6.3% of *Rattus rattus* samples, indicating that common rats might occasionally be in contact with GTOV. As *R. rattus* can live closely with the human population, these animals might represent an important source of infection, depending on their capacity to shed virus in their excreta, even for a short time [16]. More studies are necessary to test this hypothesis.

In another study, the nucleocapsid protein gene sequences from previously isolated viruses were analyzed, and it was found that viruses from *S. alstoni* correspond to the new arenavirus called Pirital virus (PIRV), and not to a strain

of GTOV [7]. These strains have also been recovered from other states besides Portuguesa, including Cojedes, Barinas, Guarico, and Lara [9]. The actual reservoir host of GTOV was thought to be *Z. brevicauda*, which is native to the plains of western Venezuela, considering that viral isolates were also recovered from this rodent species [15]. *Z. brevicauda* can reach high densities and live in areas with tall grass [15].

Another study indicated that no lethality occurs in *Z. brevicauda* when they are infected with GTOV, but they develop chronic viremia with persistent and long-term shedding of infectious viral particles in oropharyngeal secretions and urine [20]. In addition, while infection occurs particularly in young mice, which can develop chronic infection more easily, some adults clear the infection. Vertical transmission might be uncommon due to the lethality of GTOV infection in *Z. brevicauda* fetuses [8, 20]. GTOV has been isolated from *Z. brevicauda* lungs, which suggests that these rodents are infected through the respiratory tract [8].

PIRV and GTOV have both been isolated from *Z. brevicauda*, but GTOV also infects *S. alstoni*, albeit rarely, due to horizontal transmission between these two rodent species, which live in intimate contact in the wild [8, 9]. However, both rodent species have extremely limited mobility, and thus, GTOV infection might not be widespread [14]. Although both *Z. brevicauda* and *S. alstoni* have been found to be seropositive for GTOV, the virus has only been isolated from *Z. brevicauda*, suggesting that the immune response in *Z. brevicauda* controls GTOV infection but does not clear it [8, 9]. Overall, several factors, such as *Z. brevicauda* genetics, GTOV genetics, the inoculum dose, and the route of exposure, may affect GTOV infection in rodents [8].

Virology

GTOV is a single-stranded RNA virus of the genus *Mammarenavirus* that belongs to clade B of the New World arenaviruses. Its genome has two segments. The large segment (L) encodes the viral polymerase (RNA-dependent RNA polymerase) and a zinc finger motif protein (Z protein) or viral matrix protein, and the small segment (S) encodes the nucleocapsid protein (NP) and a glycoprotein precursor (GPC) [6, 21]. GTOV is part of the Tacaribe complex [22] and therefore cannot be differentiated from other members of this complex by complement fixation or indirect immunofluorescence assay due to cross-reaction [22].

In comparison with other mammarenavirus, phylogenetically, GTOV is included within clade B with Amapari, Machupo, Junin, Tacaribe, and Sabia viruses [23]. GTOV has nine distinct genotypes (Fig. 2) that differ by 4–17% in their nucleotide sequences and up to 9% in their amino acid sequences when comparing NP gene sequences obtained from 29 isolates (21 from rodents and 8 from humans) [14].

Most genotypes appear to be restricted to discrete geographic regions, and only two genotypes have been isolated from VHF cases, both of which were found in rodents outside the endemic regions. This suggests that human cases might occur in areas surrounding the endemic regions in which VHF cases have been reported [14]. Apparently, VHF is not associated with any specific GTOV genotype, and some genotypes have never been isolated from human VHF cases [14]. Although all GTOV genotypes circulate among rodents in adjacent areas of the states of Cojedes, Barinas, and Apure, due to a lack of epidemiological surveillance, it is not known if these genotypes regularly cause human disease [14]. All but genotype 1 GTOV have been isolated from *Z. brevicauda*, and genotype 1 was only isolated from *Oligoryzomys fulvescens* in Barinas State [14]. It is unlikely that exchanges of genetic elements between GTOV-like viruses and PIRV by recombination or reassortment contribute to VHF emergence, since phylogenetic analysis of S and L genome segments of GTOV has shown that its evolution is independent of that of PIRV, which forms a separate lineage together with Pichindé virus [21].

The geographical range of GTOV associated with *Z. brevicauda* includes five states in western Venezuela: Apure, Barinas, Cojedes, Guárico, Lara and Portuguesa (Fig. 1) [9, 14, 15]. Therefore, GTOV could have been enzootic in Portuguesa long before 1989, and the emergence of VHF was likely a consequence of demographic and ecological changes in rural areas that resulted from increased human activities [15]. Cases of VHF in Apure, Cojedes, Guárico and Lara may not have been recognized, since few people have been exposed to GTOV-infected rodents in these less densely populated areas. Thus, hypothetically, VHF will become a significant public health problem in these states as human population and work activities increase [15].

Pathogenesis

The pathogenesis of New World arenaviruses has not been extensively studied, but it is known that they suppress the immune response during the earliest stage of infection and that they later trigger a severe inflammatory response, which correlates with the progress of the disease [3]. Of the known pathogenic New World arenaviruses, JUNV and MACV are the best studied [24, 25]. However, although many processes that occur during the pathogenesis of New World arenaviruses might be similar, specific information on the pathogenesis of those viruses is beyond the scope of this review.

Different animal models, including rhesus monkeys and guinea pigs, have been used to study the pathogenesis of VHF. While GTOV failed to produce disease in rhesus

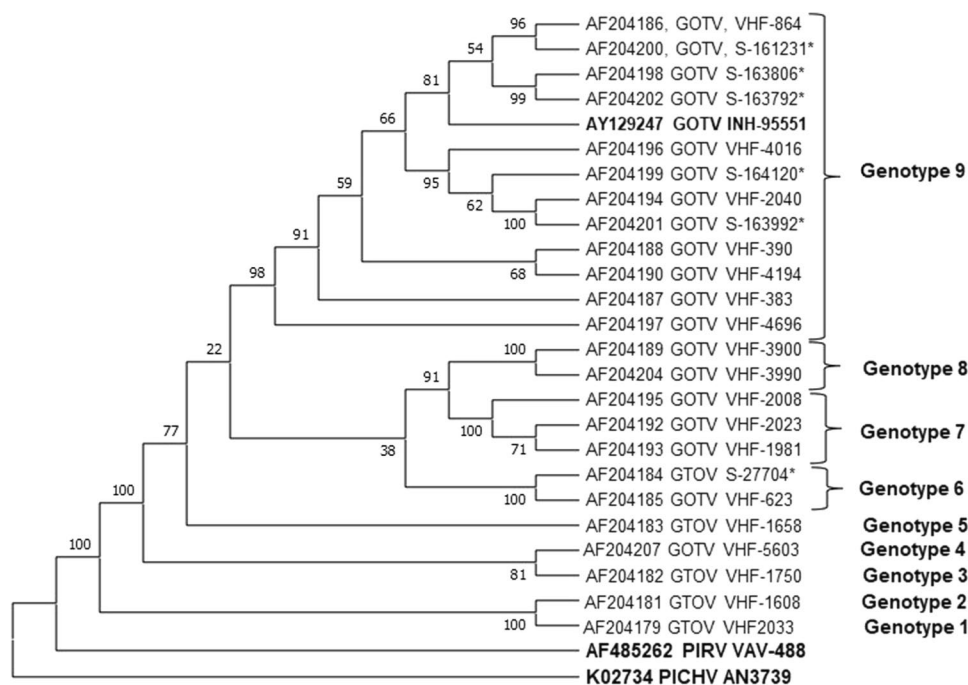


Fig. 2 Phylogenetic analysis based on 619-nt partial sequences (nt 938–1556) of the nucleocapsid genes of GTOV isolates of different genotypes. The tree was generated by the maximum-likelihood method using 10,000 replicates, with the Tamura 3-parameter plus invariant substitution model, which was selected according to the Akaike (AIC) and Bayesian (BIC) criteria. The tree was rooted using Pirital virus (PIRV) strain VAV-488, and Pichinde virus (PICHV) strain AN3739. Bootstrap values are shown at the nodes, and the GenBank accession number, species, strain, and genotype are shown at the branches. The source and region of origin of sequences for Guanarito virus (GTOV) genotypes are reported in reference 14,

and the sequencing of GTOV INH-95551, PIRV, PICHV reference strains (bold) were reported by the ICTV (genus: *Mammarenavirus* - *Arenaviridae* - Negative-sense RNA Viruses - ICTV (ictvonline.org)). All of the strains belonging to genotype 9 were identified in the state of Portuguesa, except for VHF-4016, identified in Cojedes, and VHF-2040, identified in Barinas. Genotype 8 was identified in Cojedes, genotype 7 in Barinas, genotype 6 in Portuguesa, genotype 5 in Apure, genotype 4 in Guarico, genotype 3 in Guarico, genotype 2 Apure, and genotype 1 in Marinas. * Indicates a strain detected in human samples

monkeys, guinea pigs appear to be better models for studying VHF disease [22].

Alpha-dystroglycan has been identified as the cellular receptor of Old World and clade C New World arenaviruses; however, clade A and B New World arenaviruses do not share the same receptor [26]. New World clade B arenaviruses use transferrin receptor 1 (TfR1), and development of human illness depends on the ability of some of these viruses to bind to human TfR1 [27, 28]. GTOV-TfR1 binding might be specific, since it was found that GTOV does not use TfR1 of rodent species other than *Z. brevicauda* [29].

The GPC (glycoprotein precursor complex) is split into two proteins during viral assembly: GP1 (peripheral glycoprotein) and GP2 (transmembrane glycoprotein). GP1 is known to be the viral receptor, and GP2 is structurally similar to membrane fusion proteins of other enveloped viruses [26]. GP1 facilitates host cell attachment by binding to TfR1, and GP2 has been postulated to be a fusion protein because it facilitates the fusion between the virus and the host cell [18]. After GTOV binds to its cellular receptor via GP1, it is internalized by clathrin-mediated endocytosis, and the acidic

environment in the endocytic compartment causes GP1 to be released from the virion and GP2 to mediate fusion of the viral and host membranes. This pH-dependent membrane fusion step releases the contents of the viral particle into the cytoplasm [18, 26].

Assembly and release of new enveloped virions is mainly mediated by the Z protein and by the viral NP, which enhances this process along with the host cell's endosomal sorting complex, which is required for transport and facilitates viral release [30–33]. The cellular proprotein convertase site 1 protease (S1P) has been implicated in the cleavage of the GTOV GPC, which increases the production of viral particles and cell-to-cell propagation [34].

Double-stranded RNA (dsRNA) is synthesized during infection by RNA viruses as a byproduct of replication and transcription, and this acts as a potent trigger of the innate antiviral response of the host. Viral dsRNA is recognized by RIG-I (retinoic acid-inducible gene I)-like receptors, leading to the production of type I interferon α/β [35]. The Z protein of pathogenic arenaviruses, including GTOV, inhibits RIG-I-like receptors, suppressing the innate response; thus,

the Z protein might be a potential novel target for the development of antiviral therapies [36]. However, the Z protein is not the only arenaviral protein with immunomodulatory activity, since GTOV also encodes other suppressors that interfere with the immune response. For example, the 3′–5′ exoribonuclease activity of NP results in interference with type I interferon production [35, 37].

Studies carried on in Guinea pigs as animal models of human disease have shown that the clinical course of GTOV is characterized by gastrointestinal cellular necrosis, interstitial pneumonia, lymphoid and hematopoietic cell necrosis, and the presence of platelet thrombi in blood vessels associated with hemorrhage [19]. In these animal models, VHF develops as a multi-organ disease, and viral antigen inclusions are found in multiple tissues and organs. Diffuse pulmonary edema and congestion, liver congestion with necrosis, cardiomegaly, splenic enlargement and congestion, adrenal hemorrhage, bone marrow depletion, and renal edema are some of the types of damage that have been observed in some individuals; however, liver necrosis caused by GTOV may be minimal compared with that caused by other arenaviruses (e.g., Lassa virus), which cause extensive damage [19]. Peripheral ganglia neurons can be reservoirs of the virus, creating an obstacle for GTOV treatment, since ribavirin, one of the major antivirals, cannot penetrate to neural sites [19].

Clinical manifestations

After an incubation period of 3 to 12 days, the most common initial symptoms include fever, prostration, headache, arthralgia, cough, sore throat, nausea, vomiting, diarrhea, epistaxis, bleeding gums, menorrhagia, and melena. Most patients present with thrombocytopenia and leukopenia. The platelet count has no relationship to the clinical outcome, and measurement of other laboratory parameters is unhelpful [4, 13]. Patients are usually acutely ill and often dehydrated and somnolent after the onset of severe symptoms. Those who die usually do so due to diffuse pulmonary edema and hemorrhagic congestion, and those who survive usually recover without sequelae. However, convalescence is prolonged and requires a long hospitalization [4].

Clinically, VHF cases are not different from other arenaviral hemorrhagic fevers. VHF usually begins as a mild nonspecific febrile illness that progresses in severity over around six days, when most patients report fever and progressive onset of symptoms. Patients with severe disease develop melena, hematemesis, petechiae, epistaxis, and rectal bleeding as the main hemorrhagic signs [13]. Specific clinical signs and symptoms from diagnosed VHF cases are listed in Table 1. Accurate etiological diagnosis is made in roughly 10.7% of cases, since physicians usually confuse

the diagnosis with classical dengue or severe dengue [13]. Overall, the case fatality rate is 33.3% or higher, depending on the early measures and treatment adopted, but active or recent history of convulsions worsens the prognosis [13].

Diagnosis

GTOV, a BSL-4 pathogen, is included in group I of the priority classification by the World Health Organization because of its severity and often fatal outcome due to the lack of effective treatment and preventive measures. Thus, rapid laboratory diagnosis is very important and urgent for administering adequate treatment [38].

During the first outbreaks of VHF, cases were diagnosed by isolation of GTOV, which still remains as the gold standard method for the laboratory diagnosis of many viral diseases [39]. However, viral culture is not a practical method for GTOV diagnosis because it is time-consuming and requires considerable technical expertise and a BSL-4 security level laboratory, which is rarely available [4, 39], particularly in low- and middle-income countries. Therefore, other nonculture methods for rapid viral diagnosis, such as detection of viral antigens or nucleic acids, have replaced the use of viral culture.

Serological methods can also be employed and are helpful when a paired sample is obtained to detect seroconversion. Although IgM can be detected rapidly, cross-reactions can occur between GTOV and other arenaviruses. Neutralizing antibodies, which are much more selective and would be ideal for early diagnosis, usually appear too late after infection [38]. Since GTOV and other arenaviruses are classified as BSL-4 pathogens, their diagnosis in laboratories without BSL-4 facilities is difficult, and thus, in order to overcome these difficulties, antibody detection by enzyme-linked-immunosorbent assay (ELISA) and indirect immunofluorescence assay (IFA) using recombinant viral NPs may be an option, not as much for etiological diagnosis, but for seroepidemiological surveillance, because these tests can be performed in BSL-2 laboratories, which are more accessible [40, 41].

Other important diagnostic methods include detection of neutralizing antibodies and molecular methods. The viral genome can be detected by PCR before neutralizing antibodies appear in the patient's serum. However, due to the short duration of viremia, the usefulness of PCR can be limited. Furthermore, detection of neutralizing antibodies is difficult, as it also requires a BSL-4 laboratory, which is usually unavailable in endemic areas [38]. Multiplex real-time qRT-PCR and TaqMan qRT-PCR assays for the rapid detection of viruses causing hemorrhagic fever, including GTOV, have proved to be specific and sensitive [42–44].

Table 1 Clinical features of Venezuelan hemorrhagic fever (adapted from Salas et al. 1990 and de Manzione et al. 1998)

Clinical manifestations	Frequency (n = 14) [4]	Frequency (n = 55) [13]
Fever	100% (14/14)	92.7% (51/55)
Hemorrhagic signs	92.9% (13/14)	ND
Bleeding gums	61.5% (8/13)	52.7% (29/55)
Melena	30.8% (4/13)	20% (11/55)
Petechiae	30.8% (4/13)	16.4% (9/55)
Epistaxis	23.1% (3/13)	12.7% (7/55)
Hematemesis	23.1% (3/13)	16.4% (9/55)
Hematuria	23.1% (3/13)	-
Menorrhagia*	57.1% (4/7)*	-
Prostration	78.6% (11/14)	-
Dehydration	71.4% (10/14)	29.1% (16/55)
Pharyngitis	71.4% (10/14)	12.7% (7/55)
Arthralgia	64.3% (9/14)	52.7% (29/55)
Headache	64.3% (9/14)	58.2% (32/55)
Somnolence/stupor	64.3% (9/14)	10.9% (6/55)
Conjunctivitis	50% (7/14)	14.5% (8/55)
Diarrhea	50% (7/14)	27.3% (15/55)
Cough	42.9% (6/14)	20% (11/55)
Nausea/vomiting	35.7% (5/14)	13.4% (7/55); 34.5% (19/55) ♦
Sore throat	35.7% (5/14)	36.4% (20/55)
Lymphadenopathy	21.4% (3/14)	23.6% (13/55)
Facial edema	14.3% (2/14)	-
Tonsillar exudate	14.3% (2/14)	12.7% (7/55)
Abdominal pain	7.1% (1/14)	30.9% (17/55)
Chest pain	7.1% (1/14)	-
Vertigo	7.1% (1/14)	-
Convulsions	7.1% (1/14)	18.2% (10/55)
Hepatomegaly	7.1% (1/14)	5.6% (3/55)
Rash	7.1% (1/14)	-
Malaise	-	74.5% (41/55)
Myalgia	-	30.9% (17/55)
Rectal bleeding	-	9.1% (5/55)
Splenomegaly	-	1.8% (1/55)

* Female patients

♦ Nausea, 13.4%; vomiting 34.5%

In the last decade, the use of analytical microarrays has become a promising diagnostic method that significantly reduces the time needed for analysis by allowing parallel diagnosis, testing one sample with multiple probes that discriminate among different infectious agents. For example, in one study, a prototypic microarray was developed to distinguish members of the families *Filoviridae* and *Arenaviridae*, including GTOV, and this represents an important tool for the diagnosis of VHF [38]. Nonetheless, its cost and technical requirements limit its applicability in GTOV-endemic areas.

Treatment

Even if vigorous treatment is administered, a case fatality rate of 60% among hospitalized patients can occur. Some of the therapies and supportive measures for VHF treatment include fresh convalescent plasma, platelet concentrates, fibrinogen, vitamin K, intravenous fluids, antivirals, and oxygen therapy [4].

Convalescent-phase plasma containing neutralizing antibodies has been used for mitigating the severity of VHF, and *in vitro* studies have found that it may be beneficial in the treatment of VHF. Nonetheless, concerns

about neutralizing activity of convalescent plasma against specific circulating strains in determined regions (as has been reported previously for other arenaviral diseases such as JUNV infection) has raised doubts about this option [15, 45]. The use of plasmid-DNA-based vaccine technology to express the glycoproteins of pathogenic arenaviruses in rabbits can be used to generate neutralizing antibodies, which can be included in an antibody cocktail that targets multiple arenaviruses. These neutralizing antibodies have been shown to be effective for immunotherapy of infections with GTOV and other arenaviruses in guinea pigs [45].

Ribavirin is the only licensed antiviral that has proven to be effective against arenaviral infections (e.g., Lassa virus, JUNV, MACV). Its effectivity depends on very early treatment, and its use in the late stages of the disease has only minor positive effects [46, 47]. Other antivirals, such as favipiravir, which inhibits viral replication/transcription, possibly targeting the viral RNA-dependent RNA polymerase, have also demonstrated a broad spectrum of *in vitro* activity against a great number of RNA viruses, including GTOV [46]. Its efficacy for the treatment of arenaviral hemorrhagic fever agents has shown successful results in the treatment of non-human primates infected with Lassa virus [48], but favipiravir has also been used in the treatment of other South American hemorrhagic fevers such as human cases of Argentine hemorrhagic fever [49].

Processing of GPC by S1P, also known as subtilisin-kexin-isozyme 1 (SKI-1), is a crucial step for cell-to-cell propagation of the virus; thus, inhibition of SKI-1/S1P is expected to inhibit intercellular dissemination and accelerate viral clearance [47]. *In vitro* studies have shown that SKI-1/S1P inhibitors, combined with antivirals such as ribavirin, potentiate the antiviral effect and viral clearance during chronic infection [47].

Other drugs such as amiodarone, an antiarrhythmic medicine, have proven to be potent inhibitors of cell entry by some viruses, including GTOV, at least in *in vitro* assays [50]. New technologies are now available for the identification of small molecules and other therapeutics that could be used as potential antivirals. Strategies are being developed to identify novel cell-entry inhibitors, which have become important tools for the discovery of potential treatments of VHF, including arenaviral diseases [51]. Anti-TfR1 antibodies and small molecules that inhibit viral binding to TfR1 can efficiently inhibit GTOV and other South American arenaviruses that use TfR1 as an entry receptor [52, 53]. The viral GP1 or GP2 proteins have been shown to be potential primary targets for the design of antiviral drugs [18].

Prevention measures

GTOV is classified as a high-priority category A bioterrorism agent according to the CDC [18]. Thus it can only be handled in maximum-containment laboratories (BLS-4) with all of the necessary protocols related to protective personal equipment (e.g., the use of full-body, air-supplied, positive-pressure protective suits) and facility features (e.g., strictly controlled airflow into the laboratory, airlocks within the entrances and exits, and extensive decontamination of waste materials before leaving the laboratory), in order to avoid possible contamination of workers and minimize the risk of widespread infection [19, 54].

All arenaviruses are on the priority A list of pathogens of the National Institute of Allergy and Infectious Disease (NIAID) for which vaccine development is a priority [55]. Currently, there is no FDA-approved vaccine for any pathogenic arenavirus. In Argentina, the live attenuated Candid#1 vaccine of JUNV has been shown to be effective in preventing Argentine hemorrhagic fever, and monoclonal antibodies produced from Candid#1-immunized humans neutralize MACV [56, 57]. However, the effectiveness of Candid#1 immunity for prevention of MACV infection is still not clear, as it failed to prevent lethal MACV infection in a mouse model [58]. Furthermore, the effectivity of Candid#1 as a preventive measure against other arenaviruses, including GTOV, has not yet been investigated [56].

Some epitopes on viral proteins have shown a lack of cross-reactivity with different arenaviruses, but there are some small antigenic sites that have been shown to be very conserved among many arenaviruses, making them possible molecular targets for vaccines [6]. Recently, a study based on genome and immune informatics showed that the viral NP can be a potential target for designing a cross-reactive vaccine against pathogenic arenaviruses, since NP can stimulate a humoral and cell-mediated immune response [56].

Attenuated strains can potentially be employed as vaccines. A study found that a mutation in the JUNV Z protein, which is highly conserved among mammalian arenaviruses, resulted in attenuation of the virus, raising the possibility that similar results can be achieved with closely related arenaviruses such as MACV, GTOV, and SABV [59]. In another study, an attenuated variant of MACV showed 88% protection of guinea pigs infected with GTOV [60]. Both studies suggest that a live-attenuated arenavirus vaccine can be highly effective against these pathogens.

CD4⁺ T cells are needed for optimal CD8⁺ T- and B-cell responses, playing an important role in cellular and humoral immunity in arenaviral infections. The identification of epitopes recognized by CD4⁺ T cells may be

critical for the development of a T-cell-based vaccine with the potential to target a broad range of pathogenic arenaviruses, including GTOV [61, 62].

Z. brevicauda, the reservoir host of GTOV, is commonly associated with grassy habitats in rural areas; thus, elimination of tall grass in close proximity to rural human dwellings and working areas can prevent contact with this rodent and possible GTOV infection [9].

Current challenges

Since the first discovery of GTOV in 1989, many clinical and field epidemiological studies indicated that VHF cases were being reported continuously, but a lack of interest in the disease has resulted in a lack of epidemiological studies for almost fifteen years. Clinical studies, or even field epidemiological studies involving GTOV and VHF, have not been reported since 2006. However, a recent report, using data from the Ministry of Health of Venezuela, described 118 suspected VHF cases, only 30.5% (36/118) of which had been confirmed up to epidemiological week 42 in 2021 [17].

Although confirmed cases of VHF have only been reported in the endemic region of the states of Portuguesa, Barinas and adjacent regions in Venezuela, GTOV might not be restricted to these regions. In a study performed in Colombia using GTOV antigens, antibodies were detected in *Z. brevicauda* captured from rural areas on the Caribbean coast of the department of Córdoba [63]. Although the seroprevalence in *Z. brevicauda* was much lower in Colombia than in the VHF-endemic area in Venezuela, this finding should alert local public health entities that GTOV cases might be occurring in Colombia. The geographic closeness of the Colombian and Venezuelan eastern plains, which share some common features such as climate, the presence of small mammals, social and economic conditions, and land use, suggests that it would be worthwhile to study epidemiological risk factors in Colombia that could explain the occurrence of unidentified febrile illnesses within these border regions. Although circumstantial evidence of circulation of arenaviruses in Colombia has already been documented (e.g., Pichindé virus, LCMV), the great biodiversity in this country suggests the need for field-surveillance studies that could help to determine the likely roles played by rodents in other parts of Colombia [64].

One of the most complex humanitarian crises in the western hemisphere is occurring in Venezuela, and due to this situation, the flow of Venezuelan migrants and refugees has been increasing for more than five years, principally in the nearby countries of Colombia and Brazil, representing a risk for the increase, transmission, and emergence of a great variety of infectious diseases [65]. Human mobility is an important determinant in the spread of infectious

diseases, particularly vector-borne diseases and zoonoses [12], and thus future studies should focus particularly on regions that border on the Venezuelan endemic area (the Colombian-Venezuelan border and Brazilian-Venezuelan border). Surveillance of wild rodents, particularly *Z. brevicauda* should be conducted, and cases of human febrile illness that might be attributed to arenaviral hemorrhagic fever should be investigated.

Acknowledgments We especially thank Dr. Maria Salvato for reviewing the manuscript in the English language.

Author contributions C. R. Silva-Ramos wrote the draft manuscript and reviewed the literature; C. Montoya-Ruíz, Á. A. Faccini-Martínez, and J. D. Rodas corrected the manuscript; and all authors wrote the original manuscript, critically reviewed the manuscript for relevant content, and approved the final version of the manuscript.

Funding Pontificia Universidad Javeriana, Universidad Nacional de Colombia sede Medellín, and Universidad de Antioquia provided funding. At the time of this work, Álvaro A. Faccini-Martínez was supported by the Fogarty International Center and the National Institute of Allergy and Infectious Diseases of the National Institutes of Health, under Award Number D43 TW010331. The content is solely the authors' responsibility and does not necessarily represent the official views of the National Institutes of Health.

Availability of data and material All data generated or analyzed during this review are included in the manuscript.

References

1. Radoshitzky SR, Buchmeier MJ, Charrel RN, Clegg JCS, Gonzalez JJ, Günther S, Hepojoki J, Kuhn JH, Lukashevich IS, Romanowski V, Salvato MS, Sironi M, Stenglein MD, de la Torre JC (2019) ICTV Report Consortium. ICTV virus taxonomy profile: Arenaviridae. *J Gen Virol* 100(8):1200–1201. <https://doi.org/10.1099/jgv.0.001280>
2. Hallam SJ, Koma T, Maruyama J, Paessler S (2018) Review of mammarenavirus biology and replication. *Front Microbiol* 9:1751. <https://doi.org/10.3389/fmicb.2018.01751>
3. Frank MG, Beitscher A, Webb CM, Raabe V, members of the Medical Countermeasures Working Group of the National Emerging Special Pathogens Training and Education Center's (NETEC's) Special Pathogens Research Network (SPRN) (2021) South American hemorrhagic fevers: a summary for clinicians. *Int J Infect Dis* 105:505–515. <https://doi.org/10.1016/j.ijid.2021.02.046>
4. Salas R, de Manzione N, Tesh RB, Rico-Hesse R, Shope RE, Betancourt A, Godoy O, Bruzual R, Pacheco ME, Ramos B, Taibo ME, Garcia Tamayo J, Jaimes E, Vasquez C, Araoz F, Querales J (1991) Venezuelan haemorrhagic fever. *Lancet* 338(8774):1033–1036. [https://doi.org/10.1016/0140-6736\(91\)91899-6](https://doi.org/10.1016/0140-6736(91)91899-6)
5. Bowen MD, Peters CJ, Nichol ST (1996) The phylogeny of New World (Tacaribe complex) arenaviruses. *Virology* 219(1):285–290. <https://doi.org/10.1006/viro.1996.0248>
6. Gonzalez JP, Sanchez A, Rico-Hesse R (1995) Molecular phylogeny of Guanarito virus, an emerging arenavirus affecting humans. *Am J Trop Med Hyg* 53(1):1–6

7. 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(5):548–553. <https://doi.org/10.4269/ajtmh.1997.56.548>
8. Milazzo ML, Cajimat MN, Duno G, Duno F, Utrera A, Fulhorst CF (2011) Transmission of Guanarito and Pirital viruses among wild rodents, Venezuela. *Emerg Infect Dis* 17(12):2209–2215. <https://doi.org/10.3201/eid1712.110393>
9. Fulhorst CF, Bowen MD, Salas RA, Duno G, Utrera A, Ksiazek TG, De Manzione NM, De Miller E, Vasquez C, Peters CJ, Tesh RB (1999) Natural rodent host associations of Guanarito and pirital viruses (Family Arenaviridae) in central Venezuela. *Am J Trop Med Hyg* 61(2):325–330. <https://doi.org/10.4269/ajtmh.1999.61.325>
10. Tesh RB (2002) Viral hemorrhagic fevers of South America. *Bio-medica* 22(3):287–295
11. Lindahl JF, Grace D (2015) The consequences of human actions on risks for infectious diseases: a review. *Infect Ecol Epidemiol* 5:30048. <https://doi.org/10.3402/iee.v5.30048>
12. Tuite AR, Thomas-Bachli A, Acosta H, Bhatia D, Huber C, Petrasek K, Watts A, Yong JHE, Bogoch II, Khan K (2018) Infectious disease implications of large-scale migration of Venezuelan nationals. *J Travel Med* 25(1):tay077. <https://doi.org/10.1093/jtm/tay077>
13. de Manzione N, Salas RA, Paredes H, Godoy O, Rojas L, Araoz F, Fulhorst CF, Ksiazek TG, Mills JN, Ellis BA, Peters CJ, Tesh RB (1998) Venezuelan hemorrhagic fever: clinical and epidemiological studies of 165 cases. *Clin Infect Dis* 26(2):308–313. <https://doi.org/10.1086/516299>
14. Weaver SC, Salas RA, de Manzione N, Fulhorst CF, Duno G, Utrera A, Mills JN, Ksiazek TG, Tovar D, Tesh RB (2000) Guanarito virus (Arenaviridae) isolates from endemic and outlying localities in Venezuela: sequence comparisons among and within strains isolated from Venezuelan hemorrhagic fever patients and rodents. *Virology* 266(1):189–195. <https://doi.org/10.1006/viro.1999.0067>
15. Fulhorst CF, Cajimat MN, Milazzo ML, Paredes H, de Manzione NM, Salas RA, Rollin PE, Ksiazek TG (2008) Genetic diversity between and within the arenavirus species indigenous to western Venezuela. *Virology* 378(2):205–213. <https://doi.org/10.1016/j.virol.2008.05.014>
16. Tesh RB, Wilson ML, Salas R, De Manzione NM, Tovar D, Ksiazek TG, Peters CJ (1993) Field studies on the epidemiology of Venezuelan hemorrhagic fever: implication of the cotton rat *Sigmodon alstoni* as the probable rodent reservoir. *Am J Trop Med Hyg* 49(2):227–235. <https://doi.org/10.4269/ajtmh.1993.49.227>
17. Rodríguez-Morales AJ, Bonilla-Aldana DK, Ríquez A, Paniz-Mondolfi A, Suárez JA (2021) Should we be concerned about Venezuelan hemorrhagic fever?—A reflection on its current situation in Venezuela and potential impact in Latin America amid the migration crisis. *New Microbes New* 44:100945. <https://doi.org/10.1016/j.nmni.2021.100945>
18. Parsy ML, Harlos K, Huiskonen JT, Bowden TA (2013) Crystal structure of Venezuelan hemorrhagic fever virus fusion glycoprotein reveals a class I postfusion architecture with extensive glycosylation. *J Virol* 87(23):13070–13075. <https://doi.org/10.1128/JVI.02298-13>
19. Hall WC, Geisbert TW, Huggins JW, Jahrling PB (1996) Experimental infection of guinea pigs with Venezuelan hemorrhagic fever virus (Guanarito): a model of human disease. *Am J Trop Med Hyg* 55(1):81–88. <https://doi.org/10.4269/ajtmh.1996.55.81>
20. Fulhorst CF, Ksiazek TG, Peters CJ, Tesh RB (1999) Experimental infection of the cane mouse *Zygodontomys brevicauda* (family Muridae) with guanarito virus (Arenaviridae), the etiologic agent of Venezuelan hemorrhagic fever. *J Infect Dis* 180(4):966–969. <https://doi.org/10.1086/315029>
21. Cajimat MN, Fulhorst CF (2004) Phylogeny of the Venezuelan arenaviruses. *Virus Res* 102(2):199–206. <https://doi.org/10.1016/j.virusres.2004.01.032>
22. 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(4):452–459. <https://doi.org/10.4269/ajtmh.1994.50.452>
23. 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(10):5140–5146. <https://doi.org/10.1128/jvi.76.10.5140-5146.2002>
24. Grant A, Seregin A, Huang C, Kolokoltsova O, Brasier A, Peters C, Paessler S (2012) Junín virus pathogenesis and virus replication. *Viruses* 4(10):2317–2339. <https://doi.org/10.3390/v4102317>
25. Patterson M, Grant A, Paessler S (2014) Epidemiology and pathogenesis of Bolivian hemorrhagic fever. *Curr Opin Virol* 5:82–90. <https://doi.org/10.1016/j.coviro.2014.02.007>
26. Rojek JM, Spiropoulou CF, Kunz S (2006) Characterization of the cellular receptors for the South American hemorrhagic fever viruses Junin, Guanarito, and Machupo. *Virology* 349(2):476–491. <https://doi.org/10.1016/j.virol.2006.02.033>
27. Martin VK, Droniou-Bonzom ME, Reignier T, Oldenburg JE, Cox AU, Cannon PM (2010) Investigation of clade B New World arenavirus tropism by using chimeric GPI proteins. *J Virol* 84(2):1176–1182. <https://doi.org/10.1128/JVI.01625-09>
28. Shao J, Liang Y, Ly H (2015) Human hemorrhagic fever causing arenaviruses: molecular mechanisms contributing to virus virulence and disease pathogenesis. *Pathogens* 4(2):283–306. <https://doi.org/10.3390/pathogens4020283>
29. Radoshitzky SR, Kuhn JH, Spiropoulou CF, Albariño 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 USA* 105(7):2664–2669. <https://doi.org/10.1073/pnas.0709254105>
30. 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 USA* 100(22):12978–12983. <https://doi.org/10.1073/pnas.2133782100>
31. Casabona JC, Livingston 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(14):7029–7039. <https://doi.org/10.1128/JVI.00329-09>
32. Urata S, Yasuda J, de la Torre JC (2009) The z protein of the new world arenavirus tacaribe virus has bona fide budding activity that does not depend on known late domain motifs. *J Virol* 83(23):12651–12655. <https://doi.org/10.1128/JVI.01012-09>
33. Eichler R, Strecker T, Kolesnikova L, ter Meulen J, Weissenhorn W, Becker S, Klenk HD, Garten W, Lenz O (2004) Characterization of the Lassa virus matrix protein Z: electron microscopic study of virus-like particles and interaction with the nucleoprotein (NP). *Virus Res* 100(2):249–255. <https://doi.org/10.1016/j.virusres.2003.11.017>
34. 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(12):6045–6051. <https://doi.org/10.1128/JVI.02392-07>
35. 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(4):1785–1791. <https://doi.org/10.1128/JVI.01362-09>

36. Xing J, Ly H, Liang Y (2015) The Z proteins of pathogenic but not nonpathogenic arenaviruses inhibit RIG-I-like receptor-dependent interferon production. *J Virol* 89(5):2944–2955. <https://doi.org/10.1128/JVI.03349-14>
37. Martínez-Sobrido L, Emonet S, Giannakas P, Cubitt B, García-Sastre A, de la Torre JC (2009) Identification of amino acid residues critical for the anti-interferon activity of the nucleoprotein of the prototypic arenavirus lymphocytic choriomeningitis virus. *J Virol* 83(21):11330–11340. <https://doi.org/10.1128/JVI.00763-09>
38. Zhirnov IV, Ryabinin VA, Sinyakov AN, Ternovoy VA, Shikov AN (2015) A prototype of oligonucleotide microarray for detection of pathogens relating to arena- and Filoviridae families. *Bioorg Khim* 41(1):54–66. <https://doi.org/10.1134/s1068162014050136>
39. Leland DS, Ginocchio CC (2007) Role of cell culture for virus detection in the age of technology. *Clin Microbiol Rev* 20(1):49–78. <https://doi.org/10.1128/CMR.00002-06>
40. Fukushi S, Tani H, Yoshikawa T, Saijo M, Morikawa S (2012) Serological assays based on recombinant viral proteins for the diagnosis of arenavirus hemorrhagic fevers. *Viruses* 4(10):2097–2114. <https://doi.org/10.3390/v4102097>
41. Ure AE, Ghiringhelli PD, Possee RD, Morikawa S, Romanowski V (2008) Argentine hemorrhagic fever diagnostic test based on recombinant Junín virus N protein. *J Med Virol* 80(12):2127–2133. <https://doi.org/10.1002/jmv.21265>
42. Fajfr M, Neubauerová V, Pajer P, Kubíčková P, Růžek D (2014) Detection panel for identification of twelve hemorrhagic viruses using real-time RT-PCR. *Epidemiol Mikrobiol Imunol* 63(3):238–244
43. Pang Z, Li A, Li J, Qu J, He C, Zhang S, Li C, Zhang Q, Liang M, Li D (2014) Comprehensive multiplex one-step real-time TaqMan qRT-PCR assays for detection and quantification of hemorrhagic fever viruses. *PLoS One* 9(4):e95635. <https://doi.org/10.1371/journal.pone.0095635>
44. Trombley AR, Wachter L, Garrison J, Buckley-Beason VA, Jahrling J, Hensley LE, Schoepp RJ, Norwood DA, Goba A, Fair JN, Kulesh DA (2010) Comprehensive panel of real-time TaqMan polymerase chain reaction assays for detection and absolute quantification of filoviruses, arenaviruses, and New World hantaviruses. *Am J Trop Med Hyg* 82(5):954–960. <https://doi.org/10.4269/ajtmh.2010.09-0636>
45. Golden JW, Maes P, Kwilas SA, Ballantyne J, Hooper JW (2016) Glycoprotein-specific antibodies produced by DNA vaccination protect Guinea pigs from lethal Argentine and Venezuelan hemorrhagic fever. *J Virol* 90(7):3515–3529. <https://doi.org/10.1128/JVI.02969-15>
46. Mendenhall M, Russell A, Juelich T, Messina EL, Smee DF, Freiberg AN, Holbrook MR, Furuta Y, de la Torre JC, Nunberg JH, Gowen BB (2011) T-705 (favipiravir) inhibition of arenavirus replication in cell culture. *Antimicrob Agents Chemother* 55(2):782–787. <https://doi.org/10.1128/AAC.01219-10>
47. Pasquato A, Rochat C, de la Torre JC, Kunz S (2011) Combinatorial anti-arenaviral therapy with the small molecule ski-1/s1p inhibitor pf-429242 and ribavirin. *Antiviral Res* 90(2):A62. <https://doi.org/10.1016/j.antiviral.2011.03.122>
48. Rosenke K, Feldmann H, Westover JB, Hanley PW, Martellaro C, Feldmann F, Saturday G, Lovaglio J, Scott DP, Furuta Y, Komeno T, Gowen BB, Safronetz D (2018) Use of favipiravir to treat Lassa virus infection in Macaques. *Emerg Infect Dis* 24(9):1696–1699. <https://doi.org/10.3201/eid2409.180233>
49. Veliziotis I, Roman A, Martiny D, Schuldt G, Claus M, Dauby N, Van den Wijngaert S, Martin C, Nasreddine R, Perandones C, Mahieu R, Swaan C, Van Praet S, Konopnicki D, Morales MA, Malvy D, Stevens E, Dechamps P, Vlieghe E, Vandenberg O, Günther S, Gérard M (2020) Clinical management of Argentine hemorrhagic fever using ribavirin and favipiravir, Belgium, 2020. *Emerg Infect Dis* 26(7):1562–1566. <https://doi.org/10.3201/eid2607.200275>
50. Gehring G, Rohrmann K, Atenchong N, Mittler E, Becker S, Dahlmann F, Pöhlmann S, Vondran FW, David S, Manns MP, Ciesek S, von Hahn T (2014) The clinically approved drugs amiodarone, dronedarone and verapamil inhibit filovirus cell entry. *J Antimicrob Chemother* 69(8):2123–2131. <https://doi.org/10.1093/jac/dku091>
51. Radoshitzky SR, Kuhn JH, de Kok-Mercado F, Jahrling PB, Bavari S (2012) Drug discovery technologies and strategies for Machupo virus and other New World arenaviruses. *Expert Opin Drug Discov* 7(7):613–632. <https://doi.org/10.1517/17460441.2012.687719>
52. Helguera G, Jemielity S, Abraham J, Cordo SM, Martinez MG, Rodríguez JA, Bregni C, Wang JJ, Farzan M, Penichet ML, Candurra NA, Choe H (2012) An antibody recognizing the apical domain of human transferrin receptor 1 efficiently inhibits the entry of all new world hemorrhagic fever arenaviruses. *J Virol* 86(7):4024–4028. <https://doi.org/10.1128/JVI.06397-11>
53. Lee AM, Rojek JM, Spiropoulou CF, Gundersen AT, Jin W, Shaginian A, York J, Nunberg JH, Boger DL, Oldstone MB, Kunz S (2008) Unique small molecule entry inhibitors of hemorrhagic fever arenaviruses. *J Biol Chem* 283(27):18734–18742. <https://doi.org/10.1074/jbc.M802089200>
54. Janosko K, Holbrook MR, Adams R, Barr J, Bollinger L, Newton JT, Ntiforo C, Coe L, Wada J, Pusch D, Jahrling PB, Kuhn JH, Lackemeyer MG (2016) Safety precautions and operating procedures in an (A)BSL-4 Laboratory: 1. Biosafety level 4 suit laboratory suite entry and exit procedures. *J Vis Exp* 116:52317. <https://doi.org/10.3791/52317>
55. Noad RJ, Simpson K, Fooks AR, Hewson R, Gilbert SC, Stevens MP, Hosie MJ, Prior J, Kinsey AM, Entrican G, Simpson A, Whitty CJM, Carroll MW (2019) UK vaccines network: mapping priority pathogens of epidemic potential and vaccine pipeline developments. *Vaccine* 37(43):6241–6247. <https://doi.org/10.1016/j.vaccine.2019.09.009>
56. Azim KF, Lasker T, Akter R, Hia MM, Bhuiyan OF, Hasan M, Hossain MN (2021) Combination of highly antigenic nucleoproteins to inaugurate a cross-reactive next generation vaccine candidate against Arenaviridae family. *Heliyon* 7(5):e07022. <https://doi.org/10.1016/j.heliyon.2021.e07022>
57. 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. *Nat Commun* 9(1):1884. <https://doi.org/10.1038/s41467-018-04271-z>
58. Koma T, Patterson M, Huang C, Seregin AV, Maharaj PD, Miller M, Smith JN, Walker AG, Hallam S, Paessler S (2015) Machupo virus expressing GPC of the Candid#1 vaccine strain of Junin virus is highly attenuated and immunogenic. *J Virol* 90(3):1290–1297. <https://doi.org/10.1128/JVI.02615-15>
59. Hallam SJ, Manning JT, Maruyama J, Seregin A, Huang C, Walker DH, de la Torre JC, Paessler S (2020) A single mutation (V64G) within the RING Domain of Z attenuates Junin virus. *PLoS Negl Trop Dis* 14(9):e0008555. <https://doi.org/10.1371/journal.pntd.0008555>
60. Golden JW, Beitzel B, Ladner JT, Mucker EM, Kwilas SA, Palacios G, Hooper JW (2017) An attenuated Machupo virus with a disrupted L-segment intergenic region protects guinea pigs against lethal Guanarito virus infection. *Sci Rep* 7(1):4679. <https://doi.org/10.1038/s41598-017-04889-x>
61. Kotturi MF, Botten J, Maybeno M, Sidney J, Glenn J, Bui HH, Oseroff C, Crotty S, Peters B, Grey H, Altmann DM, Buchmeier MJ, Sette A (2010) Polyfunctional CD4 + T cell responses to a set of pathogenic arenaviruses provide broad

- population coverage. *Immunome Res* 6:4. <https://doi.org/10.1186/1745-7580-6-4>
62. Kotturi MF, Botten J, Sidney J, Bui HH, Giancola L, Maybeno M, Babin J, Oseroff C, Pasquetto V, Greenbaum JA, Peters B, Ting J, Do D, Vang L, Alexander J, Grey H, Buchmeier MJ, Sette A (2009) A multivalent and cross-protective vaccine strategy against arenaviruses associated with human disease. *PLoS Pathog* 5(12):e1000695. <https://doi.org/10.1371/journal.ppat.1000695>
63. Mattar S, Guzman C, Arrazola J, Soto E, Barrios J, Pini N, Levis S, Salazar-Bravo J, Mills JN (2011) Antibody to arenaviruses in rodents, Caribbean Colombia. *Emerg Infect Dis* 17(7):1315–1317. <https://doi.org/10.3201/eid1707.101961>
64. Castellar A, Guevara M, Rodas JD, Londoño AF, Arroyave E, Díaz FJ, Levis S, Blanco PJ (2017) Primera evidencia de infección por el virus de la coriomeningitis linfocítica (arenavirus) en roedores *Mus musculus* capturados en la zona urbana del municipio de Sincelejo. *Sucre Colomb Biomed* 37(0):75–85. <https://doi.org/10.7705/biomedica.v37i2.3226>
65. Rodríguez-Morales AJ, Suárez JA, Riskey A, Cimerman S, Valero-Cedeño N, Cabrera M, Grobusch MP, Paniz-Mondolfi A (2019) In the eye of the storm: Infectious disease challenges for border countries receiving Venezuelan migrants. *Travel Med Infect Dis* 30:4–6. <https://doi.org/10.1016/j.tmaid.2019.05.014>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.