DHS SCIENCE AND TECHNOLOGY

Master Question List for Marburg Virus (MARV)

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For comments or questions related to the contents of this document, please contact the DHS S&T Hazard Awareness & Characterization Technology Center at HACTechnologyCenter@hq.dhs.gov.

Homeland Security

Science and Technology

DHS Science and Technology Directorate

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Marburg virus (MARV) – Master Question List

Table of Contents

Foreword

The following Master Question List (MQL) was developed by the Department of Homeland Security Science and Technology Directorate (DHS S&T) to present the current state of available information to Government decision makers in the operational response to outbreak of Marburg virus disease. This MQL quickly summarizes what is known and what additional information is needed to address fundamental questions such as, "What is the infectious dose?" and "How long does the virus persist in the environment?" The information provided is a succinct summary to allow structured and scientifically guided discussions across the Federal Government without burdening them with the need to review scientific reports, and to prevent duplication of efforts by highlighting and coordinating research.

NOTE: Due to the limited nature of published data on Marburg virus, this document uses data from Ebola virus to provide more relevant information to its users. Instances in which Ebola virus data are used in the absence of Marburg virus data are noted throughout the document.

Situation Overview

Marburg virus (MARV) is a zoonotic virus (a virus that originates in animals) related to the Ebola virus (EBOV) that causes Marburg virus disease (MVD), which is very similar to Ebola virus disease (EVD) and is associated with high mortality in humans and nonhuman primates. This virus is native to Africa, but cases are occasionally exported from Africa to other locations. Historically, MVD outbreaks have been small and infrequent. In fact, all but 4 of 17 total outbreaks have been fewer than 10 cases. However, the frequency of outbreaks has increased, with more than 50% of the total number occurring in the past 15 years. On March 21st, 2023, the government of Tanzania announced the country's first ever outbreak of MVD, and only one month prior to that, the government of Equatorial Guinea announced an outbreak in their country. As of the date of information gathering for this publication, there were fewer than 20 cases associated with either outbreak.

The cutoff date for information gathering related to this document was 03/26/2023.

Major Findings by Topic Area	
Topic	**Overview of Current Knowledge**
BACKGROUND	• MARV belongs to the species *Marburg marburgvirus*, the sole member of the genus *Marburgvirus* in the family *Filoviridae*, which also contains the ebolaviruses. • Filoviruses are filamentous (string-shaped) viruses with RNA genomes. • The first outbreak of MVD occurred in 1967 in Germany and Yugoslavia when MARV-infected nonhuman primates were exported from Uganda for production of vaccines. • MVD is an acute febrile illness associated with high case fatality rates. In outbreaks with more than 10 cases, the case fatality rate has ranged from 23-90%, with an average of 58%.
INFECTIOUS DOSE	• The infectious dose of MARV in humans or other animals has not been precisely defined but is not likely to be higher than 10 infectious viral particles. • A lethal dose of MARV has been established in some animal models, and indicates that in these animals, a single infectious viral particle can cause a lethal infection. • The infectious and lethal doses for EBOV vary by route of exposure, and it is likely that this is also true for MARV.
TRANSMISSIBILITY	• There are limited studies on the modes of transmission of MARV. However, EBOV is closely related and represents the best source of representative data. • Much like EBOV, MARV can be directly transmitted via blood and other bodily fluids. The virus may also transmit via aerosol and fomites. • The estimated R_0 for MARV is 1.6. The time between symptom onset in the primary and secondary cases is estimated to be 9 days. • Human index cases of MARV often occur following an extended stay in a cave or mine inhabited by *Rousettus aegyptiacus* i.e., bats. • Animals hunted for bushmeat and other human/animal contacts may also serve as intermediate hosts for transmission to humans. • For EBOV, fomites are a low-risk mechanism of transmission in public places, but contaminated Personal Protective Equipment (PPE) is highly infectious. • Transmission may occur after recovery from MVD as MARV and other filoviruses can persist in immune-privileged tissues (i.e. eyes, retina, central nervous system, sexual organs).
HOST RANGE	• The fruit bat *Rousettus aegyptiacus* is the reservoir host of MARV. • Nonhuman primates are also susceptible to infection. • MARV cannot cause disease in rodents without laboratory adaptation.
INCUBATION PERIOD	• The incubation period for MARV ranges from 2-21 days, averaging 5-9 days.

Major Findings by Topic Area	
Topic	**Overview of Current Knowledge**
	• EVD patients are not infectious during the incubation period, it is almost certain that this is the case for MVD patients as well. • In EBOV, the incubation period depends on the routes of transmission, and it is likely that this is true for MARV as well.
CLINICAL PRESENTATION	• MVD is clinically indistinguishable from EVD. Laboratory diagnostics are required to confirm the causative agent. • Initial symptoms include high fever, headache, fatigue, nausea, vomiting, diarrhea, pharyngitis, maculopapular rash, abdominal pain, conjunctivitis, and malaise. • Later in the course of the disease, patients have difficulty breathing, become increasingly weak, and may show neurological symptoms like confusion, delirium, and encephalitis. • Hemorrhagic symptoms do not always occur, but when they do, they occur late in the disease and are indicative of a severe presentation. • Patients typically begin recovery between days 13 and 21. Cases that will ultimately be fatal continue to deteriorate during this time period. Typical presentation of fatal cases includes coma, convulsions, and hypovolemic shock. Death is typically due to multiple organ failure. • MVD can be misdiagnosed for other tropical febrile diseases, which complicates and may delay outbreak identification.
CLINICAL DIAGNOSIS	• Due to similarity of clinical signs of MVD to other infectious diseases (e.g., Lassa fever, EVD, malaria, dengue, shigellosis, meningitis, typhoid fever) virus-specific diagnostics are necessary. • Detection of MARV in the blood has been the standard approach for diagnosis. • As of March 26, 2023, there are no commercial diagnostic tests approved for use in the United States. There are multiple effective diagnostic methods for confirmation of MARV infection. These include Reverse Transcription Polymerase Chain Reaction (RT-PCR) assays and various types of antigen and antibody detection-based tests. • Samples obtained for diagnostic testing are a biohazard risk and must be handled by high-containment facilities or the sample must be inactivated. State health departments should be contacted in the event of suspected MARV infection. Samples will likely be sent directly to members of the Laboratory Response Network, which was established by the Centers for Disease Control and Prevention (CDC) in 1999. • Enzyme-Linked Immunosorbent Assay (ELISA) and PCR can confirm MARV infection within days of symptom onset. • Pre-symptomatic and pre-viremic detection of MARV is limited.

Major Findings by Topic Area	
Topic	**Overview of Current Knowledge**
MEDICAL TREATMENT	• As of March 26, 2023, there are currently no MARV-specific treatments. • Both the CDC and the World Health Organizations (WHO) list supportive care including rehydration, maintaining oxygen status and blood pressure, replacing lost blood, and treating any complicating infections for treating MVD. • Antivirals are currently being developed for treating MARV infection. • Monoclonal antibody therapies are another promising area for medical intervention.
VACCINES	• There are no approved vaccines against MVD. • Three vaccine candidates are in Phase 1 clinical trials, but available doses are limited. o ChAd3-MARV (Sabin Vaccine Institute, United States), 450 doses o AD26 FILO + MVA BN FILO (Janssen, Belgium), 4,500 doses o rVSVΔG – MARV (Public Health Vaccines, United States) • The WHO-sponsored Marburg virus vaccines consortium (MARVAC) is working to coordinate vaccine development, trials, and deployment efforts.
ENVIRONMENTAL STABILITY	• MARV can be directly transmitted via blood and other bodily fluids. The virus may also transmit via aerosol and fomites; however, the stability of the virus is not well understood. • Limited data on MARV stability may be supplemented with available data pertaining to EBOV, as both viruses are members of the family *Filoviridae* and primarily transmit through direct contact. • EBOV maintains infectivity in whole blood and plasma after 5 days, even when the blood is stored at 37°C. • EBOV loses all infectivity in urine and semen at 37°C within 4-5 days and 5 days, respectively. • EBOV remains viable in wastewater for at least 8 days. • Aerosol stabilities of the EBOV Kikwit and Makona variants were determined, and similar decay rates (between 1 and 2% per minute) with an approximate half-life of 43 minutes was observed at 22°C 80% relative humidity (RH). • EBOV remains viable on some surfaces (i.e., wood, plastic, stainless steel, glass, some PPE) longer than others (i.e., cotton). EBOV remains viable potentially up to 8 days on some materials such as stainless steel, and up to 3 weeks in liquids and on plastic and glass surfaces.
DECONTAMINATION	• There is effectively no literature specific to decontamination of MARV. However, EBOV is closely related and represents the best source of surrogate data. • The most effective decontamination agents are bleach and other agents on Environmental Protection Agency's (EPA) Lists Q and L (i.e., agents for use against emerging viral pathogens, and

Major Findings by Topic Area	
Topic	**Overview of Current Knowledge**
	Ebola virus, respectively). • Filoviruses are susceptible to germicidal UV light exposure, with 3-4% of EBOV surviving after a 30 second exposure. • It is critical that the correct contact times are followed for any wipes be used, as improper use may result in transfer of the virus between surfaces.
PERSONAL PROTECTIVE EQUIPMENT (PPE)	• There are limited studies specific to PPE for MARV. However, EBOV is closely related and represents the best source of surrogate data. • CDC's PPE guidance for MARV does not differ from its guidance for EBOV. • CDC's guidance differentiates between confirmed EVD cases/patients under investigation (PUIs) for EVD who are clinically unstable or exhibiting bleeding, vomiting, or diarrhea, and PUIs that are clinically stable and not exhibiting bleeding, vomiting, or diarrhea. • CDC-recommended PPE for caring for a patient with confirmed EVD includes single-use PPE and respiratory protection in the form of a PAPR or NIOSH-certified N95 respirator. • CDC-recommended PPE for caring for a PUI who is clinically stable, and not exhibiting bleeding, vomiting, or diarrhea includes single-use, disposable PPE. Respiratory protection (PAPR or N95 respirator) is not required, but a face shield should be worn. • Detailed instructions for the recommended procedures for donning and doffing of PPE are available from CDC.
GENOMICS	• MARV and Ravn virus (RAVV) are very closely related but antigenically distinct viruses within the species *Marburg marburgvirus*. Both cause MVD in humans. • There is no cross-reactivity between MARV and RAVV. • MARV and RAVV variants associated with outbreaks do not persist in nature after the outbreak ends, and do not "spill back" into the animal reservoir of the virus. • Each outbreak has been associated with a new variant of MARV or RAVV, though some outbreaks have involved co-circulation of MARV and RAVV variants. • MVD outbreaks have originated in Uganda, Zimbabwe, Kenya, the Democratic Republic of the Congo, Angola, Guinea, Equatorial Guinea, and Tanzania.
VIRUS IMPORTATION	• Historically, MARV has been exported from Africa three times (1967 to Germany and Yugoslavia, 2008 to the US, and 2008 to the Netherlands). • It was suspected that a case of MVD was exported to Spain in February of 2023. However, it was determined that the patient was not infected with MARV. • Although there have been limited studies specifically examining the export of MARV/MVD to other countries, it can be reasonably assumed that findings for EBOV/EVD are broadly applicable.

Major Findings by Topic Area	
Topic	**Overview of Current Knowledge**
	• Air travel is the primary concern for importation of filovirus diseases from abroad to the United States. • International travel restrictions are necessary, but not sufficient to effectively prevent global spread of filovirus diseases. A more efficient control method is to attempt to prevent the spread of the disease locally during the early phase of an epidemic. • The most effective air passenger screening (such as temperature checks) occurs when applied at the embarkation airport where infected air travelers are most likely to depart. One modeling study indicated that 2.8 Ebola-infected air travelers per month departed the countries of Guinea, Liberia, and Sierra Leone during the epidemic.

Infectious Dose
How much agent will make a healthy individual ill?

What do we know?

- The infectious dose of MARV in humans or other animals has not been precisely defined but is not likely to be higher than 10 infectious viral particles.[1]
- A lethal dose of MARV has been established in some animal models, and indicates that in these animals, a single infectious viral particle can cause a lethal infection.[2-3]
  - It is not likely that that human lethal dose is this low.
- The infectious and lethal doses for EBOV vary by route of exposure,[4] and it is likely that this is also true for MARV.

What do we need to know?

- What is the infectious dose of MARV in humans by different routes?
- What is the correlation of different animal models to MARV human infection and disease?

Transmissibility
How does it spread from one host to another? How easily is it spread?

What do we know?

- There are limited studies elucidating the transmission of MARV. However, EBOV is closely related and represents the best source of surrogate data.[5]
- Much like EBOV, MARV can be directly transmitted via blood and other bodily fluids. The virus may also transmit via aerosol and fomites.[6-8]
- The estimated R_0 for MARV is 1.6, and the estimated time between symptom onset in the primary and secondary cases is 9 days.[9]
- Human index cases of MARV often occur following an extended stay in a cave or mine inhabited by *Rousettus aegyptiacus* i.e., bats.[10]
- Nonhuman primates and animals hunted for bushmeat may also become infected through contact with bat saliva, urine, feces, and contaminated fruits. These sources are also potential sources of transmission to humans.[10-13]
- Transmission may occur after recovery as MARV and other filoviruses can persist in immune-privileged tissues (i.e. eyes, retina, central nervous system, sexual organs).[9, 14]
- Limited data on MARV transmissibility may be supplemented with available data pertaining to EBOV, as both viruses are members of the family *Filoviridae*.[5]
- For EBOV, fomites are a low-risk mechanism of transmission in public places, but contaminated PPE is highly infectious.[15-18]
- Studies conducted with EBOV suggest that the route of infection has significant effects on an individual's ability to transmit the virus. Non-Human Primates (NHPs) infected via aerosol or mucosal routes transmitted the virus more efficiently via the mucosal route than intramuscular-challenged NHPs in a recently published study.[4]

What do we need to know?

- How comparable is MARV transmission to EBOV transmission?
- How long does MARV persist in immune-privileged tissues such as the testes?
- How does viral load differ among potentially infectious fluids/secretions?

Host Range
How many species does it infect? Can it transfer from species to species?

What do we know?

- An African species of fruit bat, *Rousettus aegyptiacus*, is the reservoir host of MARV.[19]
  - Bats are infected without apparent clinical disease.20
  - Majority of MARV outbreaks and individual cases have been associated with human/bat contact or human proximity to bats.
  - When infected, bats appear to tolerate viral infection by controlling viral load and

avoiding excessive inflammation and immunopathology.[20-23]
  - Bats are not chronically infected with MARV and clear the virus after approximately two weeks.[13, 20, 24]
  - Bats develop a neutralizing antibody response when infected with MARV.[20, 23]
  - It is not entirely clear how the virus is maintained in bat colonies, but it may involve transmission to newborns, and waning immunity in adults.[12-13, 19-20, 23-25]
  - Transmission between bats may occur via biting, or through mechanical transmission by arthropods.[13, 24-26]
  - Transmission to primates may occur when animals eat fruit contaminated by infected bats.[25, 27]
- Nonhuman primates are also susceptible.[2, 28]
  - MARV causes a disease very similar to human MVD in nonhuman primates, making them the ideal animal model for research purposes.[2]
- MARV cannot cause disease in rodents without laboratory adaptation, and unlike EBOV, does not appear to cause disease in ferrets.[2, 29]
  - Rodents are routinely used for MARV research, but the disease caused by adapted MARV in rodents is a poor model for human disease.[2]

What do we need to know?

- Are other species of wildlife susceptible to MARV?
- Are domestic animals susceptible to MARV?

Incubation Period
How long after infection do symptoms appear? Are people infectious during this time?

What do we know?

- The incubation period for MARV is typically stated to range from 2-21 days, averaging 5-9 days.[30] However, there is evidence that it may by up to 26 days.[31-32]
- EBOV patients are not infectious during the incubation period and the same maybe true for MARV as well.[30, 33]
- In EBOV, the incubation period varies and is dependent on routes of transmission.[34] It is likely that this is true for MARV as well.

What do we need to know?

- Are there signs or symptoms that might suggest that a patient is infected with a *marburgvirus* prior to them becoming infectious?

Clinical Presentation
What are the signs and symptoms of the infected person?

What do we know?

- MVD is an acute febrile illness associated with high case fatality rates. In outbreaks with more than 10 cases, the case fatality rate has ranged from 23-90%, with an average of 58%.[35]
- MVD can be misdiagnosed for other tropical febrile diseases, which complicates and may delay outbreak identification.[8]
- MVD is clinically indistinguishable from Ebola virus disease. Laboratory diagnostics are required to confirm the causative agent.[8]
- Initial symptoms include high fever, headache, fatigue, nausea, vomiting, diarrhea, pharyngitis, maculopapular rash, abdominal pain, conjunctivitis, and malaise.[8, 36]
  - Differential diagnosis at this phase is difficult due to the generalized nature of the symptoms.
- Later in the course of the disease, patients have difficulty breathing, become increasingly weak, and may show neurological symptoms like confusion, delirium, and encephalitis.[8]
- Hemorrhagic symptoms do not always occur, but when they do, they occur late in the

disease and are indicative of a severe presentation.[8]
  - Typical hemorrhagic symptoms include petechiae, ecchymoses, blood in diarrhea and vomit, and some bleeding from mucosa.
  - Hemorrhages are typically small in volume, and blood loss is not a cause of death.
- Patients typically begin recovery between days 13 and 21. Fatal cases will continue to deteriorate. Typical presentation of fatal cases includes coma, convulsions, and hypovolemic shock. Death is typically due to multiple organ failure.[8]

What do we need to know?

- Is fever a useful indicator for the ability of a patient to transmit the virus? Is a patient able to transmit the virus after developing less noticeable symptoms (such as headache and low-grade fever), but prior to becoming noticeably febrile?
- Is there a naturally immune human population?

Clinical Diagnosis
Are there tools to diagnose infected individuals? When during infection are they effective?

What do we know?

- Due to similarity of clinical signs of MVD to other infectious diseases (e.g., Lassa fever, EVD, malaria, dengue, shigellosis, meningitis, typhoid fever) virus-specific diagnostics are necessary.[6, 37]
- Detection of Marburg virus in the blood has been the standard approach for diagnosis.[38]
- As of March 26, 2023, there are no commercial diagnostic tests approved for use in the United States. Confirmation of MARV infection can be achieved using the following methods:
  - antibody-capture enzyme-linked immunosorbent assay (ELISA)6, 37
  - antigen-capture detection tests6
  - serum neutralization test6
  - reverse transcriptase polymerase chain reaction (RT-PCR) assay6, 37
  - electron microscopy6
  - virus isolation by cell culture6, 37
- Samples obtained for diagnostic testing are a biohazard risk and must be handled by high-containment facilities or the sample must be inactivated.[6] State health departments should be contacted in the event of suspected MARV infection.[39] Samples will likely be sent directly to members of the Laboratory Response Network.[40]
- ELISA and PCR can confirm MARV infection within days of symptom onset.[37]
  - A Taq-Man based RT-PCR assay is able to detect and differentiate between EBOV and MARV. The limit of detection is 40 copies/ml and 100 copies/ml for EBOV and MARV, respectively.41
  - Due to the similarity of symptoms between hemorrhagic fever viruses, an oligonucleotide microarray was developed to detect and distinguish between 16 different pathogens, including MARV.42
- Pre-symptomatic and pre-viremic detection of MARV is limited.
  - Detection of changes in gene expression in an infected individual may allow for earlier detection of infection as demonstrated in one study utilizing an NHP model.[38]

What do we need to know?

- How can the availability of diagnostic testing and relevant training be improved, especially in response to an outbreak?
- Do current assays lose sensitivity due to viral mutations?
- Can the period between initial infection and diagnostic detection be shortened?

Medical Treatment
Are there effective treatments?

What do we know?

- As of March 26, 2023, there are currently no MARV-specific treatments.[6, 43]
- The CDC and WHO list treatment for MVD as supportive care including rehydration,[6] maintaining oxygen status and blood pressure, replacing lost blood, and treating any complicating infections.[43]
- Antivirals such as Remdesivir, Favipiravir, Galidesivir, NP-718-LNP, AVI-7288, and AVI-6003 are in development for treating MARV infection.[39] The WHO notes that Remdesivir and Favipiravir may be used under compassionate use/expanded access because of their use in treating EVD;[6] however, clinical trials for treatment of EVD with Remdesivir and Favipiravir have generally found limited or inconclusive evidence for efficacy.[44-45]
- A study in an NHP model of MVD showed that Remdesivir and human monoclonal antibody (mAb) MR186-YTE could both protect against fatal disease if initiated within 5 days of infection. By day 6 post infection, significant protection was only achieved when the two treatments were administered in combination.[46] These results also highlight the importance of early detection of infection.

What do we need to know?

- Are other therapeutics currently in development for EVD that can be identified as effective treatments for MVD?
- Can methods be developed to address the gap between successful animal trials and efficacy in humans?
- What additional non-specific treatments (i.e., supportive care measures) can be implemented to improve patient outcomes?

Vaccines
Are there effective vaccines?

What do we know?

- There are currently no regulatory agency-approved vaccines for MVD, however several vaccine candidates are showing promise in NHP preclinical studies and are advancing through Phase I clinical studies in humans.[47-48] A comprehensive list of all Marburg vaccine candidates is maintained by the WHO.[49]
- In August 2021, the WHO convened a consortium called MARVAC, composed of international experts in vaccine development from academia, government, nonprofit organizations, and industry, with the goal of sharing knowledge to promote the development of MVD vaccines.[48] The group held an urgent meeting on February 14, 2023, due to the new outbreak in Equatorial Guinea, and discussions included the current vaccine landscape as well as protocols for vaccine clinical trials.[50]
- Vaccine candidates in Phase I clinical trials include:
  - Sabin Vaccine Institute' ChAd3-MARV, a single-dose adenovirus-based vaccine. Preclinical NHP studies showed 100% protection one week after vaccination when challenged with Marburg.51 Early Phase I results in humans show the vaccine is safe with no severe adverse events, and immune responses are in the range that correlates with protection in NHPs in preclinical studies. 95% of participants showed an antibody response with 70% still displaying immunogenicity at 48+ weeks post vaccination.52 Phase II clinical trials are planned in Africa in 2023.48, 50 There are 450 doses ready for emergency use if needed, and a finished vaccine ready for filling is available for another 18,000 doses.48
  - Janssen's AD26 FILO + MVA BN FILO is a two-dose adenovirus-based vaccine. Doses are administered 56 days apart.[48, 50, 53] While Janssen is not actively pursuing further development of this vaccine they contribute to MARVAC and have offered support with

their existing stock.[48, 50] There are over 3,000 doses available for emergency use if needed. This vaccine design follows Janssen's strategy for their European Medicines Agency-approved Ebola vaccine against the related filovirus.[48]
  - Public Health Vaccine's rVSVΔG – MARV, a single dose rVSV vaccine that is not yet in Phase I clinical trials but has shown promising results in NHP preclinical studies has been cleared by the US Food and Drug Administration (FDA) to begin human testing.[54]

What do we need to know?

- How effective are the available vaccines in real-world conditions?
- What is the efficacy of the vaccines when used for post-exposure prophylaxis?
- What is the actual efficacy of the vaccines for pre-exposure prophylaxis?
- What is the impact and safety of vaccines on pregnant or lactating women?
- How long are the vaccines effective? What is the onset and duration of protection?
- What are the correlates and thresholds of protection? In other words, what types of vaccine induced immune responses are responsible for preventing infection and disease, and how strong do these responses have to be?
- How often do breakthrough infections occur?

Environmental Stability
How long does the agent live in the environment?

What do we know?

- MARV can be directly transmitted via blood and other bodily fluids. The virus may also transmit via aerosol and fomites; however, the stability of the virus is not well understood.[6-7]
  - A study determined the aerosol decay curves for two MARV variants Angola and Popp, to have an approximate half-life of 35 min at ambient temperature (19-21°C) and relative humidity (42-57%).[55]
- Limited data on MARV stability may be supplemented with available data pertaining to EBOV, as both viruses are members of the family *Filoviridae* and primarily transmit through direct contact.[5]
- EBOV maintains infectivity in whole blood and plasma after 5 days, even when the blood or plasma are stored at 37°C.[56]
  - EBOV remains infectious in liquid blood in syringe needles up to 190 days.57
  - EBOV in blood also remains infectious on banknotes for up to 6 days.57
- EBOV loses all infectivity in urine and semen at 37°C within 4-5 days and 5 days, respectively.[56]
- EBOV remains viable in wastewater for at least 8 days.[58]
  - EBOV remains viable in laboratory-grade water for 3 (27°C) to 6 (21°C) days, depending on the temperature of the water.[59]
- Aerosol stabilities of the EBOV Kitwit and Makona variants were determined, and similar decay rates (between 1 and 2% per minute) with an approximate half-life of 43 minutes was observed.[55]
- EBOV remains viable on some surfaces (wood, plastic, stainless steel, glass, some PPE) longer than others (cotton). EBOV remains viable potentially up to 8 days on some materials such as stainless steel, and up to 3 weeks in liquids and on plastic and glass surfaces.[59-63]
  - At an Ebola treatment center (ETC) in Sierra Leone, EBOV RNA was detected on materials and surfaces that were in direct contact with patients (clothing, blankets, pit latrines). No RNA was detected on chlorine tap handles and ceiling fan blades. RNA was also found in bodily fluids and visibly bloodied soaker pads.64
  - A 4-log inactivation of EBOV on glass (22°C, 30-40% humidity, no light) required 5.9 days.62
  - EBOV persisted on steel surfaces for 1-3 days (27°C 80% RH).61

  - There was no difference in the stabilities of aerosolized Mayinga 1976 EBOV and Makona 2014 EBOV over 3 hours at 22°C and 80% RH. Both viruses remained viable, and was comparable to the stability of EBOV dried on surfaces at 27°C.[65]

What do we need to know?

- How stable is Marburg virus on porous and non-porous surfaces?
- How long is Marburg virus infectious on the bodies of the deceased?
- What are the best surrogates for persistence testing?
- Are surrogates used for decontamination testing acceptable surrogates for use in persistence testing?

Decontamination
What are effective methods to kill the agent in the environment?

What do we know?

- There is effectively no literature specific to decontamination of MARV. However, EBOV is closely related and represents the best source of surrogate data.[5]
- The most effective disinfecting agents are bleach and other agents listed on the US EPA's Lists Q and L (Agents for use against emerging viral pathogens and Ebola virus, respectively).[66-67]
- Filoviruses are susceptible to germicidal ultraviolet exposure, with 3-4% of EBOV surviving after a 30 second exposure.[68]
- It is critical that correct contact times for wipes are used, as improper use may result in transfer of virus between surfaces.
  - Accelerated hydrogen peroxide-impregnated wipes demonstrated secondary transfer of EBOV up to 0.5 $\log_{10}$ $TCID_{50}$/mL when contaminated steel surfaces were wiped for 30 seconds. Wipes containing a single quaternary ammonium compound transferred up to 0.8 $\log_{10}$ $TCID_{50}$/mL EBOV when wiped for 5 seconds, but EBOV was undetectable when wiped for 15 seconds or longer.[69]
- Androx 6092 and sodium hypchlorite (1:2 and 1:10 dilutions respectively) are effective disinfectants capable of inactivating Ebola virus-Ecran with a 10-minute exposure time on both porous and nonporous surfaces.[70]
- Chlorine disinfectants are effective and widely used for routine/daily disinfection of non-porous surfaces (floors, bedside surfaces, equipment).
  - At least 0.5% sodium hypochlorite and a contact time of at least 5 minutes to achieve at least 4 $\log_{10}$ reduction in titer.[60, 70-73]
  - Contact time and concentration is key for effective disinfection. Even a high concentration (1%) of sodium hypochlorite did not decontaminate EBOV-contaminated surfaces within 1 minute of contact time but was effective after 5 minutes.[60]
- Other commonly used disinfectants have shown varying effectiveness of EBOV inactivation on non-porous surfaces (e.g., stainless steel, aluminum).
  - 67-70% ethanol is effective at inactivating EBOV within 5-10 minutes.60, 72
  - Chloroxylenol (≥ 0.12%) is effective at inactivating EBOV within 5 minutes.74-75
  - Commonly used military aircraft disinfectants showed varying effectiveness at EBOV inactivation on seat belts and aluminum surfaces.70
  - Povidone iodine (PVP-I) formulations (e.g., 7.5% PVP-I surgical scrub, 10% PVP-I solution, or 3.2% PVP-I and 78% alcohol solution) are > 99.99% effective against EBOV at a 15-second exposure time.76
- Chlorine dioxide,[77] vaporized hydrogen peroxide fumigation,[78-79] or UV germicidal irradiation[68] can be used to decontaminate medical equipment and isolation units.Degree of soiling of material can reduce effectiveness of fumigation methods; prior physical cleaning is required.[68]
  - The process of decontamination requires nearly one week from the time the patient exits the room to when personnel can enter without PPE.[80]

  - Decontamination with vaporized hydrogen peroxide fumigation can be completed in 3 working days – approximately half the time of formaldehyde decontamination procedures.[78]
  - Field decontamination kits utilize chlorine dioxide and can sterilize ebolavirus-contaminated medical equipment at remote clinical sites over a 30 to 60-minute period.[77]
  - Surrogate studies suggest that chlorine dioxide gas may not be effective at inactivating EBOV present in body fluids.[81]
- When left at ambient temperature and 30% relative humidity, Ebola virus-Makona suspended in organic soil is capable of surviving on plastic gowns, respiratory masks, and stainless steel surfaces for up to 192 hours, while virus on cotton gowns does not survive beyond 24 hours.[60]

What do we need to know?

- Does MARV differ from EBOV in disinfection of any contaminated materials?
- What are best practices for disinfection and decontamination of textile or cloth materials common in hospitals for MARV?

Personal Protective Equipment (PPE)
What PPE is effective and who should be using it?

What do we know?

- There are limited studies specific to PPE for MARV. However, EBOV is closely related and represents the best source of surrogate data.[5]
- CDC's PPE guidance for MARV does not differ from its guidance for EBOV.[82]
- CDC's guidance differentiates between confirmed EVD cases/PUIs for EVD who are clinically unstable or exhibiting bleeding, vomiting, or diarrhea,[83] and PUIs that are clinically stable and not exhibiting bleeding, vomiting, or diarrhea.[83]
  - Variations in PPE should be avoided within a specific facility.
- CDC-recommended PPE for caring for a patient with confirmed EVD[83] includes single-use, disposable PPE and respiratory protection in the form of a PAPR or NIOSH-certified N95 respirator.[84-85]
  - Single-use disposable impermeable gown or coverall, examination gloves with extended cuffs (two pair), boot covers that extend to at least mid-calf, and an apron that covers the torso to the level of mid-calf should be used over the gown or coveralls if the coverall has an exposed, unprotected zipper in front.
  - Standardized attire should be worn under PPE (e.g., scrubs and dedicated washable footwear).
- CDC-recommended PPE for caring for a PUI who is clinically stable, and not exhibiting bleeding, vomiting, or diarrhea includes single-use, disposable PPE. Respiratory protection (PAPR or N95 respirator) is not required, but a face shield should be worn.[83]
- Detailed instructions for donning and doffing of PPE are available from CDC.[83, 86]
  - A trained individual should observe donning and doffing to confirm and document that each step has been completed correctly.
  - Designated areas separate from the patient care area should be dedicated to donning or doffing of PPE.
  - Use of a checklist and closed loop communication strategy can result in a more deliberate and mindful doffing process.87-88
  - It is crucial that facial and respiratory protection is removed last for safe doffing.89

What do we need to know?

- How can PPE be improved to reduce occupational risks (e.g., heat stress, dexterity)?
- Are there improved PPE designs to allow for easier removal without touching the outside of the PPE?

- Can a standardized simulation system for training clinical workers in PPE usage for care of MVD patients be devised?

Genomics
How does the disease agent compare to previous strains?

What do we know?

- MARV, along with Ravn virus (RAVV) are members of the species *Marburg marburgvirus*, the sole member of the genus *Marburgvirus* in the family *Filoviridae*, which also contains the ebolaviruses.[5]
- Members of the species *Marburg marburgvirus* have single stranded negative sense RNA genomes approximately 19 kB in length.[5]
- MARV and RAVV are antigenically distinct, but not sufficiently distinct to be placed in separate species.[5]
- There is no cross-reactivity between MARV and RAVV.[5]
- MARV and RAVV variants associated with outbreaks do not persist in nature after the outbreak ends, and do not "spill back" into the animal reservoir of the virus.[5]
- Each outbreak or MARV or RAVV has been associated with a new variant.[5]
- The mutation rate of MARV appears to be similar to that of EBOV,[90] which suggests that mutations that enhance transmission between humans are unlikely to occur during small outbreaks, as this process has required a large number of human-to-human transmission events during EBOV outbreaks.[91]
- There appears to be more phenotypic diversity between MARV variants than between ebolavirus variants. This is best illustrated by the large variation in case fatality rates between MARV outbreaks with at least 10 cases.[35]
- MVD outbreaks have originated in Uganda, Zimbabwe, Kenya, the Democratic Republic of the Congo, Angola, Guinea, Equatorial Guinea, and Tanzania.[35]
  - The majority of transmission events to humans have occurred in Uganda, and outbreaks in West Africa have only occurred within the past five years.[35]

What do we need to know?

- Why do MARV variants appear to be more phenotypically diverse than ebolavirus variants?
- What mutations or types of mutations should prompt concern that a new variant may exhibit enhanced transmission?
- What is the diversity of MARV within its bat reservoir?

Virus Importation
What are the main routes of entry into the United States?

What do we know?

- Historically, MARV has been exported from Africa three times.[35]
  - The first outbreak of MVD occurred in 1967 when MARV-infected nonhuman primates were exported from Uganda to Germany and Yugoslavia for production of vaccines.[35]
  - The other two instances were travelers from the United States and the Netherlands who were exposed to MARV in a cave in Uganda in January and July of 2008, respectively.[35]
- Although it was suspected that a case of MVD was exported to Spain in February 2023, it was determined that the patient was not infected with MARV.[92]
- There are limited studies specifically examining the export of MARV/MVD from Africa. However, it can be reasonably assumed that findings for EBOV/EVD are broadly applicable.[93]
- Air travel is the primary concern for importation of filovirus diseases from abroad to the United States.[93-94]
- International travel restrictions are necessary, but not sufficient to effectively prevent global spread of filovirus diseases. A more efficient control method is to attempt to prevent the

spread of disease locally during the early phase of an epidemic.[94-95]

- The most effective air passenger screening (such as temperature checks) occurs when applied at the embarkation airport where infected air travelers are most likely to depart. One modeling study indicated that 2.8 Ebola-infected air travelers per month departed the countries of Guinea, Liberia, and Sierra Leone during the epidemic.[96]
- Successful cross-border viral surveillance was implemented to prevent imported EVD cases in Uganda in 2019. Three EVD cases crossing into Uganda from the Democratic Republic of the Congo (DRC) were detected at the time of first contact with a healthcare facility and a fourth case was detected at point of entry by temperature screening.[97]

What do we need to know?

- How can response times be improved to implement protective measures more rapidly?
- Can an effective screening methodology be developed for inbound international travelers?
- How can monitoring be more sensitive, cost-effective and efficient with personnel resources?

Commonly Used Acronyms and Abbreviations

Acronym/Term	Definition	Description
BSL	Biosafety Level	N/A
CDC	Centers for Disease Control and Prevention	N/A
DHS S&T	U.S. Department of Homeland Security Science and Technology Directorate	N/A
EBOV	Ebola virus	N/A
ELISA	Enzyme-Linked Immunosorbent Assay	Assay used to detect the presence of antibodies to a specific protein
EPA	U.S. Environmental Protection Agency	N/A
EUA	Emergency Use Authorization	Provisional FDA approval granted for pharmaceuticals and other medical products under emergency conditions
FBS	Fetal Bovine Serum	Media additive used in tissue culture to facilitate cell growth
FDA	U.S. Food and Drug Administration	N/A
HHS	U.S. Department of Health and Human Services	N/A
Ig	Immunoglobulin	Antibodies (glycoprotein molecules produced by white blood cells)
IND	Investigational New Drug	FDA designation allowing for limited/controlled use of an unapproved pharmaceutical under specific conditions
LD_{50}	Median Lethal Dose	Dose required to cause a lethal effect in 50% of subjects
MARV	Marburg virus	N/A
MVD	Marburg virus disease	The disease caused by Marburg virus
MQL	Master Question List	N/A
NHP	Non-Human Primate	N/A
NIOSH	National Institute for Occupational Safety and Health	N/A
PCR	Polymerase Chain Reaction	Assay used to determine the number of RNA or DNA molecules representing a specific sequence target are present in a sample
PEP	Post-Exposure Prophylaxis	N/A
PFU	Plaque Forming Unit	Unit representing a single infectious viral particle derived from viral quantification via plaque assay
PPE	Personal Protective Equipment	Equipment intended to protect individuals against hazardous environments
RAVV	Ravn virus	A close relative of Marburg virus

Acronym/Term	Definition	Description
R_0	Basic Reproductive Number	Average number of new infections that each case is expected to generate in a population where all individuals are susceptible to infection
SNS	Strategic National Stockpile	Stockpile of drugs, tests, vaccines, and equipment maintained by the federal government for pandemic and biothreat response
$TCID_{50}$	Median Tissue Culture Infectious Dose	Dose necessary to infect 50% of tissue cells.; used as a standard measure of infectivity (e.g., it required 10^3 $TCID_{50}$ to produce clinical signs in exposed chickens)
UV	Ultraviolet	Light with wavelength in the 100-400 nm range

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