Mini Review Article

Marburg Virus: Unveiling the Complex Dynamics of Reservoir Hosts and its Far-Reaching Threats to Global Public Health

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Abstract | Marburg virus disease (MVD) is a rare but severe illness caused by the highly pathogenic Marburg virus, an RNA virus, and a member of the *Filoviridae* family. First identified in 1967 in Germany and Serbia, MVD has since been responsible for sporadic outbreaks in Central and East Africa, with the fruit bats of the genus *Rousettus* as the primary reservoir. The largest recorded outbreak occurred in Angola in 2005, resulting in 374 cases and 329 deaths, giving rise to a staggering case mortality rate of approximately 88%. This highlights the global public health significance of MVD. Despite its devastating impact, MVD has not received the attention it deserves, compared to Ebola. The geographical spread of MVD into new regions, including Guinea, Equatorial Guinea, Ghana, and Tanzania, underscores its growing threat. The sporadic outbreaks of this deadly pathogen necessitate continued investment in research, surveillance, and public health preparedness to mitigate its impact on global health security. This review aims to provide a brief overview of our current knowledge of Marburg virus and the threats on global health, which is crucial for the development of effective countermeasures against this devastating disease.

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Introduction

Marburg virus (MARV) is a one single-stranded, negative-sense RNA virus that causes hemorrhagic fever and belongs to the family of viruses known as the *Filoviridae* (Kuhn *et al.*, 2010; CDC, 2021). It is a lipid (fatty) membrane-encased filovirus that can take on a variety of forms. The virus is classified as a Risk Group 4 Pathogen and Category A Bioterrorism agent by the World Health Organization (WHO) and Centers for Disease Control and Prevention, respectively, (USDHHS, 2020; CDC, 2011), hence the challenge in carrying out research with the virus due to availability of only

few category 4 laboratories.

Marburg virus belongs to the genus *Marburgvirus*, family *Filoviridae*, and order *Mononegavirales*, the virus is one of two members of the species *Marburg marburgvirus*. The word "Marburg virus" is a combination of the taxonomic suffix "virus" and the German city of Marburg, where the virus was initially identified (Kuhn *et al*., 2010). Marburg virions, like those of other *Mononegaviruses,* are composed of non-infectious, linear, non-segmented, single-stranded RNA genomes of negative polarity, with inverse-complementary 3' and 5' termini, lacking in 5' cap, polyadenylation, and covalent protein attachment (Pringle, 2005; Srivastava *et al*., 2023). The Marburg virus genomes is made up of seven genes in the sequence encoding for seven open reading frames: nucleoprotein NP, virion protein (VP) 35, VP40, glycoprotein GP, VP40, VP24, and viral polymerase L with a total length of approximately 19 kilobase pairs (Shifflett and Marzi, 2019). The Marburg virus virion containing the filovirus genome is characterized by its filamentous structure with a diameter of 80 nm and a length of 790 to 970 nm contains the filovirus genome (Geisbert and Jahrling, 1995; Abir *et al*., 2022). In addition, its replication and transcription closely resemble those of rhabdoviruses with structural proteins involved in particle maturation and budding, while four are involved in virus transcription and replication (Kolesnikova *et al*., 2002).

Humans and non-human primates alike are susceptible to the uncommon but severe hemorrhagic fever known as Marburg virus disease (MVD), a genetically distinct zoonotic (or, animal-borne) RNA virus of the filovirus family the pathogen that cause MVD. Around 50% of MVD cases end in death on average. In previous epidemics, case fatality rates ranged from 24% to 88% depending on the strain of the virus and case care (WHO, 2021). Both Marburg and Ebola disease are rare but present with similar clinical features and have the potential to cause large-scale outbreaks with a high mortality rate (WHO, 2021).

Healthcare workers have regularly become infected while treating patients with suspected or diagnosed with MVD, especially when infection control protocols are not strictly followed. Transmission via contaminated equipment or needle-stick injuries is related to more severe illness, quicker deterioration, and, presumably, a greater fatality rate (WHO, 2021). The incubation phase lasts for 5 to 10 days, although it could extend up to 20 days. Symptomatic individuals must be regarded as contagious because of the high viral concentrations in their blood, hence the use of suitable safety measures while attending to them (Ulrich *et al*., 2012).

History of marburg virus

In 1967, it was widely believed that MARV originated in Africa, this position was disputable due to lack of credible evidence to support the claim because the virus had not yet been isolated from the implicated monkeys. Doubts on the origin of MARV persisted as interactions between humans and the *C. aethiops* monkeys housed at London's animal shelter did not result in any infections (Werner and Hans, 2007). A comprehensive and in-depth field research conducted in East Africa, the monkeys' original home did not yield any viral recoveries. Subsequently, serologic research was conducted in numerous areas on rodents, humans, and primates samples (Downs, 1993).

Scientific evidence on the source of Marburg virus first came to limelight in 1967 at Philipps University in Marburg, Germany in scientists and laboratory personnel handling samples from African green monkeys which were mainly carriers, but not susceptible to the disease presented with hemorrhagic fever symptoms. Subsequently, samples were collected from the sick people and tested (Takada, 2022), but suspected to be yellow fever (Casals, 1971). To prepare cell cultures, the kidneys of *Cercopithecoid* (Green African; vervet) monkeys were excised and in the process twenty-seven laboratory employees became seriously ill, seven of whom died. A total of four secondary infections were recorded. Further investigation on the identity of the isolates using electron micrographs showed a virus with a distinct form was observed (Peters *et al*., 1971). The illness was then called Marburg disease, and the pathogen, *Marburg virus*. Containment strategies such as strict quarantine of imported monkeys resulted in no new case of the disease among the laboratory employees (Downs, 1993).

The first cases of Marburg outbreak was reported in Africa in February 1975 when a young Australian couple traveling through Rhodesia (Zimbabwe) arrived in South Africa sick (Gear *et al*., 1975). The young male passed away and the young woman recovered after they

were admitted to the Johannesburg hospital. While tending to them, a nurse was infected but recovered afterwards (Downs, 1993). MARV was discovered at a time when the development of biochemical tools greatly increased our understanding of the structure and reproduction of numerous viruses. However, due to its high pathogenicity, such investigations on MARV were challenging to conduct. Only 20 years later, with the development of recombinant DNA technology and the availability of suitable biosafety containment measures, was a breakthrough achieved (Werner and Hans, 2007).

Marburg virus epidemiology

Following simultaneous outbreaks in numerous European laboratories, including one in Marburg, Germany, in 1967, the Marburg virus, which is the first filovirus was identified. It was inferred that contact with sick African green monkeys was the most likely source of initial transmission to humans. The initial diagnosis was followed by 31 cases that were attributed to human-to-human transmission. According to various reporting sources, there have been between 500 and 600 human cases documented worldwide as of today (WHO, 2021; CDC, 2023a; UKHSA, 2023).

African countries such as Angola, the Democratic Republic of the Congo, Equatorial Guinea, Kenya, Ghana, Uganda, South Africa and Tanzania have all reported outbreaks and sporadic cases; the largest outbreak was the Angolan case in which 374 cases and 329 fatalities were reported (UKHSA, 2023). There were a few rare occurrences in addition to two epidemics, one in the Democratic Republic of the Congo from 1998-2000, with 154 deaths, and in Angola occurring from 2004-2005 with 252 deaths recorded. Between 2007 and 2017, Uganda also saw a few minor epidemics (WHO, 2017, 2021).

Recently, MVD outbreaks were reported in February and March of 2023 in Equatorial Guinea and Tanzania, respectively. A total of 8 laboratory-confirmed cases, one probable case that resulted to 6 deaths were reported in Tanzania (WHO, 2023a). In Equatorial Guinea, a total of 17 confirmed and 23 suspected cases with 35 fatalities were reported (WHO, 2023b). In 2022, Ghana reported its first-ever confirmed cases. Between June 28 and August 5, 2022, there were three documented cases, including two fatalities all of which occurred at the same home. On September 16, 2022, a pandemic was declared over (WHO, 2022). In August 2021, the first documented case in West African was reported in Guinea as a single case report, however the patient died afterwards (WHO, 2021).

Transmission and symptoms

The virus is known to be transmitted to humans through direct or indirect contact with Rousettus bats (Towner *et al*., 2008). Direct human exposure can occur through contact with infected bats, their tissues, or contaminated materials (Nyakarahuka *et al*, 2017). The virus is transmitted through semen as it can remain up to 7 weeks after clinical remission. Subsequent human to human transmission occurs through direct contact with the infected person's bodily fluids such as blood, saliva, vomit, urine, feces, respiratory secretions, and semen (WHO, 2021). Marburg virus Reservoirs, like African fruit bats, can transmit the disease to one another by direct contact, sexual transmission, or biting. Humans and non-human primates (NHPs) may become infected with the virus through direct contact with reservoir hosts or through eating virus infested fruit (Abir *et al*., 2022). Direct contact between NHPs and humans can result in transmission, and ingestion of poorly processed wild animals harvested for consumption can result in direct contact between NHPs and humans (Abir *et al*., 2022).

Subsequent to infection, the virus incubation period is around 2–21 days after which clinical manifestation begins with high fever (> 39°C) accompanied by intense chills, headache, muscle, joint pain and profound malaise. Gastrointestinal symptoms: Nausea, vomiting, and diarrhea can lead to dehydration, exacerbating disease severity, and respiratory symptoms: Chest pain, cough, and sore throat may occur. Severe Cases and Neurological Symptoms may include hemorrhagic manifestations, such as bleeding, which informed the earlier name as Marburg hemorrhagic fever. Neurological symptoms: Confusion, agitation, and seizures may occur in advanced stages, and multi-organ dysfunction may lead to shock, with a case fatality rate ranging from 24% to 88% (Srivastava *et al* 2023). Early detection and isolation of cases are essential to prevent further spread of the virus in affected communities.

Reservoir hosts for marburg virus

A species that a disease such as Marburg Virus endemically circulates in and is thought to have

coevolved with is referred to as a reservoir host (Ghai *et al*., 2021). The Marburg virus (MARV) natural reservoir has been identified as Egyptian *rousette* bats (ERBs, *Rousettus aegyptiacus*), cave-dwelling fruit bats that are widespread in sub-Saharan Africa and portions of the Middle East (Jonathan *et al*., 2021). It has been proven beyond reasonable doubts that the majority of MARV strains were found in *Rousettus aegyptiacus* species. Example, in 2005, three MARV strains were isolated from Gabon (Towner *et al*., 2007); one from Kenya (Kuzmin *et al*., 2010) and ten from Uganda in 2007 (Towner *et al*., 2009); and most recently, five- from Sierra Leone (Amman *et al*., 2020).

Other bat species in which filovirus genome RNAs including Marburg viruses have been recovered are *Miniopterus inflatus* and *Rhinolophus eloquens* (Katendi *et al*., 2014). Although the ecology of filoviruses is still poorly known and bats are seemingly a major source of filovirus transmission (Katendi *et al*., 2014) the involvement of other animal species, such as pigs, dogs (Allela *et al*., 2005), duikers and non-human primates (Leroy *et al*., 2004a, b) are undoubtedly real. Monkeys can be infected by Marburg virus, become ill and most often die very quickly, they are seldom thought of as reservoir hosts. In addition, pigs are surprisingly susceptible to the filovirus, Reston virus (RESTV) that is non-pathogenic in humans but can shed the virus thereby increasing the potential risk of spillover of pathogens to human populations especially to pig farmers (Lewis *et al*., 2024).

Generally speaking, it is believed that bats and a variety of possible infectious organisms have co-evolved and circulated for thousands of years, with more zoonotic pathogens recently spreading to humans owing to human invasion into previously uninhabited places (Smith and Wang, 2013; Wibbelt *et al*., 2010). Climate change, urbanization, agricultural and mining activities amongst other factors impact wildlife habitats and densities in a manner that drive the risk of human exposure to reservoir hosts of viral pathogens (Mills *et al*., 2010).

Marburg virus disease (MVD): An emerging public health threat

Marburg virus disease is a significant public health threat because it is highly contagious, fatal, and does not currently have approved treatment modalities. The difficulty in establishing the danger posed by Marburg virus disease threatens early detection and diagnosis, and its symptoms are similar to those of malaria, typhoid, and other febrile illnesses Florez-Alvarez et al. (2022). However, early detection is pivotal to successful and effective management of this disease. World globalization with increasing mobility of people and goods heightens the risk of Marburg virus transmission beyond the African continent posing threat to global health security. There are currently limited known medical countermeasures, diagnostic test kits are not readily available, including a lack of bio-containment laboratories. However, the virus infection can be detected in blood samples through various ways such as antibody-capture enzyme-linked immunosorbent assay, antigen-capture detection tests, serum neutralization test, reverse transcriptase polymerase chain reaction assay, electron microscopy, and perhaps virus isolation by cell culture.

There are currently no licensed vaccines or treatments, making outbreak response and control challenging. The treatment of the infected person is mainly supportive and symptomatic. The absence of specific treatment measures and the high fatality rate make the virus a global health threat. Similarly, due to the virus's high virulence and pathogenicity and potential for aerosol transmission, the virus is considered a potential bioterrorism.

Marburg virus: Pathogenesis and zoonotic threat with pandemic potential

The Marburg virus, a member of the Filoviridae family, is a zoonotic virus that poses a significant threat to global health. The Marburg virus is a single-stranded, enveloped, non-segmented, negative-sense RNA virus that belongs to the genus Marburg virus. Its genome encodes for seven structural proteins, including nucleoprotein (NP), virion protein 35 (VP35), VP40, VP30, VP24, glycoprotein (GP), and large (L) viral polymerase (Muhlberg, 2007). Each gene has conserved transcriptional start and stop signals and plays important roles in viral replication and pathogenesis. NP binds to viral RNA, forming the nucleocapsid, while VP35 aids in RNA synthesis and immune evasion. VP40 facilitates viral assembly and budding, whereas GP mediates host cell attachment and entry and is a potential vaccine and therapeutic target. VP30 assists in transcription activation, VP24 regulates viral replication, and L functions as the RNA-dependent RNA polymerase for genome replication and transcription.

During replication, MARV initially attaches to host cells *via* GP, enters through endocytosis or membrane fusion, and releases its genomic RNA into the cytoplasm. The polymerase then transcribes mRNA from the negative-sense genome, which is translated into viral proteins by the host cell machinery. The viral genome replication occurs through the production of positive-sense RNA intermediates, which act as a template for generating new viral genomes and mRNA. Viral assembly takes place at the host cell membrane, facilitated by VP40 and GP, leading to budding of mature virions (Abir *et al*., 2022; Schmidt and Muhlberger, 2016). As an RNA virus, Marburg virus is prone to mutation, which can increase its: virulence; transmissibility; mortality rate; and morbidity rate. The recent outbreak in Equatorial Guinea, which spread to Tanzania, highlights the virus's potential to spread across borders. The Marburg virus's genetic similarity to the lethal Ebola virus and its high mortality rate makes it a concern for healthcare professionals and the general population worldwide (Scarpa *et al*., 2023). The Marburg virus poses a significant threat to global health due to its zoonotic nature, mutation potential, transmission dynamics, and pandemic potential. Enhanced surveillance, rapid response, and international collaboration are essential to preventing the spread of this deadly virus.

Diagnosis and treatment

Given the high risk of rapid disease progression and severe outcomes associated with Marburg virus (MARV) infection underscores the significance of timely diagnosis for effective patient management and infection control. The diagnosis of MVD can be challenging due to: (i) Presentation of non-specific symptomatology that is similar to those of other infectious diseases, (ii) Limited diagnostic capabilities in the resource-limited settings, diagnostic capabilities pose a serious challenge to early detection, may be limited, making it difficult to confirm MVD diagnosis, (iii) High chance of misdiagnosis exists without laboratory testing, which may lead to delayed or inappropriate treatment.

Laboratory testing for MVD includes: (1) Reverse transcription polymerase chain reaction (RT-PCR): A molecular test that detects MARV RNA in blood or tissue samples. (2) Enzyme-linked immunosorbent assay (ELISA): A serological test that detects MARV-specific antibodies in blood samples. (3) Virus isolation: A test that involves growing the virus in a laboratory using blood or tissue samples. The samples should be tested in maximum biological containment conditions. The rapid diagnostic tests are essential for early disease identification and outbreak control in resource-limited settings. There are no specific antiviral therapies or vaccines approved presently for MARV, fluid and electrolyte replacement, symptomatic relief, and intensive care support may improve the condition significantly. Advances in supportive care have improved the survivability of the infected individuals. Although there is no authorized vaccination against MVD (Rougeron *et al*., 2015), phase 1 clinical trials are underway for some potential MARV vaccines, including cAd3, MVA-BN-Filo, and MARV DNA. There is an urgent need to develop of effective and potent vaccines and treatment for this deadly disease in light of the documented outbreaks and potential future risks (Dulin *et al*., 2022).

Prevention and control of marburg virus disease

Marburg Virus Disease (MVD) is a rare viral hemorrhagic fever caused by a family of viruses known as *marburgviruses* and can cause severe disease (CDC, 2023b). In the absence of vaccines for MVD, implementing preventive measures is crucial to combat outbreaks of Marburg virus infection. Since research on Marburg virus transmission from wildlife to humans is evolving, preventive methods against infection are not well characterized and understood. To prevent person-to-person transmission, healthcare professionals must take stringent precautions when interacting with infected patients. Infection Prevention and Control (IPC) measures include: quick isolation: Immediately isolating infected patients to prevent further transmission; personal Protective Equipment (PPE): Wearing masks, gloves, and gowns to create a barrier against viral transmission; proper use of protective materials: Ensuring the correct use of protective materials, such as disposable sheets and towels; disinfection and sterilization: Regularly disinfecting and sterilizing equipment, surfaces, and instruments to prevent viral contamination; safe disposal of infectious waste: Properly disposing of infectious waste, including needles, syringes, and other contaminated materials. The cornerstone of preventing filovirus disease is limiting contact with contaminated objects, animal vectors, and human Marburg virus disease cases' bodily fluids, such as breast milk and semen from survivors, which can carry the infection for weeks or months after recovery (Heeney, 2015). One major strategy to guard against

infection is to stay away from fruit bats and ill non-human primates around the central part or the entire continent of Africa (Africa CDC, 2023), avoiding handling human remains after they have died from suspected or confirmed cases of Marburg virus disease and cremating them instead of burying them are two examples of this strategy (CDC, 2022).

The prevention of healthcare workers from the Marburg virus infection is critical to entire public health ecosystem, and this involves: Adherence to established IPC protocols and guidelines, use of PPE correctly and consistently when interacting with infected or suspected patients, and practicing of regular hand hygiene using soap and water or hand sanitizer. By implementing these preventive measures, healthcare professionals can reduce the risk of MVD transmission and protect themselves, patients, and communities from this deadly disease.

Few laboratories are equipped to conduct basic and applied research to create prophylactics and treatments against the deadly virus MARV, as the virus can only be handled in BSL-4s. Therefore, it is essential to equip research facilities with maximum containment laboratories to manage MARV. Everyone should take away One Health lesson from the SARS-CoV-2 pandemic- that the environment, animals, and humans are the three interconnected factors essential to the prevention and control of zoonotic diseases like MVD (Islam *et al*., 2023). In light of the COVID-19 pandemic and the present global health issue of the rising occurrences of monkeypox, a quick, interdisciplinary effort is needed to incorporate and investigate such incidences of MVD before an unwanted fast spread of MARV happens in other regions and countries (Islam *et al*., 2022).

Conclusion

The recent MVD outbreak with a high case fatality rate is a threat to the global public health, even as the world is already struggling to cope with the recent COVID-19 pandemic. The rapid human-to-human spread makes it a potential global pandemic virus. Therefore, the world health organization, Healthcare authorities, and policymakers need to take urgent and appropriate countermeasures to prevent and control the spread of MVD. Major stakeholders across global public health experts, including researchers, should remain actively vigilant about potential MVD outbreaks and conduct research to create effective treatments and suggest preventative practices. Above all, public health experts should engage in community outbreak preparedness, apart from healthcare professionals.

Novelty Statement

This mini-review highlights the global public health significance of MVD, prevalence and the application of such knowledge in the development of effective countermeasures against this devastating disease.

Author's Contribution

All Authors contribute equally.

Ethical approval
Not applicable.

Conflicts of interest
The authors have declared no conflict of interest.

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