Past and current advances in Marburg virus disease: a review

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SUMMARY

Marburg Virus (MARV), along with the Ebola virus, belongs to the family of *Filovirus* and is cause of a lethal and severely affecting hemorrhagic fever. The *Marburgvirus* genus includes two viruses: MARV and Ravn. MARV has been recognized as one of utmost importance by the World Health Organization (WHO). The case fatality rate of the virus ranges from 24.0 to 88.0% which demonstrates its lethal nature and the need for its widespread information.

The first case of the Marburgvirus disease (MARD) was reported in 1967 when lab personnel working with African green monkeys got infected in Germany and Serbia simultaneously. Following the initial case, many more outbreaks occurred around the world such as Uganda, Angola, Congo, Kenya and even in the United States in 2008. It was soon found out that the MARV was a zoonotic virus and mainly contracted from animal-to-human contact and further transmitted via human-to-human contact. The Egyptian fruit bat (*Rousettus aegyptiacus)* is known to be one of the significant sources of the infection and tourists visiting caves inhabited by these bats or workers accessing mines, populated by the bats, are at an increased risk of contracting the illness. The incubation period ranges from 2-21 days and the clinical outcome can be broken down into three phases: initial generalized phase (day 1-4), early organ phase (day 5 to 13) and either a late organ/convalescence phase (day 13 onwards).

Furthermore, the treatment of MARD is solely based on supportive care. Much has been investigated in over the past half-century of the initial infection but only a few treatment options show promising results. In addition, special precaution is advised whilst handling the patient or the biospecimens. Disease-modifying agents and inhibitors of viral replications show constructive outcomes.

It is crucial to identify the host of the virus and educate the populations that are greatly at risk of the disease. While much is being investigated to devise a vaccine, it is important to educate Health Care Workers (HCWs) and close contacts facing the illness. Stopping the transmission remains the best measure that can be taken.

Keywords: Marburg virus, Ebola virus

INTRODUCTION

Marburg virus (MARV) is one of two viruses of the *Filovirus* family in the order of Mononegavirales which, along with Ebola virus (EBOV), can cause a severe and fatal Marburg disease (MARD). The genus *Marburgvirus* includes a single species, *Marburg marburgvirus*, which is represented by two distinct viruses, MARV and Ravn virus (RAVV) [1]. Both the EBOV and MARV genomes are about 19,000 nucleotides long and are transcribed into eight major sub-genomic messenger RNAs (mRNAs), encoding for seven structural proteins [2]. Both viruses are classified as category A pathogens by the Centers for Disease Control and Prevention (CDC) and select agents, with Marburg being rated as a Risk Group 4 Pathogen (requiring biosafety level 4-equivalent containment) by the

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World Health Organization (WHO) [3]. The morphological traits of MARV when studied under a transmission electron microscope showed pleomorphism, with filamentous, rod-shaped, cobra-like, spherical, and branch-shaped particles of uniform diameter but different lengths [4]. MARV was first discovered in 1967, when outbreaks of hemorrhagic fever occurred simultaneously in laboratories in Marburg and Frankfurt in Germany, and Belgrade in Yugoslavia (now Serbia). A total of 31 people became ill, including 25 laboratory workers, and medical personnel and a family member who had cared for them [5].

MARV is a zoonotic (animal-borne) virus and its reservoir is the Egyptian fruit bat (*Rousettus aegyptiacus*) [6]. Previous literature shows that majority of the primary infections of natural MARV disease outbreaks so far have been linked to human entry into caves inhabited by bats (*e.g.*, cave visitors, mine workers) [7]. After an initial zoonotic transmission from an infected animal to a human occurs, the transmission is then amplified through close human-to-human contact. This can be through direct contact with bodily fluids and through contact with contaminated fomites (objects or materials that are likely to carry infection) [8].

Its clinical manifestations include abrupt onset of high fever, severe headache and severe malaise followed by severe watery diarrhea, abdominal pain with cramping, nausea and vomiting. Many patients develop severe hemorrhagic manifestations later as the disease progresses. Both the *Filoviridae* are associated with high case fatality rates (CFR). WHO reports that the CFR of EBV ranges from 25.0 to 90.0% while that of MARV ranges from 24 to 88% [9]. As of March 2018, there have been thirteen outbreaks of MARV disease, most occurring in sub-Saharan Africa; the largest of which occurred in Angola during 2004-2005 and had a case-fatality rate of 90% [10, 11]. In lieu of MARV posing a potential and severe threat to public health and safety, systemic surveillance is required to overcome its recurrence and rising mortality rates.

Considering the regular epidemics and a very recent pandemic, it is important to highlight the burden even of the rare diseases, primarily diseases such as MARD, which do not have a definitive treatment with a high case-fatality rate. Hence, rare diseases with the possibility of frequent outbreaks must be addressed, so more clinical trials must be conducted to make a treatment regimen for such life-threatening diseases. Therefore, we wrote this comprehensive review of viral pathogenesis, clinical manifestation, management, and the advances made in efforts to combat such a disease.

METHODS

A literature search was conducted using PubMed and Google Scholar from their inception to November 2019 for this review. In order to refine the search, the following keywords were incorporated: (Marburg virus) AND ("epidemiology" OR "virology" OR "pathogenesis" OR "vector" OR "transmission" OR "reservoir" OR "symptom" OR "diagnosis" OR "management" OR "treatment" OR "vaccination" OR "prevention"). Any articles that were found in languages other than English were excluded.

The initial search included 2245 articles. 578 duplicates found between Google Scholar and PubMed were excluded. A total of 1667 titles and abstracts were scanned, of which 156 studies were found relevant. A further 14 exclusions were made as full texts were not available for these. 142 full texts were then retrieved, and a further 84 exclusions were made as the studies were beyond the scope of this review. A total of 58 studies were included in this final review.

Epidemiology

The first outbreak of the MARD was reported in 1967 when the laboratory personnel working with grivets (*chlorocebusaethiops*) which are green African monkeys, brought in from Uganda, got infected in Germany and Serbia (former Yugoslavia). The grivets were reported to have been infected with MARV and the personnel mainly contracted the disease due to the direct handling of the tissue and organs of these infected wild animals [12, 13]. A total of 31 patients [25 primary, six secondary infections] developed a severe illness which then resulted in seven of the patients dying of the disease [14]. Following the initial outbreak, more cases emerged. Some of these cases supported nosocomial transmission whilst only one case was reported that showed seminal transmission. A woman was reportedly infected from her hus-

band's semen, who was already infected for almost three months [12].

In 1975, in Zimbabwe/South Africa three new cases were reported. Amongst them was the index patient who was on a visit to the Senoia caves in Rhodesia (now Zimbabwe) with other tourists slept in places where insectivorous bats resided [13, 15]. Their itinerary was a leading evidence for bridging direct contact of bats and their discharge as the cause of contraction of the disease [12].

In 1980, another outbreak was reported in Kenya. The infected patient was known to have visited many small forested areas on multiple occasions and fed the forests' mammals and birds. Prior to the development of the illness, the patient had also visited the Kitum cave with a huge population of bats live at Mount Elgon National park. The forest visits were most likely the reason of his direct contact and exposure to wild animals and bats that are known to be the reservoirs of the virus. Furthermore, while the doctor was resuscitating the patient, he slightly developed certain symptoms but eventually recovered [12].

After seven years, another outbreak took place in 1987 in Western Kenya. The index case was a 15-year-old Danish boy who visited the Kitum cave and was suspected to have direct contact with bats or to bat discharge which led to him getting infected [12, 13, 16]. This fatal case was characterized by the appearance of fever and anorexia within 4-7 days and death by day 11 [17].

Additionally, in 1988, 1991 and 1995 due to laboratory accidents in Russia known MARD cases occurred and 1 out of 3 cases lead to death [12, 18-20].

From 1998-2000 another outbreak occurred in the Durba-Watsa region of Democratic Republic of Congo, in a forsaken gold mine due to multiple genetically distinct virus strains [7, 13]. The primary cases for the aforementioned infection were the miners who worked in an underground mine instead of on the open land mines. A total of 154 patients was affected by the disease (106 suspected cases) and the disease fatality estimated out to be 83%. The infection was transmitted from the miners to close family contacts and occasionally to healthcare workers. Most cases appeared in Durba village, but few cases were also reported from nearby villages and areas where patients received their treatment. The short chain of human-human transmission and reports of even sporadic cases supported the theory that there were repeated infections. This was further confirmed when 9 genetically different virus strains were found out to be the source of transmissions of the disease during the outbreak. Furthermore, it became apparent that there had been cases in association with the mine in 1987 that were unreported. These cases were previously called as 'hemorrhagic syndrome' of Durba [7, 21]. According to Languon S. et al., gold mine workers had exposure to the fauna littered near the mine. The mine had variety of animals such as rodents, bats, frogs, shrews, cockroaches, and mouth flies; however, there was no report of direct exposure or insect bites in the mine to the infected individuals [12, 21]. Moreover, the work environment of the miners was unhygienic, they had simple hand tools and no protective gear as such and were in direct contact with the surroundings stained with human and bat excreta [12].

The largest MARD outbreak till date has been reported between the years 2004 and 2005 in Western Africa, notably in Uige, Angola [12, 13, 15]. Around 252 cases were reported, out of which 227 died [13]. However, the origin was not known, and the efforts put into the study have been unsuccessful [12].

In 2007, in Uganda, an outbreak took place where only four cases were confirmed. The patients were workers of Kitaka mine, in the Ibanda District. The two co-workers were infected by sharing a tent camp in the Kashoya-Kitomi Central Forest Reserve near the mine with the index case. The fourth patient got the communicable disease by working in the mine without any Personal Protective Equipment (PPE) at the time of outbreak. The tunnel of mine was encompassed by the bats and the PPE was only by gloves, with no provision of masks, respirators or goggles. The cause behind the contraction was mainly the direct contact with the bats or bat discharge. During this outbreak, the first definitive Filovirus reservoir was identified through the sampling of bats and MARV was isolated from *Rousettusaegyptiacus* [12].

In 2008, one fatal case of MARD occurred in the United States of America (USA) and the Netherlands. In both the cases, it was an imported infection from the visit to a cave in western Uganda [7, 13, 22]. The US tourist indulged in activities related to camping, visiting local villages

and visiting of wildlife. The infection could have most likely been due to the exposure to wild animals. Similarly, a Dutch tourist was infected by visiting two caves in Uganda, the Python cave and a cave without bats - and had an experience to come as close as 5 mm to gorillas in the wild. Her visit to the Python Cave where bats species resided was speculated to be the cause of her infection [12].

On 29th November 2012, in Uganda, MARV infection was reported by the Ugandan Ministry of Health. Approximately 15 deaths and 8 probable cases in the Kabale, Ibanda, Mbarara and Kampala districts of Uganda were documented [14]. This outbreak in the Ibanda district was the same where the Kitaka mine was and 2007 outbreak of MARV disease occurred. This led to the linkage of the *Rousettus aegyptiacus* bats again to the outbreak in 2012. Interestingly enough, the outbreak took place at the time when the bat populations, *Rousettus aegyptiacus*, had the second bi-annual virus circulation [12].

In 2014, a new case was again reported in early October. Three weeks after the first case that proved fatal, a total of 197 new cases were reported. Among them, 8 cases developed symptoms similar to Marburg but when tested they were declared to be negative at the Uganda Virus Research Institute with the support from CDC. On 13th November 2014, the Ministry of health of Uganda declared Uganda free of MARV. Three years later, on 19 October 2017, in Kween District, Eastern Uganda, the Ministry for Health once again declared an outbreak [23]. This was the first outbreak of MVD that was detected in this part of the country, since the rest of them were reported in the western part of Uganda [24]. In addition, this outbreak was geographically linked to the 1980 outbreak and occurred within a single family; out of four people infected, three of them died of the disease. However, within a month, due to the trained and professional national and district teams, this outbreak was handled. Through investigations it was known that the index case was a herdsman who used to be involved in hunting games in a sub-country and that area had caves encompassed by huge populations of Egyptian fruit bats [12]. The global distribution and outbreaks of MARV have been shown in Table 1 and Figure 1.

Table 1 - Outbreaks of MARV from 1976 to 2017.

Location	*Year*	*Cases/ Deaths*	*Epidemiology*
Germany/ Serbia	1976	32/7	Infection occurred due to the tissues from monkeys imported for research from Uganda.
Zimbabwe	1975	3/1	Unknown origin, the lethal index case was infected in Zimbabwe on a visit to the Senoia caves in Rhodesia.
Kenya	1980	2/1	Unknown origin, Index case, visited the Kitum cave before the illness which had a huge population of bats.
Kenya	1987	1/1	Expatriate traveling in western Kenya.
Russia	1988	1/1	Laboratory accident
Russia	1991	1/1	Laboratory accident
Russia	1995	1/0	Laboratory accident
Democratic of the Congo Angola	1998-2000	154/128	Infections were related to mining.
Angola	2004-2005	252/227	Unknown cases: cases linked to Uige Hospital
Uganda	2007	4/1	Workers of Kitaka mine
USA	2008	1/0	Infection from the visit to a cave in Western Uganda.
The Netherlands	2008	1/1	Infection from the visit to a cave in Western Uganda.
Uganda	2012	32/15	Outbreak in the Ibanda district same as where the Kitaka mine was
Uganda	2014	198/1	Unknown origin.
Uganda	2017	–	Unknown origin.

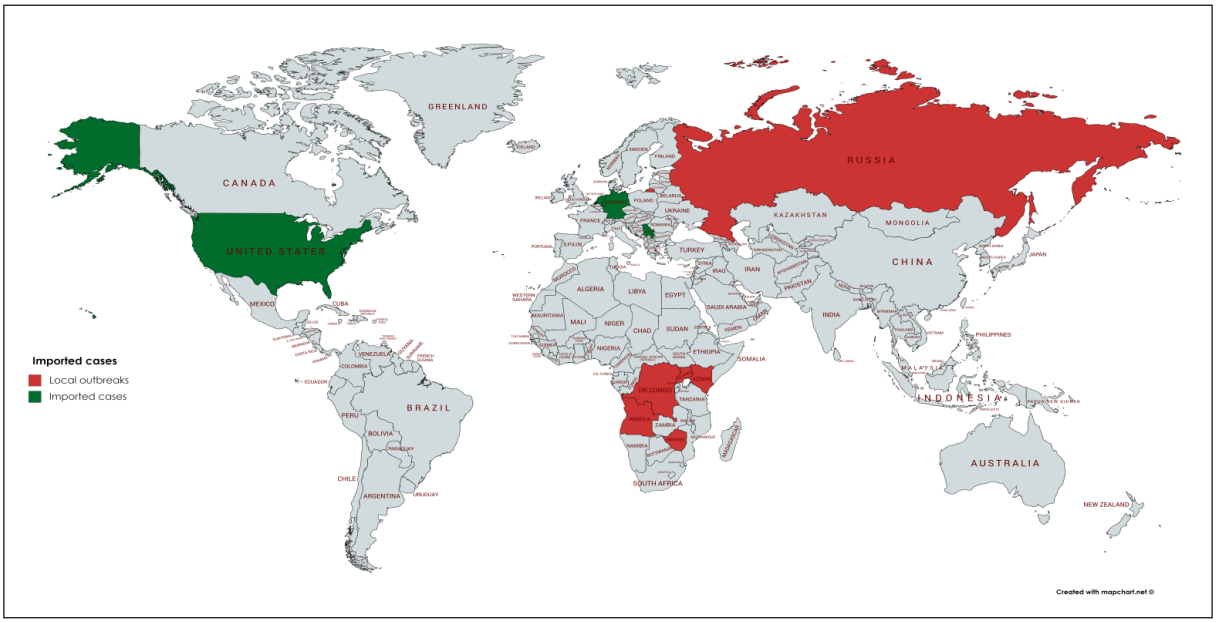

Figure 1 - Global distribution of MARV.

Vectors, transmission and reservoirs

The first case of the MARV emerged to the surface in 1967 amongst three people working in a factory where vaccines and sera were produced and, therefore, had a history of direct contact with blood, parts of the organs or cell cultures of green monkeys or their organs. Consequently, three other patients in Frankfurt, Germany were also reported to contract a similar disease by handling material from the same group of monkeys from Uganda. An additional number of people infected with the virus came upfront and, by tracing their contact history, it was established that some of these patients had assisted to the killing or post-mortem dissecting of the monkeys; others had trephined their skulls and the remaining also had some kind of contact with infected monkeys. Surprisingly, a female patient was infected by her husband via sexual intercourse as was proved by the antibody fluorescence that the sperm hosted infectious material [25]. Unfortunately, WHO has also highlighted this rampant issue of sexual transmission as the disease remains transmissible by infected semen up to seven weeks after clinical recovery. Moreover, a transmission that occurs via contaminated injection equipment or through needle-stick injuries is associated with a significantly greater proportion of severe disease [26].

Moving on, Brainard J. et al. have discussed major aspects of behavioral and cultural practices that have evolved as an evident contributory factor for MARV transmission. The findings of his review reinforce three behaviors that add to the virus spread:

1) close contact in the later stages of infection;
2) caring for a sick person;
3) when preparing the recently deceased for burial.

Furthermore, to clear the air, the article stated that firstly, there is no evidence of risk associated with casual contact with asymptomatic individuals outside the home. Secondly, household contacts with no direct physical contact, the risk imposed on disease transmission are 1%. Additionally, there is a negligible risk of contracting the disease during the in cubation period and only low risk in the first week of symptomatic illness. Another crucial aspect of disease transmission is associated with funerals, which appears to most often follow after touching the body of an infected case [27]. Lastly, the presence of MARV in the area of Durba/Watsa was also investigated which exemplified that the preponderance of antibody in male miners without obvious evidence for person-to-person transmission directed towards the local mines as a site of primary infection with MARV, most likely through exposure to the primary zoonotic reservoir [28].

Virology

MARV is an enveloped, non-segmented, negative-sense, single-stranded RNA (ssRNA) virus with an average particle length of 795-828 nm [29]. The virions are said to be pleomorphic as they can appear either in long filamentous forms or in short U-shaped, six-shaped, or circular configurations. Genomes of the various MARV isolated range in size from 19,111 to 19,114 nts and contain seven monocistronic genes in a linear order that encode for seven structural proteins, namely, nucleoprotein (NP), polymerase cofactor (VP35), matrix protein (VP40), glycoprotein (GP), replication-transcription protein (VP30), matrix protein (VP24), and RNA-dependent RNA polymerase (L) [7].

At the core of the virion lies the helical ribonucleoprotein complex, which consists of the genomic RNA molecule wrapped around by NP, which is linked to the inner matrix proteins VP30, VP35, and the L protein. This complex is involved in the transcription and replication process. Furthermore, the ribonucleoprotein is embedded in a matrix, formed by VP40 (major) and VP24 (minor) matrix proteins whose roles include viral nucleocapsid formation, viral budding of assembly, and host range determination [30]. Finally, a host-derived envelope surrounds the nucleocapsid that has spikes like protrusions on the surface of 5-10 nm in length, which are formed by trimers of the GP. GP is an integral membrane protein responsible for virus entry into susceptible cells through attachment, receptor binding, and fusion. Moreover, it also plays a key role in the pathogenesis, by affecting the immunogenicity and inducing neutralizing antibodies [31, 32].

Pathogenesis

MARV primarily causes hemorrhagic fever which is remarkably severe and associated with high case fatality rates often exceeding 80 percent. In addition to humans, these viruses have annihilated Nonhuman Primates (NHP) causing a similar lethal presentation [33, 34]. Thus, owing to this emergent call, MARV was quickly highlighted as a pathogen of utmost global importance and is currently classified as a Risk Group 4 pathogen by the WHO and as a Select Agent by the CDC [34].

MARV and disease modeling with laboratory animals are a widely known technique to study the pathogenesis and host immunological responses. Currently, four MARV disease models have been developed using immunocompetent animals; NHPs (mainly cynomolgus and rhesus macaques, African green monkeys and baboons), hamsters, guinea pigs, and mice. Of these models, NHPs are the 'gold standard'; as they are highly susceptible to MARV infections, with almost 100% lethality, and display hallmark pathological features similar to those seen in human infections. Furthermore, direct transmission of MARV between NHPs by close contact has been reinforced [35].

In 1987, Kenyan case immunohistochemical and electron, microscopic examinations detected viral antigen and virions in both circulating and tissue-associated macrophages, as well as flow cytometric analyses, which revealed MARV infection in macrophages of the peripheral blood mononuclear cell population of infected macaques [13]. Therefore, these have postulated the base that the cells of the mononuclear phagocytic system, including macrophages, monocytes, Kupffer cells, and dendritic cells are the early target cells of MARV infection. Moreover, most severe necrotic lesions were observed in the lymph nodes, liver, and spleen. Since the aforementioned organs contain high numbers of reticuloendothelial cells, migration of infected cells facilitates the dissemination of multiple organs cultivating a systemic infection. Other cell types infected include hepatocytes, adrenal cortical and medullary cells and fibroblasts; endothelial cells are late target cells during MARV infection in multiple tissues [7].

At the organ level, liver and lymphoid tissues are the main targets for MARV; liver is also an important site for MARV replication [34]. The lymphatic tissue exhibits plasma cellular, monocytoidal transformation. Adjacent to the areas of necrosis basophilic bodies are also found along with necrotic cells or as inclusion bodies in parenchymal cells. However, none of the other organs are spared and present with pathological changes upon infection, such as focal or disseminated necrosis with a lack of significant inflammatory responses. Renal dysfunction presenting as proteinuria is frequently observed in MARD patients. Grossly, the affected kidneys are pale, swollen and indicate grave parenchymal damage associated with signs of tubular insufficiency. The mucous membranes of the stomach and intestines have a high number of plasma cells and monocytes. The alveoli of the lungs are congested, hemorrhaged, and contain alveolar macrophages surrounded by fibrin, that

occasionally stain positive for viral antigen. Few cases of orchitis have also been reported along with necrosis in testicles and ovaries. Furthermore, clouded consciousness associated with aggressiveness and glial nodule encephalitis are also reported in MARD patients [34, 36].
In humans, microscopically there is marked necrosis of the follicles and medulla of the lymph nodes and the red pulp of the spleen, as well as depletion of lymphocytes. Surprisingly, the virus does not infect lymphocytes, the phenomena of bystander apoptosis supports the depletion of lymphocytes. Moreover, the asialoglycoprotein receptor is a liver-specific receptor capable of enhancing MARV infection, thus the elevation in liver enzymes such as aspartate aminotransferase, alanine aminotransferase, serum glutamic oxaloacetic transaminase, and serum glutamic pyruvic transaminase is characteristic of MARV infections. Also, since multiple clotting factors are synthesized in the liver, the pathological liver changes probably contribute to the coagulation defects observed during MARV infection. Thus, it potentiates the mass effect of the lethal virus that triggers multiorgan failure observed in severe MARD cases. Besides, the involvement of the adrenal gland and its failure with impairment of steroid-synthesizing enzyme production predisposes to hypotension and hypovolemia, leading to shock [13].
This noxious hemorrhagic fever shows signs of hemorrhagic diatheses in the skin and mucous membranes which is not due to jaundice. The histopathological changes in the skin tissue consist mainly of various degrees of dermal edema, focal hemorrhage as well as endothelial cell swelling and necrosis. Abundant antigens are demonstrated via immunohistochemical stains in the epidermal dendritic cells, endothelial cells, and connective tissue fibroblasts. These antigens are also found in the epithelium of sweat and sebaceous glands. By using electron microscopy, viral inclusions and viral particles can be seen within endothelial cells and connective tissue. Additionally, it has been established that the MARV successfully evades host immune response to IFN by its structural protein VP40 [37].

Symptoms
Both EBOV and MARV infections present with a similar clinical picture and are known to cause the most severe hemorrhagic fever syndromes with case fatality rates in humans of up to 90% [2, 15, 25, 38, 39]. MARV has an incubation period ranging from 2 to 21 days (mean 4 to 9 days), which can be modified by factors such as infectious dose and possibly by route of infection [7]. MARV disease has a clinical course that can be conventionally broken down into three phases namely, an initial generalization phase, an early organ phase, and either a late organ phase or convalescence phase depending upon disease outcome; details of which are displayed in Table 2 [40]. The presentation of MARD is similar to that of malaria or typhoid fever and hence, clinical diagnosis of the disease can be difficult, especially if only a single case is involved. The disease appears first with non-specific symptoms which are followed by multi-organ involvement. Hemorrhagic manifestation, although an important indicator of the disease, appears only in 1/3 of patients during the peak of the illness [2]. More specific signs in immune privileged sites such as the eyes and testicles have been reported which are associated with transient persistence of the virus [15, 41].
The MARV disease outbreak in the Democratic Republic of Congo (1998-2000) showed that mortality rates were higher in patients exhibiting conjunctival injection and hiccups [20]. Deaths associated with the virus are usually due to tachypnea, coma, convulsions, severe metabolic disturbance and shock. In fatal cases, death usually occurs within nine days from the time of onset of signs and symptoms [41].
Previous literature shows evidence of the typical clinical picture of MARV infection. For example, in 2014, a healthcare in Kampala, Uganda was diagnosed with MARV disease and displayed hemorrhagic signs, notably profuse bleeding from body orifices; along with clinical findings mentioned in Table 2 [42].

Diagnosis
Diagnosis of MARD is not solely possible on the basis of clinical assessment as it mimics not only the other Filovirus hemorrhagic fevers, but also presents with signs and symptoms similar to other common infectious diseases such as malaria and typhoid [43]. However, if a patient is susceptible to MARD, he should be immediately isolated, and samples should be collected for laboratory investigations to confirm. The current laboratory diagnostics of MARD includes Reverse Tran-

Table 2 - Clinical signs and symptoms due to MARV infection.

Generalization Phase (Day 1 to 4)	*Early Organ Phase (Day 5 to 13)*	*Late Organ/Convalescence Phase (Day 13+)*
- Flu-like symptoms; high fever (39–40 0C), severe headache, chills, myalgia, prostration, and malaise. - Followed by gastrointestinal symptoms: anorexia, abdominal pain, severe nausea, vomiting, and watery diarrhea. - Enanthem, dysphasia, and pharyngitis (Day 4 to 5). - Characteristic maculopapular rash. - Others; lymphadenopathy, leukopenia, and thrombocytopenia.	- High fever - Neurological symptoms; encephalitis, confusion, delirium, irritability, and aggression. - Dyspnea, abnormal vascular permeability, particularly conjunctival injection and edema. - Hemorrhagic manifestations; petechiae, mucosal bleeding, melena, bloody diarrhea, hematemesis, and ecchymoses. - Multiple organ involvement; pancreas, kidney, and liver.	- Restlessness, obtundation, confusion, dementia, convulsions, reduced circulation due to severe dehydration, metabolic disturbances, severe diffuse coagulopathy, multiorgan failure, shock, and coma. - Extensive convalescent period; myalgia, exhaustion, sweating, peeling of the skin at the sites of rash, partial amnesia, and secondary infections (non-fatal cases).

scriptase-Polymerase Chain Reaction (RT-PCR), and antigen detection Enzyme-Linked Immunosorbent Assay (ELISA). These two tests have a high positive background rate. The detection of anti-filoviral antibodies based on ELISA has been the primary investigation since the 1995 Kikwit outbreak [41, 44]. The most frequently used assays for antibody detection are direct IgG and IgM ELISA. An IgM response indicates early illness (forms during the 1st week of illness and peaks during the 2nd week), while virus-specific IgG appears soon after IgM [45, 46]. Other sensitive tests include virus isolation and antigen detection using immunohistochemical analyses, particularly important for post mortem diagnosis [47]. Nevertheless, it is essential to use multiple specific, sensitive, and reliable diagnostic methods rather than a single test for confirming the diagnosis because misdiagnosis of MARD can be a major threat to the patient and society.

Management and treatment

Treatment of individuals infected with MARD is currently limited to supportive care measures such as maintenance of blood volume, electrolyte balance, psychosocial treatment and palliative management [13]. MARD is a highly contagious infection that affects multiple organs. As hemorrhage is an important symptom that presents late in the clinical course and leads to serious morbidity and mortality, early diagnosis is a crucial step. Human to human transmission of the virus necessitates practicing extra precautionary measures, especially, by the hospital staff as they are particularly exposed to it. Treatment of the patient must be in isolation and frequently monitored [48]. The clinical presentation greatly resembles other infections such as Ebola, and electron microscopy along with serology is important in determining the causative agent.

Many post-exposure treatment experiments have been carried out on animal models consequently to determine a promising treatment regimen. Much of these still show questionable efficacies but many have been adopted into the management regimens of the patients with MARD. These experiments provide an idea of approach to affected patients. The initial approach of the experiments was to focus on supportive therapies. The supportive care mainly comprises of antibiotics, anticoagulative therapies, antiviral therapies, hydration regimens, antipyretics, analgesics, and careful monitoring of the patient. In this regard, antibiotics (amoxicillin, cotrimoxazole, cefixime or ciprofloxacin) were given initially to prevent secondary infections as well as coagulation factor transfusion to revert thrombocytopenia and enhance the coagulation profile were used. Antipyretic drugs were added to the supportive care; their target was to manage fever. Furthermore, as a replacement therapy, electrolyte solutions were included in the treatment regimen of the patients. Albumin was used for the reversal of hypoproteinemia. These three approaches were also a part of the intensive care management of MARD. Antibiotics, electrolyte replacement treatment, antipyretics and coagulation components still make a substantial part of the regimens followed today especially in the intensive care protocol for MARD. Heparin was used for the prevention of

Diffuse Intravascular Coagulation (DIC) and this treatment did show some efficacy in the Guinea pig. Lastly, hemodialysis was performed as part of supportive treatment and a renal replacement therapy. However, it was tested in only one patient so its efficacy is yet questionable [13].

The second approach of the experiments has been the direct antiviral mechanism. These approaches included human convalescent; its aim was to directly treat the virus and was used in some secondary cases which were not very severe. In addition, immunoglobulin G (IgG) have also been tried from equine antiserum having same target as the above. It demonstrated efficacy in Guinea pig. However, it was found out that a specific titer of about >1:2408 is needed for such results. Another IgG from vaccinated NHPs also targeted virus neutralization had efficacy in NPH and was used as rhesus macaque model. Furthermore, monoclonal antibodies were used, its mechanism of action was to target VP40 to induce antibody mediated complement lysis and it showed efficacy in Guinea pigs and targeted mucin-like domain of glycoprotein (GP) with partial efficacy in Guinea pigs. Ribavirin with a broad-spectrum antiviral effect owing to increased virus mutation rate and/or Guanosine-triphosphate (GTP) pool depletion was used. Phosphorodiamidatemorpholino oligomers-plus, PMO-Plus (NP+VP24); that blocks translation of both VP24 and NP mRNA transcripts, were also included and showed efficacy in mouse, guinea pig and NHP; however, this approach was expensive.

Another experiment was carried out using suppression of deleterious host responses through various drugs. For instance, the use of anti-Tumor Necrosis Factor-alpha (TNF-α) antibody, the use of this earlier in the course did not show significantly positive results whereas it resulted in beneficial outcomes at day 3 of treatment. Desferal was also used and its mechanism of action is suppression of TNF-α and Inter Leukin-1(IL-1) production. It demonstrated efficacy in guinea pigs. Moreover, IL-1 receptor antagonist, rNAPc2, interferon, prednisone and ridostin were also used, all of which have different mechanism of action. An unknown FG-103 was also used with unknown MAO which showed efficacy in mouse [13].

Most experiments have been carried out in animal models that have mostly focused on evaluation of MARD of either the viral or host response. A broad-spectrum drug, ribavirin, synthetic guanosine analog has virustatic activity against a variety of DNA and RNA viruses, but unfortunately it has not shown any fruitful effect on MARV infection.

One approach for post-exposure treatment, determined through experiments, is with recombinant vesicular stomatitis virus [VSV] - based vaccine expressing MARV glycoprotein. This has exhibited efficacy when given once up to 48 h post-infection in NHPs. However, the mechanism still remains unknown and has certain safety concerns related to the use of live attenuate viruses [13].

Furthermore, some post-exposure prophylaxes have achieved reassuring results in NHPs and other animals. This treatment can be included in as an innovative component to human treatment. Therefore, the innovative treatment is divided into 2 categories:

1) disease-modifying agents;
2) inhibitors of viral replication [13, 48, 49].

Disease modifying agents

According to Roddy et al., the pathophysiology of FHF resembles sepsis and septic shock. This leads to the incitation for animal-model testing of recombinant human activated protein C, which is considered as a licensed therapy for severe sepsis in humans and has been successfully used for EHF in NHPS as post-exposure prophylaxis [49].

Inhibitors of viral replication

Antisense phosphorodiamidatemorpholino oligomers [PMOS] and short-interfering RNA [siRNA] molecules interfere with filovirus replication [49].

A study was conducted among healthy Uganda adults, which was also the first Ebola or Marburg vaccine trial done in Africa. According to this study, both vaccines were highly compatible and provoked antigen-specific humoral and cellular immune responses, whether used separately or together. However, these findings were inclined towards the development of more potent Ebola vaccine [50].

Prevention

Keeping in view the above-stated implications and endangers associated with the lethal virus, we must keep a strict check and control policy to effectively deal with the so-called: 'Marburg threat'. However, effective control is challenging

because no general treatment and a licensed vaccine are currently available. Thus, to break the vicious cycle of MARV dissemination we target the secondary transmission.

To begin with, the orientation of isolation wards in hospitals will aid in the rapid isolation of MARV-infected patients and prevent person-to-person transmission. In addition to this, the establishment of reliable laboratory diagnosis of suspected cases will further promote the idea of stopping transmission. The use of barrier nursing methods and education of health care professionals have benefitted the mass and reduced the incidence of nosocomial infections contrary to the previous outbreaks. As highlighted previously, close contact with the body of infected cases also contributes to the spread of infection; thus, the execution of safe burial, disinfection techniques and information campaigns to educate the local population are needed for the containment of the virus [7].

Moreover, it is important to avoid direct contact with blood, saliva, vomit, urine and other bodily fluids of infected people. Vegetative objects like infected needles and pins must also be handled with care. Furthermore, avoidance of contact with potential carriers (monkeys, chimpanzees, gorillas, fruit bats, pigs), both live and dead should also be fended off [51]. Besides, for tourist visits in mines or caves inhabited by fruit bat colonies, it must be assured that people wear gloves and other protective clothing (including masks). Also, based on WHO guidelines it is recommended that male survivors of MARV disease practice safe sex and hygiene for 12 months from onset of symptoms or until their semen twice tests negative for MARV [52].

To control this lethal virus, CDC in collaboration with the WHO has developed practical, hospital-based guidelines, titled: Infection Control for Viral Hemorrhagic Fevers in the African Health Care Setting. This manual aims to help healthcare facilities recognize cases and prevent nosocomial disease transmission using locally available materials and limited resources [53].

Although an absolute effective solution to prevent highly infectious diseases, group 3 and 4 pathogens, infections is not available, the European Network for Infectious Diseases (EUNID) suggests several ways, in addition to the standard precautionary methods, in which containment of these lethal pathogens can be achieved. In this regard, it is recommended to take extensive precautions whilst handling HID specimens in laboratories or treating infected persons in the hospitals. As MARV is a group 4 pathogen, the EUNID recommends that when caring for infected patients in the hospital treatment should commence in a high-level isolation unit (HLIU). The development of a high-level isolation unit (HLIU) is EUNID's utmost priority. HLIU are safe rooms or isolation areas that allow cautious care of one or more patients infected with a HID. A patient who is only a suspected MARV case should also be admitted in these units. If a patient with MARV presents in the emergency department (ED), standard precautions especially concerning cough and respiratory transmission control protocol should be enforced. The emergency staff should be well trained, proper communication between HLIU should be maintained and at least one separate room should be isolated with a dedicated access or route for the patients referred from other hospitals. Moreover, laboratory personnel are advised to take specific precautions whilst obtaining samples from patients, in an isolation room of ED or HLIU. The samples should be inactivated with formalin first and then subsequent testing performed in a group 3-4 pathogens' specific laboratory. If feasible, carry out point-of-care bedside laboratory testing. The protocol of admitting MARD patients in intensive care units also require special measures advising that such practices must either be carried out in HLIU or in the ICUs extensively supported with a negative-pressure and frequent air change. The choice of either using non-invasive positive pressure ventilation (NPPV), mask therapies or careful intubation depends on aerosol generation and careful handling is necessary in all cases. NPPV is preferred over mask therapies and, if carefully possible intubation is advised over NPPV. Furthermore, manual ventilation procedures especially during resuscitation should be as minimum as possible, endotracheal intubation should be performed by the most trained professionals who are advised to be covered with appropriate PPE and ventilated patients with HID must undergo highly precautionary tracheostomy and bronchoscopy either with or without bronchoalveolar lavage. Pediatric population infected with MARD must also be handled with great precaution as infection spread due to improper precautionary methods can oc-

cur. To avoid dissemination of infection, family communication must be minimized, and affected children admitted to HLIU and ICUs must be provided with adequate facilities such as nursing care keeping in consideration of parents' and patients' privacy. The EUNID also recommends meticulous ways to minimize transmission whilst carrying out investigational interventions. Unless deemed absolutely necessary, these interventions are advised to be avoided however, certain precautions must be considered if these procedures are sought. These procedures are mostly performed at the bedside making sure that patient movement is avoided. Enforcement of these measures is absolutely essential when carrying out bronchoscopy and endoscopy.

Furthermore, radiological imaging should be as precise and short as possible, performed at bedside and interpreted by only professional radiologists with assigned time-periods. Moreover, renal dialysis machines should be specific for MARD patients and must be decontaminated where possible. Lastly, specified protocols must be followed if post-mortem is performed in any MARD patient. Although, limited autopsies with little blood collection might be beneficial, these should be avoided as much as possible [54].

Vaccination

Along with efforts to curtail Marburg transmission, it is equally important to develop vaccines to particularly target four groups of people: the general population during outbreaks in susceptible areas or related to imported cases of MARV infection, healthcare workers in these same areas, laboratory workers researching MARV, and military and other service personnel susceptible to the virus. Despite this targeted vaccination approach, an ideal MARV vaccine must be able to provide long term protection against diverse strains of MARV after a single administration. The conventional approaches to viral vaccines investigated the use of protein subunits, whole-killed virions and live attenuated viruses. The drawbacks to this approach include multiple injections to elicit protective responses and despite this, they are unable to elicit strong cellular immune responses [55].

Currently, on-going researches aim to develop vaccines by concentrating on the use of various recombinant vectors for the delivery of genes expressing Filovirus proteins to induce protective immunity. Delivery systems used to express filovirus proteins for these purposes include vaccinia viruses, Venezuelan Equine Encephalitis Virus (VEEV) replicons, DNA-based vaccines, adenoviruses, Vesicular Stomatitis Virus (VSV), Human Parainfluenza Virus type 3 (HPIV3) and Virus-Like Particles (VLPs) [56].

The recombinant vaccinia virus vector system is the most commonly studied vector system with its major disadvantage being associated with the live, replication-competent virus in immuno-compromised individuals. Moving on, the VEEV replicon system also poses some challenges including failure of cross-protection between strains of MARV and even protection against homologous MARV required a series of three injections over 17 weeks. Although this might be improved by increasing the vaccine dose, even with a high dose, monkeys developed VEEV-neutralizing antibodies after two injections. Furthermore, the DNA based vaccine system hosts a variety of comparable advantages; they are amenable to rapid assemblage and large-scale production, ability to stimulate both humoral and cell-mediated immune responses and being reusable systems. However, similar advantages fail to confer when limited to humans along with the risks and challenges for the possible induction of autoimmune disease and integration into the host genome. Virus-like particles (VLP) is a specific type of subunit vaccines that structurally mimics authentic virions, its composition includes glycoprotein, nucleoprotein and VP40, but do not contain infectious genetic material, and are thus safe [13]. Additionally, they are not subject to problems associated with pre-existing lentivector immunity; but, this too presents with a challenge as the need to include numerous antigens is required to afford broad protection, this imposes a more significant engineering challenge and also the current laboratory process employing eukaryotic cells does not readily produce a compositionally - and structurally -consistent product when scaled-up. Adenoviruses have also been investigated for similar purposes and the obstacles associated with it are high prevalence of pre-existing immunity to the adenoviruses that limit their immunogenicity and clinical effectiveness. Unsurprisingly, the prevalence of anti-adenovirus antibody is up to 60% in the general human population and up to 85% in

Africa, where a MARV vaccine would be most needed and thus ineffective. Lastly, the most successful recombinant system that has proven to be the most successful vaccine platform for MARV to date is the vesicular stomatitis virus system. A single intramuscular vaccination of cynomolgus monkeys with an rVSV vector elicited complete protection against a high dose intramuscular challenge of homologous MV given 28 days later. The animals were also protected upon re-challenge 113 days later. Also, the same vaccine conferred protection against all the diverse strains of MARV, including the genetically divergent Ravn strain and Angola strain, which is considered the more virulent strain. The vaccine was also effective as post-exposure prophylaxis, affording complete protection in rhesus monkeys when administered 20-30 min after MARV challenge. The results for nosocomial acquired infections although have not been tested yet but appear to be promising as well [55].

Regrettably, we still do not have a clear understanding of the immunological correlates of Filovirus vaccine protection [57]. Hence, even though several various approaches are being entailed, the lack of concrete results that promise hope for the Marburg stricken endemic areas prevails.

CONCLUSIONS

Many outbreaks of the MARD have been documented ever since the first-ever case in 1967, following the initial contact with wild animals and contraction of the disease. Despite, a wide range of medicines have been tried to date, no constructive outcome has been achieved. Therefore, the most reliable method of treatment remains supportive with careful monitoring and isolation of the patient. Disease-modifying drugs and inhibitor of viral protein have shown some promising results in many patients and can be given to those affected; however, they might not be the final answer to the lethal virus. There is sheer need for devising a better option such as a safe and reliable vaccine to protect people especially vulnerable to the disease. Although much has been done to develop a fully credible vaccination, there are still many aspects regarding it that need to be dealt with before we proceed to the great invention.

Thus, by providing a thorough review on MARD, we aim to focus on its related developments along with its influence on the health care system. It adds value to medical literature by compiling the necessary information about MARD and helps directing health policies to constraint its affect. In view of the recent Ebola outbreak in the Democratic Republic of Congo, it is imperative for health authorities to devise a plan keeping in view the current times, where an uncontrolled endemic and pandemic can rapidly transgress an epidemic causing havoc on the already limited health facilities and innumerous challenges [59].

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