Tissue and cellular tropism, pathology and pathogenesis of Ebola and Marburg Viruses

Abstract

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Abstract

Ebolaviruses and marburgviruses include some of the most virulent and fatal pathogens known to humans. These viruses cause severe haemorrhagic fevers with case fatality rates ranging from 25% to 90%. The diagnosis of filovirus using formalin-fixed tissues from fatal cases poses a significant challenge. The most characteristic histopathological findings are seen in the liver: however findings overlap with many other viral and non-viral haemorrhagic diseases. The need to distinguish filovirus infections from other haemorrhagic fevers, particularly in areas with multiple endemic viral haemorrhagic agents, is of paramount importance. In this review we discuss the current state of knowledge of filovirus infections and their pathogenesis, including histopathological findings, epidemiology, modes of transmission, and filovirus entry and spread within host organisms. The pathogenesis of filovirus infections is complex and involves activation of the mononuclear phagocytic system with release of proinflammatory cytokines, chemokines and growth factors; endothelial dysfunction; alterations of the innate and adaptive immune systems; direct organ and endothelial damage from unrestricted viral replication late in infection; and coagulopathy. Although our understanding of the pathogenesis of filovirus infections has rapidly increased in the past few years, many questions remain unanswered.
Introduction

Viral haemorrhagic fevers (VHFs) is a term used for severe systemic infections characterized by fever and haemorrhage that are caused by four distinct families of small, lipid-enveloped RNA viruses, including Arenaviridae, Filoviridae, Bunyaviridae and Flaviviridae. All are negative-stranded viruses with the exception of flaviviruses, which are positive-stranded. Each of these viruses is maintained in its own particular transmission cycle, which may involve non-human primates (NHP), bats, rodents, domestic ruminants, humans, mosquitoes, and ticks [1, 2].

The family Filoviridae consists of marburgviruses and ebolaviruses [3]. Since the discovery of Marburg virus (MARV) in 1967 and Ebola virus (EBOV) in 1976, these viruses have traditionally been associated with sporadic outbreaks predominantly in geographically remote areas of Central Africa [4]. The largest recorded Marburg outbreak to date occurred in Angola in 2004-2005, with 227 reported deaths [5,6]. At the time of writing this article, the largest reported outbreak of EBOV is ongoing and is the first outbreak ever to occur in West Africa, affecting Guinea, Liberia, Sierra Leone and Nigeria. As of August 31, 2014, there have been 3,707 suspect and confirmed cases with 1,848 deaths [7, 8]. Without additional interventions, the projected estimate of total cases in Liberia and Sierra Leone by January 20, 2015 is approximately 550,000 cases, or 1.4 million when corrected for underreporting [9].

Pathology has traditionally played a critical role in the discovery and advancement of our knowledge of emerging infectious diseases [10, 11, 12]. Autopsies can determine the pathological features of the disease, help identify the causative...
pathogen, and provide new insights into the pathogenesis of infections. Filoviruses are highly virulent and are classified as Biosafety Level (BSL) 4 pathogens—that is, they are potentially transmissible via aerosols and cause life-threatening disease for which there is no vaccine or therapy [13]. Biosafety is critical for autopsy personnel, particularly when dealing with infectious agents. Standard precautions for infection control, as outlined by the Center for Disease Control (CDC), integrates hand hygiene, use of personal protective equipment – such as gloves, gowns, masks, face masks, respirators, goggles and face shields – safe injection practices, safe handling of potentially contaminated equipment or surfaces, and respiratory hygiene [14]. The World Health Organization recommends that post-mortem examinations of VHF patients should be performed by trained personnel and limited to essential evaluations only [15]. The 2014 CDC recommendation for Ebola patients in U.S. hospitals and mortuaries is that autopsies should be avoided and that, if an autopsy is necessary, the state health department and CDC should be consulted regarding additional precautions [16].

Numerous reports using animal models and in vitro studies to explore the pathogenesis of filoviruses have been published. These have made significant strides in our understanding of filoviruses and will be referenced throughout the article. On the other hand, human studies have been relatively few because of: 1) biosafety concerns, 2) occurrence of the disease in remote areas with little modern infrastructure, 3) sporadic and unpredictable timing of outbreaks and 4) poor filovirus surveillance. Furthermore, efforts to explain the pathogenesis of Ebola and Marburg haemorrhagic fevers must consider a multitude of factors, including clinicopathological and epidemiological data. We review how experimental animal
and human pathology studies have contributed to a better understanding of filovirus pathogenesis and to our ability to diagnose filovirus infections. This review will also attempt to identify the gaps in our knowledge that represent critical challenges for the future.

**The agents**

Filoviruses are filamentous, enveloped, single-stranded, negative-sense RNA viruses. The family *Filoviridae* includes two distinct genera: marburgviruses and ebolaviruses, which differ in their glycoproteins at both the nucleotide and amino acid levels [3, 17]. Their genomes have a length of approximately 19kb, which encodes seven structural proteins (3′-UTR-NP-VP35-VP40-GP-VP30-VP24-L-5′-UTR), and one non-structural protein. These pleomorphic filamentous viruses enter the host cell through membrane-embedded glycoproteins and then replicate using seven genes encoded in their negative sense RNA genome. The genus *Ebolavirus* includes five species; three are highly pathogenic in humans: *Zaire ebolavirus* has case fatality rates (CFR) ranging from 70%–90%; *Sudan ebolavirus* CFR of about 50%; and *Bundibugyo ebolavirus* CFR of about 25% [2, 3, 18]. Only one patient who recovered from a *Taï Forest ebolavirus* infection has been reported [19]. Although *Reston ebolavirus* causes disease in non-human primates and pigs, it appears to be non-pathogenic in humans [20]. The genus *Marburgvirus* contains only one species of virus and has a variable case fatality rate; for example, a CFR of 25% was noted in 1967 in Germany, while CFRs of 90% were seen in more recent outbreaks in Africa [5, 21, 22].
Transmission, ecology and epidemiology

Filoviruses are zoonotic diseases transmitted through direct contact with infected live or dead animals. The main means of transmission in humans is contact with the blood, secretions or bodily fluids of an infected human, non-human primate or reservoir host animal (Figure 1 A, B) [7, 20, 23, 24, 25]. Humans can transmit the virus as soon as they are febrile and continue to be infectious during the later stages of the disease as well as after death e.g., during ritual body washing [6, 7, 22, 26, 27]. Rare human infections have been documented associated with accidental laboratory exposure or during conduction of a necropsy Figure 2 [8, 19, 28, 29].

Several studies have attempted to identify the natural reservoir of filoviruses. Epidemiological data from numerous EBOV outbreaks, in combination with molecular and virological analyses, suggest that fruit bats, including Hypsignathus monstrosus, Epomops franqueti, Rousettus aegyptiacus and Myonycteris torquata are likely to be reservoirs of these viruses [25,30,31,32,33] (Figure 1 A). On the other hand, Rousettus aegyptiacus, fruit bats of the Pteropodidae family, have been definitively identified as the natural reservoir of Marburg virus (Figure 1 B) [34, 35]. In several cases, epidemiological evidence suggests a link between bats and Marburg virus infection, and includes a French engineer who became infected by Marburg virus after visiting caves with large bat populations in Kenya in 1980 [24] and 4 miners in Uganda in 2007 who likely acquired Marburg virus through exposure to bats or bat secretions in a mine [36]. However, many questions regarding the mechanism by which bats shed and transmit the viruses remain unanswered.
Since the discovery of Marburg virus in 1967, there have been 11 documented outbreaks of Marburgvirus; since the discovery of Ebola virus in 1976, there have been 24 Ebola outbreaks, confirmed by virus isolation or by molecular and biological methods [7, 20, 29, 37, 38]. A summary of all known filoviruses outbreaks and CFRs can be found in Table 1. Reston virus was introduced into quarantine facilities in the United States and Italy in 1989, 1990, 1992 and 1996 from non-human primates imported from primate facilities in the Philippines. These occurrences were linked to Reston virus isolated from primate export facilities in the Philippines. In 2008, pigs were identified as a host for Reston virus in the Philippines [39]. There have been 13 asymptomatic and serologically confirmed human cases associated with these different events since 1989. Geographical distribution of Marburg virus and Ebola virus outbreaks, since 1967 to 2014 are illustrated in Figure 2.

**Clinical presentation and laboratory diagnosis**

Early on in the clinical course, the presentation of filovirus-infected patients is non-specific and difficult to distinguish from other endemic diseases, such as Lassa fever, malaria, cholera or typhoid fever. Patients can also develop simultaneous infections with other pathogens, thereby further complicating the diagnosis [38, 40, 41]. The asymptomatic incubation period of filoviruses is 2-21 days. The disease manifests abruptly with non-specific flu-like symptoms, including chills, fever, myalgia, and general malaise. This is followed by lethargy, nausea, vomiting, abdominal pain, anorexia, diarrhoea, coughing, headache, and hypotension. Haemorrhagic manifestations vary in severity and are seen in 30-50% of patients.
These include a maculopapular rash and mucosal bleeding, especially in the gastrointestinal and genitourinary tracts [6, 7, 29, 31].

In severe and fatal forms, death occurs between 6 and 16 days after the onset of symptoms as a result of hypotensive shock and multi-organ failure, including hepatic damage and renal failure [29, 40]. Features of illness seen in fatal cases include a high viral load in blood, a marked rise in blood neutrophil count, a fall in lymphocytes and platelets, and coagulopathy [42, 43].

Several diagnostic methods to detect filoviruses are available, including reverse transcription polymerase chain reaction (RT-PCR), serology, virus isolation, electron microscopy (EM), histopathology and immunohistochemistry (IHC) (Table 2, Figure 3 A). Because of the biosafety hazards associated with filoviruses, these assays can be performed in only a few specialized laboratories worldwide. Such testing requires that potentially dangerous biological specimens be transported to these laboratories from remote sites, necessitating preservation of a cold chain and rigorous international packaging and shipping procedures [5, 44].

Ebola virus and Marburg virus are detectable in blood only after the onset of symptoms, most notably fever. Viral antigen and nucleic acid can be detected in blood from day 3 up to 7–16 days after onset of symptoms. IgM antibodies can appear as early as 2 days post onset of symptoms and disappear between 30 and 168 days after infection. Specific IgG antibodies can develop between day 6 and 18 after onset and persist for up to 3-5 years [45]. Detection of filoviruses by PCR is
available and is able to distinguish between the different viral variants in a range of tissues and fluid specimens [46]. Virus is generally detectable by real-time RT-PCR from 3-10 days after the onset of symptoms, and has even been detected several months after infection in certain secretions (Figure 3 A) [7, 26, 46]. More recently, in outbreak settings, mobile laboratories commonly use PCR and/or ELISA analysis for rapid screening.

Ebola virus and Marburg virus antigens can be detected by IHC on formalin-fixed tissues by using specific polyclonal and monoclonal antibodies. These methods have a unique role in cases in which formalin-fixed tissues are the only specimens available for diagnostic testing. Formalin-fixed biopsy specimens are not infectious and may be sent without special precautions or refrigeration and may be obtained in the most basic field conditions [44, 47].

In situ hybridization has been used in reports characterizing Ebola virus infections in animal models [48, 49, 50]. In cultures of alveolar macrophages, a notable increase in the amounts of Ebola virus RNA and protein in the cytoplasm of infected cells has been observed [51]. Experimental studies with guinea pigs have detected macrophages containing EBOV RNA in lymph nodes, spleen, hepatocytes, Kupffer cells, circulating mononuclear cells, gastrointestinal tract, adrenal gland, kidney and urinary tract, lung, reproductive organs, and heart [48].

Electron microscopy (EM) has long been used in the discovery and description of infectious diseases, particularly in cases where agents are unknown or unsuspected...
EM was contributory in elucidating the presence of a novel virus in the first outbreak of Marburg in Germany in 1967, in the first outbreak of Ebola virus in Zaire in 1976 [53, 54, 55] and in identifying the Reston virus infection of a monkey colony in Reston, VA, in 1989 [56]. Several studies have demonstrated ultrastructural differences between MARV and EBOV, and have established EM to be a critical and definitive tool in the diagnosis of filoviruses (Figure 3B) [47, 57, 58].

Microarray analysis is a relatively newer research technique that was critical in the identification of pigs as a host for Reston virus in 2008. These pigs were co-infected with Porcine Reproductive and Respiratory Syndrome virus [39].

Pathology

Although more than 3,000 fatal cases with documented filovirus infection have been recognized in the past 40 years, autopsies and postmortem biopsies have been performed on a very small subset of these patients. This relatively small number can be partially attributed to filoviruses being category A pathogens, which have the potential to be transmitted to humans performing autopsies and necropsies [44, 59, 60]. Transmission may occur by percutaneous inoculation (i.e., injury) and by splashes to unprotected mucosa [44, 61]. In contrast, aerosol transmission of filoviruses is highly controversial; a recent study in a BSL-4 laboratory found no evidence of aerosol transmission among non-human primates [13].

Marburg haemorrhagic fever (MHF) and Ebola haemorrhagic fever (EHF) should be considered in patients with the appropriate clinical symptoms and exposures, such
as recent travel in an endemic area, particularly during seasonal or epidemic disease activity. Clinical suspicion of filovirus infection based on clinical history and presenting symptoms can be supported histopathologically and confirmed by using immunohistochemistry [62].

Gross autopsy findings include petechiae or ecchymoses of skin or mucous membranes, and hemorrhage of internal organs [63, 64, 65, 66, 67]. However, these pathological features are seen in many other diseases and raise a broad differential diagnosis, which includes not only other VHF s but also rickettsial, bacterial, other viral and even noninfectious causes of haemorrhage. More definitive diagnosis can be made by laboratory tests such as PCR, antigen-capture enzyme-linked immunosorbent assay (ELISA), virus isolation, IgM and IgG antibodies, histopathology, immunohistochemistry (IHC), and electron microscopy [44,46, 47,58].

In this article we review the histopathology, immunohistochemical, in situ hybridization and ultrastructural findings of fatal human cases caused by Zaire Ebola virus (EBOV), Sudan virus (SUDV), Bundibugyo virus (BDBV), and Marburg virus (MARV) at Centers for Disease Control and Prevention, from 1995 to 2007. A total of 89 fatal cases of filoviruses were analyzed, from skin biopsies, post-mortem biopsies and autopsies; 74 ebolaviruses (28 EBOV; 45 SUDV; 1 BDBV) and 15 Marburgvirus (14 skin biopsies and 1 autopsy) were evaluated. A review of the literature identified only 30 fatal cases where an autopsy or post-mortem biopsies were performed and
pathological descriptions were reported. This article will present our findings on the 89 cases and compare them with the reports in the literature.

In general the overall histopathological features are similar in Marburg virus and Ebola virus infections, with necrosis seen in many organs, including liver, spleen, kidney and gonads, the gastrointestinal tract and endocardium [47, 68].

Liver

The liver has the most characteristic histopathological features in filovirus infections. Hepatocyte necrosis ranges from focal to widespread, often with minimal inflammation. Mild to moderate small droplet steatosis and Kupffer cell hyperplasia are also seen. In EBOV, the portal tracts usually exhibit extensive karyorrhexis and mononuclear inflammatory cell infiltrates (Figure 4 A). Hepatocytes may show characteristic intracellular eosinophilic and filamentous or oval virus inclusions, which are predominantly found in periportal zones and surrounding areas of necrosis [44, 47, 69]. Ultrastructurally, the inclusions are composed of aggregates of viral nucleocapsids (Figure 4 B) [44, 47, 53, 58, 66, 70]. The inclusions are usually more readily identified in EBOV infections than in MARV infections. EBOV infections appeared to contain the most frequent hepatocyte inclusions; SUDV infections demonstrated an intermediate number of inclusions, while no inclusions were identified in a single case of BDBV evaluated (Figure 5, 6 and 7). Although the literature describes hepatocyte inclusions in Marburg virus infections [58, 66, 71], we did not observe hepatocyte inclusions in the one case of Marburg virus infection examined (Figure 8 A, B).
**Lung**

Microscopic examination of lung from fatal Ebola cases shows congestion, focal intra-alveolar oedema, and haemorrhage with no significant inflammation. Immunohistochemical staining shows viral antigens in alveolar macrophages, endothelial cells, fibroblasts, and others interstitial cells [47, 62]. By careful review of the H&E sections using high-power, viral inclusions can be seen within the intra-alveolar macrophages (Figure 9 B) and more readily identified by using IHC (Figure 9 C). Replication of the virus in these macrophages is evidenced by ISH staining (Figure 9 D). These inclusions within macrophages can also be seen by EM (Figure 9 E). Free viral particles can be seen within the alveolar spaces (Figure 9 F). In the few case reports, Marburgviruses do not demonstrate significant lung pathology [65, 67]. Only one description was associated with diffuse congestion, haemorrhage and suppurative pneumonia, but the patient had a bacterial coinfection [58]. Experimental studies show variable lesions, which range from oedema, focal haemorrhage, and necrosis in non-human primates to pneumonia in pigs [48, 72, 73, 74, 75, 76, 77].

**Spleen and lymph nodes**

In EHF, widespread lymphoid depletion associated with necrotic debris and apoptosis have been observed in the spleen and lymph nodes. Viral immunostaining is seen throughout the spleen, and involves cells of the mononuclear phagocytic system, dendritic cells, and fibroblasts (Figure 10 A-F) [47, 58, 65, 67]. Additionally, large amounts of antigen are present extracellularly, in association with necrotic cells
and debris. Marburg virus infection shows depletion of the white pulp, perifollicular congestion, and centrifollicular necrosis [66].

Comparisons between the splenic histopathology of different virus strains is extremely difficult given that many of the samples are core needle biopsies and limit the ability to assess architectural changes. However, our overall impression is that EBOV infections tend to have greater degrees of lymphoid depletion and necrosis and have comparatively larger amounts of viral antigen by using IHC. BUDV infection appears to show the least amount of lymphoid depletion and antigen, while the changes seen in SUDV cases is somewhat intermediate.

**Skin**

In EHF and MHF, signs of haemorrhagic diatheses are commonly seen in the skin and mucous membranes, and are not associated with jaundice. Cutaneous manifestations usually appear 4-5 days after initial symptom onset [78]. The histopathological changes in the skin tissue are minimal and consist mainly of various degrees of dermal oedema, focal haemorrhage as well as endothelial cell swelling and necrosis. Fibrin thrombi are rare, and vascular necrosis is absent [44, 78]. Immunohistochemical stains reveal abundant antigens in epidermal dendritic cells, endothelial cells, and connective tissue fibroblasts. Viral antigens can also be detected 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 (Figure 11 A-E). Immunohistochemistry on post-mortem skin
punch biopsies has been used for diagnosis, evaluation of transmission, and surveillance [44].

**Gastrointestinal tract**

In cases of EHF, the gastrointestinal tract shows mild focal mononuclear infiltrates in the lamina propria. By using immunohistochemistry, viral antigens can be seen in mononuclear cells within the lamina propria of gastric, small intestinal and colonic mucosa (Figure 12 A, B). Ultrastructurally, Marburg-like inclusions in macrophages and fibroblast- like cells, and virions in necrotic cells and reticular fibres, have been described [58]. These findings correlate with human-to-human transmission resulting from exposure to bloody stools.

**Kidney**

Kidneys frequently show evidence of acute tubular necrosis with no significant inflammation [47, 58]. Immunohistochemistry shows endothelial (including glomerular capillary loops), focal interstitial, and tubular staining. Apoptotic debris is frequently seen in the areas of interstitial staining (Figure 13 A-D). These findings correlate with studies that found evidence of virus in urine by EM and PCR [79].

**Testis**

No significant inflammation was observed in the testicular tissue from the one EBOV case examined. Immunohistochemical staining of viral antigens was seen focally within seminiferous tubules, extensively in the endothelium, and in fibrin clots within
blood vessels (Figure 14 A-F). Fibrin clots associated with Ebola antigens in the testis and other tissues support the conclusion that thrombocytopenia is likely a result of platelet consumption. Experimental infection in non-human primates shows testicular inflammation and haemorrhage. Electron microscopy showed numerous Ebola virus particles in interstitial cells, endothelium and monocytes [75]. Ebola virus was detected in semen for up to 82 days in one report, and Marburg virus for up to 13 weeks in another [26,80] after the onset of illness. These findings along with reported sexual transmission in the latter study indicate that these viruses can be sexually transmitted [26]. The presence of viral antigens in seminiferous tubules supports the possibility of sexual transmission of filovirus, which has been previously reported.

**Bone marrow**

The morphological changes in the bone marrow in MHF and EHF are non-specific. The findings include a normocellular bone marrow with focal necrosis. In EBOV cases, abundant inclusions in mononuclear cells as well as extracellular antigens can be seen by using IHC. Normal megakaryocyte number, and the absence of immunostaining in megakaryocytes, support the conclusion that the thrombocytopenia observed in these infections is not due to reduction in platelet production (Figure 15 A, B).

**Heart and the Central Nervous System**

Morphological myocardial damage in EBOV and MARV autopsy cases is not significant. Immunohistochemistry shows abundant endocardial, endothelial and
extracellular antigens (Figure 15 C, D). Histological examination of the central nervous system in EHF has never been performed, and is limited in MHF, with only two cases reported in the literature. The patients were described to have subacute encephalitis; histologically, the brain showed panencephalitis with glial nodules and a slight perivascular lymphocytic infiltration [63, 67].

Pathogenesis of Filoviruses: Current hypothesis

In the following sections the pathogenesis of filovirus infections including viral entry, early cellular targets, immunopathology, endothelial cell dysfunction and coagulopathy will be discussed (Figure 16).

Filovirus entry

Filoviruses are capable of infecting and replicating in a wide range of tissues and cells. Filoviruses enter the host through mucosal surfaces, breaks, and abrasions in the skin, or by accidental injection. Filovirus entry involves three distinct phases: cellular attachment, endocytosis, and fusion. There are multiple proposed mechanisms of cell entry, which include: clathrin-mediated endocytosis, macropinocytosis [81], and glycoprotein-facilitated receptor binding [81, 82, 83, 84, 85]. Several cellular molecules have been proposed to be receptors or cellular mediators of viral entry including C-type lectins, tyrosine kinase receptors, and more recently Niemann Pick C1 [84, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100]. A wide range of cell types, including hepatocytes and adrenal cortical and medullary cells, have been found to be permissive to filovirus infection [44, 58, 101, 102, 103]. The interaction of filovirus with many different cellular proteins may
explain the broad tropism seen in filovirus infection. Organ tropism may also be enhanced by direct access of viral particles to the cells of the mononuclear phagocytic system without the need to penetrate cellular or tissue barriers [104].

**Early targets: Macrophages and dendritic cells**

Macrophages, Kupffer cells and dendritic cells (DCs) have been identified as major early and sustained targets of EBOV and MARV infection [1, 47,48,49,58, 105, 106]. Moreover, monocytes, macrophages and DCs are thought to responsible for spreading the virus from the initial site of infection to regional lymph nodes via lymphatics, and to liver and spleen via blood. EBOV subsequently infects tissue macrophages, including Kupffer cells, DCs, and fibroblastic reticular cells [105, 107].

**Immunopathology**

The tissue damage seen by histological examination may be interpreted as morphological evidence of the virus’ ability to subvert both innate and adaptive immune responses. Despite having high viral load and necrotic lesions in fatal filovirus cases, only minimal inflammation is observed in infected tissues and organs, indicating a dysregulated immune response. Electron microscopic, immunohistochemical, and in situ hybridization studies have also shown a strong association of parenchymal necrosis in organs and the presence of abundant virus particles, antigens, and nucleic acids with minimal inflammation [44, 47, 58].
Marburg virus and Ebola virus utilize multiple mechanisms to evade detection and undermine innate immune responses. The structural proteins VP24 and VP35 are central to EBOV’s ability to evade host innate immunity and inhibit type I interferon (IFN) responses [108, 109, 110, 111, 112, 113, 114, 115, 116, 117]. The Marburg viruses also diminish host cellular responses to IFNs. The mechanism is thought to be dependent on the structural protein VP40 [118, 119].

Several studies have shown that, in cases of severe infection, there is a massive release of proinflammatory mediators and vasoactive substances, which promotes inflammation and coagulation, but renders the immune system unable to effectively prevent systemic spread of the virus [120, 121, 122]. Associations between multiple proinflammatory cytokines and increased death rates are seen in human studies [122, 123, 124, 125]. Monocytes and macrophages release numerous proinflammatory cytokines (IL-1β, IL-1RA, IL-6, IL-8, IL-15 and IL-16), chemokines and growth factors (MIP-1α, MIP-1β, MCP-1, M-CSF, MIF, IP-10, GRO-α and eotaxin). Tumour necrosis factor and reactive oxygen and nitrogen species are also thought be involved [119, 120, 121, 122, 123, 124, 125, 126, 127]. A recent study noted that the inflammatory response profile in paediatric patients who survived is different from the profile in those who died. Fatal paediatric cases tended to have lower levels of RANTES and higher levels of IL-10 [128]. DCs secrete interleukins and cytokines, which provide a critical link between innate and adaptive immune responses but also act as antigen-presenting cells and initiate adaptive immune responses through T-cell activation [106, 119, 122]. Infected DCs secrete only a limited range of chemokines, fail to express co-stimulatory molecules or upregulate major
histocompatibility complex (MHC) and are unable to induce differentiation of allogenic lymphocytes [84, 105, 119, 128].

Although lymphocytes are not targets of infection, natural killer (NK) cells and T-lymphocytes undergo ‘bystander’ apoptosis relatively early in the course of the disease [2, 122, 123, 130]. Depletion of NK cells and T-lymphocytes may be seen histologically in the spleen and lymph node, and further impair the immune response. Infected macrophages and DCs demonstrate increased expression of TNF, Fas and Fas ligand, TNF-related apoptosis-inducing ligand (TRAIL) and nitric oxide (NO), which have all been implicated in inducing apoptosis of host lymphocytes [120, 130]. One study observed decreased circulating CD4+ and CD8+ T-lymphocytes, which was associated with increased in Fas in the T-lymphocytes, suggesting that the apoptosis is mediated by a Fas-FasL mechanism [123].

*Endothelial cell dysfunction*

Ultrastructural analyses in fatal cases show numerous inclusions and viral particles within endothelial cells. Although immunostaining with EBOV antibody is seen in endothelial cells and endocardium, these cells often remain intact and show minimal signs of injury. Cardiac endothelial cell, along with systemic endothelial cell, immunostaining and inclusions seen by EM suggest their involvement in haemorrhage, fluid and electrolyte imbalance and cardiovascular failure. One study showed that endothelial cells in non-human primates are not early targets of EBOV infection [1]. However, abundant EBOV viral particles have been detected in the endothelium of fatal cases [44, 47] and may be related to the high viral titres and
severity of symptoms seen in these cases. Several studies suggest that endothelial impairment is caused by an indirect immune-mediated mechanism. NO, prostacyclin, interferons (IFNs), interleukins and chemokines can modify vascular tone and permeability, thrombosis, and inflammation, and may contribute to the pathogenesis of hypotensive shock and coagulopathy seen in filovirus infections [131, 132, 133].

Coagulopathy

The coagulopathic state seen in EHF is multifactorial and appears to be caused by a combination of activation of the mononuclear phagocytic system, platelet aggregation and consumption, activation of the coagulation cascade, deficiency of coagulation factors due to liver damage, and endothelial damage. Elevated levels of proinflammatory cytokines, particularly IL-6, trigger the coagulation cascade [133, 134]. Moreover, infected monocytes and macrophages upregulate expression of tissue factor (TF) mRNA and production of cell surface TF protein; one study also detected increased TF-expressing membrane microparticles in macaques infected with EBOV [129, 131, 133]. Extensive viral replication also causes hepatic necrosis/apoptosis, which further impairs synthesis of critical coagulation factors. Decreased levels of protein C, protein S and fibrinogen have also been observed [135]. Aberrant activation of both fibrogenic and fibrinolytic pathways leads to disseminated intravascular coagulation (DIC). Markers of fibrinolysis, such as D-dimers, are detectable on day 1 in EBOV-infected non-human primates [129, 130].

Treatment and Vaccines

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The large 2014 outbreak in West Africa highlights that no licensed vaccines or post-exposure treatments against Ebola are available. The first Ebola treatment recently approved by the WHO uses convalescent sera from people who recovered from infection with Ebola virus [136]. Another promising treatment is a combination of monoclonal antibodies (ZMapp), which was tested in rhesus macaques with 100% protection when it was administered as late as five days after Ebola exposure. [137,138]. These antibodies have already been used on a few patients during this outbreak. The WHO also declared that two candidate vaccines could be introduced as early as this year when safety studies have been concluded [136, 139].

Conclusions

Human histopathological studies and ancillary techniques such as IHC, PCR and EM are important in the diagnosis of these infections, and for enhancing our understanding of their pathogenesis. Additional post-mortem biopsies and autopsy studies will undoubtedly further elucidate the pathology and pathogenesis of filovirus infections in humans.

Haemorrhagic fevers have a complex pathogenesis that involves activation of the mononuclear phagocytic system with release of proinflammatory cytokines, chemokines and growth factors, endothelial dysfunction, alterations of the innate and adaptive immune system, direct organ and endothelial damage from unrestricted viral replication late in infection and coagulopathy. Viral particles and antigens have been detected in the blood, skin, kidney, and seminiferous tubules and explain the role of bodily fluids in human-to-human transmission. Aerosol transmission is
extremely controversial but the finding of free viral particles in alveoli and within intra-alveolar macrophages warrants further study.

Targeted therapies and vaccines are an immediate and top priority. The highly virulent and infectious nature of filoviruses, increasingly porous geographic borders, breakdown of public health measures, poverty and social inequity [140], create significant and daunting challenges ahead. Resolving these issues will require long-term commitment and collaboration from the world community and international partners.

**Ethical Statement:**

Tissues used in this study were obtained at autopsy or by post-mortem biopsies and were submitted to the CDC for laboratory evaluation. This activity is not considered research that requires review by an institutional review board or informed consent from patients.

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Author Contributions

All coauthors on this manuscript have been responsible for:

1) Substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data;

2) drafting the article or revising it critically for important intellectual content;

3) final approval of the version to be published.
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Figure Legends

**Figure 1. Filovirus ecology.** Illustrations detailing both enzootic and epizootic cycles of filoviruses and transmission to humans.

*Illustrations courtesy of Craig Manning, Viral Special pathogens Branch and Alissa Eckert, Medical Illustrator, CDC.*
Enzootic Cycle
New evidence indicates that cave-dwelling African fruit bats (Rousettus aegyptiacus) are the reservoir hosts for Marburg virus. How the virus is maintained and transmitted within fruit bat populations remains unknown.

Ebolaviruses:
- Ebola virus (formerly Zaire virus)
- Sudan virus
- Tai Forest virus
- Bundibugyo virus
- Reston virus (non-human)

Epizootic Cycle
Epizootics caused by ebolaviruses appear sporadically, producing high mortality among non-human primates and diukers and may precede human outbreaks. Epidemics caused by ebolaviruses produce acute disease among humans, with the exception of Reston virus which does not produce detectable disease in humans. Little is known about how the virus first passes to humans, triggering waves of human-to-human transmission, and an epidemic.

Human-to-human transmission is a predominant feature of epidemics.

Following initial human infection through contact with an infected bat or other wild animal, human-to-human transmission often occurs.

It is possible that Marburg virus can be transmitted within secondary wild animal hosts, such as non-human primates. Humans might become infected through contact with these secondary hosts.

Humans may become infected when they enter caves or mines containing populations of infected bats. Following the initial human infection through contact with virus from infected bat or other wild animal, human-to-human transmission can occur.
Figure 2. Worldwide geographic distribution of Filovirus Haemorrhagic Fever cases, 1967 – 2014.

(Illustration courtesy of Elizabeth Ervin, Viral Special Pathogens Branch, CDC)
Figure 3. Laboratory diagnosis of Filovirus Hemorrhagic Fever. (A) IgM antibodies can appear as early as 2 days after onset of symptoms and disappear after up to 3-6 months. Specific IgG antibodies can develop between day 6 and 18 after onset of symptoms and persist for several years. RT-PCR detects the virus generally 3-10 days after the onset of symptoms. (B) EM photomicrograph showing three Ebola virions, measuring between 500 nm and 1 µm. Surface spikes composed of glycoprotein (arrow), 7-10 nm in length, can be seen extending from the membrane of the nucleocapsid. Scale bar, 0.1 µm.

(A) Courtesy of Viral Special Pathogens Branch, CDC; (B) Negative-stained EM courtesy of Maureen G. Metcalfe, Infectious Disease Pathology Branch, CDC.
Figure 4. Hepatic histopathological and ultrastructural findings in a fatal case of Ebola virus (EBOV) infection. (A) Low-power photomicrograph showing hepatocellular necrosis, sinusoidal dilatation and congestion. Note numerous intracytoplasmic eosinophilic inclusions (arrow). (B) EM photomicrograph shows hepatic viral inclusions (arrow) within infected hepatocytes. Note the extracellular sinusoidal virus particles (arrowhead).

(A) Haematoxylin and eosin stain; (B) Thin-sectioned EM courtesy of Cynthia S. Goldsmith. IDPB, CDC.
Figure 5. Hepatic histopathological features and viral immunostaining in a fatal case of Ebola virus (EBOV) infection. (A) Hepatocellular necrosis with intracytoplasmic eosinophilic and filamentous inclusions. (B) Extensive immunostaining of EBOV antigens is seen throughout, in sinusoids, sinusoidal lining cells, and hepatocytes.

(A) Haematoxylin and eosin stain; (B) Immunoalkaline phosphate staining using a rabbit anti-Ebola antibody, naphthol fast red substrate with light haematoxylin counterstain.
Figure 6. Hepatic histopathological features and viral immunostaining in a fatal case of Sudan virus (SUDV) infection. (A) Rare intracytoplasmic eosinophilic and globular inclusions (arrows). (B) Abundant SUDV antigens are diffusely distributed in Kupffer cells. Note the intracytoplasmic immunostaining of an inclusion in a hepatocyte (arrow).

(A) Haematoxylin and eosin stain; (B) Immunoalkaline phosphate staining using a rabbit anti-Ebola antibody, naphthol fast red substrate with light haematoxylin counterstain.
Figure 7. Hepatic histopathologic features and viral immunostaining in a fatal case of Bundibugyo virus (BDBV) infection. (A) Small droplet steatosis and focal hepatocyte necrosis (arrow). Note the absence of inclusions. (B) BDBV antigens are seen focally.

(A) Haematoxylin and eosin stain; (B) Immunoalkaline phosphate staining using a rabbit anti-Ebola antibody, naphthol fast red substrate with light haematoxylin counterstain.
Figure 8. Hepatic histopathological features and viral immunostaining in a fatal case of Marburg virus (MARV) infection. (A) Extensive hepatocellular necrosis adjacent to relatively intact hepatocytes. No distinct viral inclusions are present. (B) Serial section showing diffuse immunostaining of MARV antigens in sinusoidal lining cells in the necrotic areas observed in A.

(A) Haematoxylin and eosin stain; (B) Immunoalkaline phosphate staining using a rabbit anti-Ebola antibody, naphthol fast red substrate with light haematoxylin counterstain.
Figure 9. Pulmonary histopathological, immunohistochemical, in situ hybridization and ultrastructural characteristics of fatal Ebola Haemorrhagic Fever. (A) Low-power image of lung shows congestion and absence of significant inflammatory cell infiltrate. (B) High-power image shows an intra-alveolar macrophage with a cytoplasmic eosinophilic inclusion (arrow). (C) Ebola virus antigens in intra-alveolar macrophages and interstitium. Note immunostaining of globular inclusions in intra-alveolar mononuclear cells (arrow). (D) In situ hybridization showing evidence of viral replication in intra-alveolar macrophages. (E) EM photomicrograph showing Ebola virus inclusion (arrow) in an intra-alveolar macrophage, suggesting that viral replication occurs within the macrophage. (F) EM
photomicrograph showing extracellular Ebola virus particles (arrowhead) free-floating within the alveolar space.

(A, B) Haematoxylin and eosin stain; (C) Immunoalkaline phosphate staining using a rabbit anti-Ebola antibody, naphthol fast red substrate with light haematoxylin counterstain; (D) In-situ hybridization using digoxigenin-labeled riboprobe, immunoalkaline phosphate staining and naphthol fast red substrate with light haematoxylin counterstain; (E, F) Thin-sectioned EM (courtesy of Cynthia S. Goldsmith, IDPB, CDC).
Figure 10. Splenic histopathological features and immunohistochemical characteristics in fatal cases of Filovirus infection. Various degrees of lymphoid depletion and congestion are seen in Ebola virus (EBOV), Sudan virus (SUDV) and Bundibugyo virus (BDBV) infection. (A) EBOV case with severe widespread lymphoid depletion and congestion. (B) Immunostaining of Ebola antigens in capsular dendritic cells (arrow) and macrophages in the red pulp. (C) SUDV case showing a moderate degree of depletion of the germinal centres, haemorrhage and congestion. (D) Immunostaining in macrophages in red pulp associated with karyorrhexis and apoptosis. (E) Low-power photomicrograph of BDBV case showing a mild degree of lymphoid depletion, (F) Note the rare immunohistochemical staining of a macrophage in the red pulp from the same case as in E.

(A, C, E) Haematoxylin and eosin stain; (B, D, F) Immunoalkaline phosphate staining using a rabbit anti-Ebola antibody, naphthol fast red substrate with light haematoxylin counterstain.
Figure 11. Cutaneous immunohistochemical and ultrastructural features in fatal cases of Ebola virus (EBOV), Sudan virus (SUDV), and Marburg virus (MARV) infection. (A) Extensive amounts of EBOV antigens are seen primarily within fibroblasts in the dermis. Note rare immunostaining of a dendritic cell in the surface epithelium (arrow) (B) EBOV immunostaining in connective tissue surrounding sweat glands. Note nuclear staining in a sweat gland duct (arrow). (C) SUDV case showing immunostaining of endothelial cells in the superficial dermis. (D) Abundant MARV antigen in sebaceous glands. (E) Ebola virus inclusion (arrow) in an endothelial cell and extracellular virus particles (arrowhead) in dermal collagen; RBC, red blood cell.

(A-D) Immunoalkaline phosphate staining using a rabbit anti-Ebola antibody, naphthol fast red substrate with light haematoxylin counterstain; (E) thin-sectioned EM courtesy of Cynthia S. Goldsmith, IDPB.
Figure 12. Gastrointestinal tract histopathological features and immunohistochemistry in a fatal case of Ebola Haemorrhagic Fever. (A) Photomicrograph shows a mild inflammatory infiltrate in the lamina propria of the small intestine. Note the incidental schistosoma ovum in the lamina propria (arrow). (B) Immunopositive cells are seen in the lamina propria in the same case as A.

(A) Haematoxylin and eosin stain; (B) Immunoalkaline phosphate staining using a rabbit anti-Ebola antibody, naphthol fast red substrate with light haematoxylin counterstain.
Figure 13. Renal histopathological features and immunohistochemistry in Ebola and Marburg Haemorrhagic Fevers. (A) Photomicrograph showing vascular congestion and karyorrhectic debris in a fatal case of Ebola virus infection. (B) High-power image showing karyorrhexis and apoptosis. (C) Immunostaining of tubular epithelial cells in association with karyorrhectic debris, in the same case as A. (D) Focal staining in renal tubular epithelial cells in a fatal case of Marburg virus infection.

(A-B) Haematoxylin and eosin stain; (C-D) Immunoalkaline phosphate staining using a rabbit anti-Ebola antibody, naphthol fast red substrate with light haematoxylin counterstain.
Figure 14. Histopathological and immunohistochemical features in the testis of a fatal case of Ebola virus infection. (A) Photomicrograph showing interstitial congestion and seminiferous tubules with no significant histological changes. (B) Low-power photomicrograph of the same area as A showing immunostaining of viral antigens in seminiferous tubules and interstitium, despite the absence of any histological lesions. (C) High-power photomicrograph showing immunostaining of viral antigens in the seminiferous tubules and interstitium. (D) Immunostaining of viral antigens in endothelial cells (E) Fibrin mesh in a small interstitial blood vessel. (F) Section of same blood vessel as seen in E, showing viral immunostaining within fibrin mesh, endothelial cells (arrow head), and a circulating monocyte (arrow).

(A, E) Haematoxylin and eosin stain; (B,C,D,F) Immunoalkaline phosphate staining using a rabbit anti-Ebola antibody, naphthol fast red substrate with light haematoxylin counterstain.
Figure 15. Bone marrow in a fatal case of Ebola virus infection. (A) Normocellular bone marrow with unremarkable megakaryocyte (arrow). (B) Abundant cellular and extracellular staining in the same bone marrow as seen in A. Note the lack of megakaryocyte staining (arrow). (C) Endocardium with no significant histopathology. (D) Abundant immunostaining of Ebola virus antigens in the endocardium and extracellularly in the subendocardium.

(A, C) Haematoxylin and eosin stain; (B, D) Immunoalkaline phosphate staining using a rabbit anti-Ebola antibody, naphthol fast red substrate with light haematoxylin counterstain.
Figure 16. Diagram illustrating filovirus entry, cellular tropism and pathogenesis.
Table 1: Chronology and location of human Filovirus outbreaks (1967-2014), including all laboratory confirmed Ebola and Marburg Haemorrhagic Fever cases. Table modified from [5, 7]

<table>
<thead>
<tr>
<th>Year(s)</th>
<th>Country</th>
<th>Number of human cases</th>
<th>Fatal cases and CFR</th>
<th>Filovirus</th>
</tr>
</thead>
<tbody>
<tr>
<td>1967</td>
<td>Germany and Yugoslavia</td>
<td>31</td>
<td>7 (23%)</td>
<td>MARV</td>
</tr>
<tr>
<td>1975</td>
<td>Johannesburg, South Africa</td>
<td>3</td>
<td>1 (33%)</td>
<td>MARV</td>
</tr>
<tr>
<td>1976</td>
<td>Zaire (Democratic Republic of the Congo - DRC)</td>
<td>318</td>
<td>280 (88%)</td>
<td>EBOV</td>
</tr>
<tr>
<td>1976</td>
<td>Sudan (South Sudan)</td>
<td>284</td>
<td>151 (53%)</td>
<td>SUDV</td>
</tr>
<tr>
<td>1976</td>
<td>England</td>
<td>1</td>
<td>0 (0)</td>
<td>SUDV</td>
</tr>
<tr>
<td>1977</td>
<td>Zaire</td>
<td>1</td>
<td>1 (100%)</td>
<td>EBOV</td>
</tr>
<tr>
<td>1979</td>
<td>Sudan (South Sudan)</td>
<td>34</td>
<td>22 (65%)</td>
<td>SUDV</td>
</tr>
<tr>
<td>1980</td>
<td>Kenya</td>
<td>2</td>
<td>1 (50%)</td>
<td>MARV</td>
</tr>
<tr>
<td>1987</td>
<td>Kenya</td>
<td>1</td>
<td>1 (100%)</td>
<td>MARV</td>
</tr>
<tr>
<td>1990</td>
<td>Russia</td>
<td>1</td>
<td>1 (100%)</td>
<td>EBOV</td>
</tr>
<tr>
<td>1994</td>
<td>Gabon</td>
<td>52</td>
<td>31 (60%)</td>
<td>TAFV</td>
</tr>
<tr>
<td>1994</td>
<td>Ivory Coast</td>
<td>1</td>
<td>0 (0)</td>
<td>EBOV</td>
</tr>
<tr>
<td>1995</td>
<td>DRC (formerly Zaire)</td>
<td>315</td>
<td>250 (81%)</td>
<td>EBOV</td>
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<tr>
<td>1996</td>
<td>Gabon</td>
<td>37</td>
<td>21 (57%)</td>
<td>EBOV</td>
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<tr>
<td>1996-1997</td>
<td>Gabon</td>
<td>60</td>
<td>45 (74%)</td>
<td>EBOV</td>
</tr>
<tr>
<td>1996</td>
<td>South Africa</td>
<td>2</td>
<td>1 (50%)</td>
<td>EBOV</td>
</tr>
<tr>
<td>1996</td>
<td>Russia</td>
<td>1</td>
<td>1 (100%)</td>
<td>EBOV</td>
</tr>
<tr>
<td>1998-2000</td>
<td>DRC</td>
<td>154</td>
<td>128 (83%)</td>
<td>MARV</td>
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<tr>
<td>2000-2001</td>
<td>Uganda</td>
<td>425</td>
<td>224 (53%)</td>
<td>SUDV</td>
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<tr>
<td>October 2001- March 2002</td>
<td>Gabon</td>
<td>65</td>
<td>53 (82%)</td>
<td>EBOV</td>
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<tr>
<td>October 2001- March 2002</td>
<td>Republic of Congo</td>
<td>57</td>
<td>43 (75%)</td>
<td>EBOV</td>
</tr>
<tr>
<td>December 2002- April 2003</td>
<td>Republic of Congo</td>
<td>143</td>
<td>128 (89%)</td>
<td>EBOV</td>
</tr>
<tr>
<td>November-December 2003</td>
<td>Republic of Congo</td>
<td>35</td>
<td>29 (83%)</td>
<td>EBOV</td>
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<tr>
<td>2004</td>
<td>Sudan (South Sudan)</td>
<td>17</td>
<td>7 (41%)</td>
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<td>2004-2005</td>
<td>Angola</td>
<td>252</td>
<td>227 (90%)</td>
<td>MARV</td>
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<tr>
<td>2004</td>
<td>Russia</td>
<td>1</td>
<td>1 (100%)</td>
<td>EBOV</td>
</tr>
<tr>
<td>2007</td>
<td>Uganda</td>
<td>4</td>
<td>1 (25%)</td>
<td>MARV</td>
</tr>
<tr>
<td>2007</td>
<td>DRC</td>
<td>264</td>
<td>187 (71%)</td>
<td>EBOV</td>
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<tr>
<td>December 2007- January 2008</td>
<td>Uganda</td>
<td>149</td>
<td>37 (25%)</td>
<td>BDBV</td>
</tr>
<tr>
<td>2008</td>
<td>USA ex Uganda</td>
<td>1</td>
<td>0 (0)</td>
<td>MARV</td>
</tr>
<tr>
<td>2008</td>
<td>Netherlands ex Uganda</td>
<td>1</td>
<td>1 (100%)</td>
<td>MARV</td>
</tr>
<tr>
<td>December 2008-February 2009</td>
<td>DRC</td>
<td>32</td>
<td>15 (47%)</td>
<td>EBOV</td>
</tr>
<tr>
<td>May-2011</td>
<td>Uganda</td>
<td>1</td>
<td>1 (100%)</td>
<td>SUDV</td>
</tr>
<tr>
<td>2012</td>
<td>Uganda</td>
<td>15</td>
<td>4 (27%)</td>
<td>MARV</td>
</tr>
<tr>
<td>June-October 2012</td>
<td>Uganda</td>
<td>11*</td>
<td>4* (36.4%)</td>
<td>SUDV</td>
</tr>
<tr>
<td>June-November 2012</td>
<td>DRC</td>
<td>36*</td>
<td>13 (36.1%)</td>
<td>BDBV</td>
</tr>
<tr>
<td>November 2012-January 2013</td>
<td>Uganda</td>
<td>6*</td>
<td>3* (50%)</td>
<td>SUDV</td>
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<tr>
<td>March 2014 - Present</td>
<td>Guinea, Liberia, Sierra Leone, Nigeria</td>
<td>3017*</td>
<td>1513(50%)*</td>
<td>EBOV</td>
</tr>
</tbody>
</table>

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DRC - Democratic Republic of the Congo; MARV- Marburg virus; EBOV- Ebola virus; SUDV- Sudan virus; TAFV- Tai Forest virus; BDBV - Bundibugyo virus

* - Numbers reflect laboratory confirmed cases only.
Table 2: Laboratory diagnostic arrays are available at Centers for Disease Control and Prevention. Table modified from [7].

<table>
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<tr>
<th>Timeline of infection</th>
<th>Diagnostic tests available</th>
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<tbody>
<tr>
<td>Within a few days after symptoms begin</td>
<td>• Antigen-capture enzyme-linked immunosorbent assay (ELISA) testing</td>
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<tr>
<td></td>
<td>• IgM ELISA</td>
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<td></td>
<td>• Polymerase chain reaction (PCR)</td>
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<td></td>
<td>• Virus isolation</td>
</tr>
<tr>
<td>Later in disease course or after recovery</td>
<td>• IgM and IgM antibodies</td>
</tr>
<tr>
<td>Retrospectively in deceased patients</td>
<td>• Histopathology</td>
</tr>
<tr>
<td></td>
<td>• Immunohistochemistry testing</td>
</tr>
<tr>
<td></td>
<td>• PCR</td>
</tr>
<tr>
<td></td>
<td>• Virus isolation</td>
</tr>
<tr>
<td></td>
<td>• Electron microscopy</td>
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