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Ebola: Facing a New Transboundary Animal Disease?

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Abstract

Ebola viruses are zoonotic pathogens with the potential of causing severe viral hemorrhagic fever in humans and nonhuman primates. Bats have been identified as a reservoir for Ebola viruses but it remains unclear if transmission to an end host involves intermediate hosts. Recently, one of the Ebola species has been found in Philippine pigs raising concerns regarding animal health and food safety. Diagnostics have so far focused on human application, but enhanced pig surveillance and diagnostics, particularly in Asia, for Ebola virus infections seem to be needed to establish reasonable guidelines for public and animal health and food safety. Livestock vaccination against Ebola seems currently not justified but proper preparedness may include experimental vaccine approaches.

Keywords

Ebola; hemorrhagic fever; pigs; livestock; diagnostics; vaccines

Introduction

Ebola viruses (EBOV) are members of the genus *Ebolavirus* in the family *Filoviridae* of the order *Mononegavirales*. The genus is subdivided into five species, *Zaire ebolavirus*, *Sudan ebolavirus*, *Tai forest ebolavirus*, *Reston ebolavirus*, and the recently discovered tentative species *Bundibugyo ebolavirus* [1]. With the exception of *Reston ebolavirus*, which originates from the Philippines and has not been associated with human disease, all other four species are human pathogens found across Central Africa in a region of approximately 10° north and south of the equator [2].

EBOV causes a disease designated Ebola hemorrhagic fever (EHF) and most outbreaks have been reported from Sudan (*Sudan ebolavirus*), Uganda (*Sudan ebolavirus*, *Bundibugyo ebolavirus*), Democratic Republic of the Congo (*Zaire ebolavirus*, *Bundibugyo ebolavirus*),

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Republic of the Congo (*Zaire ebolavirus*) and Gabon (*Zaire ebolavirus*) [2]. *Tai Forest ebolavirus* has so far caused only one reported severe human infection in West Africa and was retrospectively diagnosed in a survivor in the same region. This leaves three species as the main cause of human EHF, *Zaire*, *Sudan* and *Bundibugyo ebolavirus*. Case fatality rates range between 35–90% based on the species, with *Zaire ebolavirus* being the most virulent one followed by *Sudan* and *Bundibugyo ebolavirus*. Therefore, diagnostic and countermeasure development (antivirals, therapeutics, vaccines) are largely focused on these three EBOV species [2].

EHF is a systemic infection starting with a flu-like disease and often gastrointestinal symptoms. The incubation period ranges from a few days to three weeks with an average of about a week. Infected individuals develop more serious clinical symptoms by the end of the first week after disease onset, which can include multiple forms of hemorrhages accumulating in multiorgan failure and shock. Hemorrhages are not always obvious but their appearance is associated with a bad prognosis. Convalescence can be long with survivors suffering from multiple sequelae, which are not well studied [2, 3].

Transmission among humans is associated with close contact to infected individuals and deceased people during burial procedures. Nosocomial infections occur often due to lack of proper hygiene and safety precautions in hospitals and health care centers in endemic regions [2].

EHF is a zoonosis and certain fruit bat species are thought to be the reservoir [4]. It remains unknown if there are additional reservoirs or a yet unidentified primary reservoir. End hosts for EBOV are largely humans and nonhuman primates with a similar disease outcome. Therefore, hunting and bush meat preparation/ consumption are other risk factors for exposure to EBOV [2]. It remains unclear if intermediate or amplifying hosts play a role in the transmission cycle.

Ebola and Livestock

Until very recently, EBOV was only described as a pathogen of humans and nonhuman primates. However, in 2008 *Reston ebolavirus* was discovered in the Philippine pig population causing concerns of food safety and fears of human exposure through livestock [5]. In the meantime a report from China seems to confirm the existence of *Reston ebolavirus* in pigs and expands the region of potential endemicity in Asia [6]. As mentioned before, *Reston ebolavirus* has not yet caused symptomatic infections in humans [2], but infections have been documented in a few individuals involved in handling of nonhuman primates or pigs. Laboratory infection of pigs with *Reston ebolavirus* led to virus replication and shedding from mucosal membranes, but did not cause symptomatic disease in the animals [7]. On the contrary, experimental infection of pigs with *Zaire ebolavirus* resulted in an age-dependent severe disease in pigs with a fulminant lung involvement [8] indicating that EBOV can be pathogenic for this livestock species. Currently, the role of pigs or other livestock species in the transmission of EBOV is unknown, but a matter of great concern to public and animal health. Current *Reston ebolavirus* isolates may be non-pathogenic to humans and pigs, but their close genetic relationship to highly human pathogenic EBOV

species and the mutation frequency of an RNA virus, such as EBOV, may well generate and select mutants of a higher virulence for mammals. However, the food safety concern has not been well addressed and urgently needs further attention, particularly if *Reston ebolavirus* is distributed more widely in pig populations throughout Asia.

Diagnostics

The difficulty with EBOV diagnostics is the identification of individual/index cases. The first clinical presentation of disease is rather unspecific and mimics multiple infectious disease causes in tropical Africa as well as developed countries. This calls for a large list of differential diagnostic pathogens and without exposure history to bats, certain wildlife or a known case, single or initial cases are often missed. Therefore, the foremost priority is education of medical personnel to consider EHF in the differential diagnosis and to initiate proper laboratory testing. If case history and clinical presentation are coherent with EHF, proper case patient management including patient isolation may have to be initiated prior to laboratory confirmation [9].

Laboratory diagnostic testing is largely unavailable in endemic and non-endemic areas, but a limited number of reference centers do exist in Africa and worldwide. The use of reference centers delays laboratory confirmation and adds the complication of specimen transport, but in general the diagnostic service is more reliable. Laboratory testing largely follows standard viral diagnostic procedures and is targeted towards detection of the pathogen and the host immune response (see Table 1). Nowadays, molecular detection of viral genome is the assay of choice and multiple systems and approaches are available ranging from RT-PCR/nested PCR to real-time RT-PCR to Loop-Mediated Isothermal PCR (LAMP) and to microarray detection methods. The assays are rapid and highly sensitive, but sometimes prone to failure when it comes to virus variants or new species due to high specificity of primers and probes. The proper specimen sources for molecular detection are whole blood and tissue. Alternatively, swabs derived from mucosal membranes can be used if taken during the symptomatic phase or from deceased people. Antigen detection, usually performed on an enzyme-linked immunosorbent assay (ELISA) platform, is often used in combination with molecular detection assays and serves confirmatory purposes; specimen sources are the same as for molecular detection methods. Detection of the host immune response focuses on antibodies. The assays of choice are IgM-capture ELISA and IgM or IgG direct ELISA. The presence of EBOV-specific IgM antibodies and a four-fold rise of EBOV-specific IgG antibodies are in general taken as confirmation for a recent infection [9, 10].

All these assays can be performed safely in most diagnostic laboratories on inactivated specimens; the use of high containment (biocontainment level 4 -BSL4) is not necessary for these procedures. Proper inactivation of specimens can be achieved through treatment with chaotropic buffers, irradiation, and a combination of heat/detergent. Virus isolation should certainly be attempted on single or index cases. This, however, requires BSL4 containment and can only be performed in a few facilities worldwide [9, 10]. For outbreak support, mobile laboratories have been deployed that mainly operate on molecular detection technologies and serology but also perform antigen detection ELISA [11].

Current issues with laboratory diagnostics for EBOV are largely related to the unavailability of proper assay systems and control reagents in regular microbiology diagnostic laboratories in endemic areas and worldwide. The easiest and safest methodology is molecular detection and could be established in almost any diagnostic setting. Problems arise with assay evaluation, quality assurance and test performance. For single/index cases it is therefore highly recommended to seek independent confirmation by a reference laboratory, given the tremendous impact of a false-positive or false-negative result. It should be mentioned that with the exception of one assay (Ebola abicap antigen detection; Senova), no other assays are commercially available.

Vaccines

The development of countermeasures against pathogens of bioterrorism, of which EBOV is a prime example, has dramatically improved over the past decade due to increased interest and funding by governments of developed countries. This work is highly dependent on animal infection/disease models which are important components of the licensing procedure for modalities against pathogens for which human efficacy trials cannot be performed. Multiple animal models have been developed for EBOV [12, 13]. The rodent models (mouse, guinea pig and hamster) are dependent on in vivo adaptation. Adapted strains usually show mutations in proteins that interfere with the interferon response of the host, such as the EBOV VP30 and VP24 proteins [2, 13]. The rodent models are disease models but lack certain key characteristic features of human EHF. Among the rodent models, the mouse is a good screening model due to ease of handling and the availability of immunological tools. The predictive value for efficacy testing, however, is low which might be explained by a lack of several hallmark features of human EHF. The guinea pig model has a higher predictive value for efficacy testing of countermeasures likely due to more similar clinical features with human EHF, but it is limited in its application by the general lack of tools available for this animal species. The recently developed hamster model, for which tools are currently under development, shows great potential, because it seems to mimic human EHF best of all rodent models, including the development of coagulation abnormalities that are not observed in mice and guinea pigs. This model, however, has not yet been used for extensive efficacy testing of countermeasures. The ultimate animal model is the nonhuman primate, in particular cynomolgus and rhesus macaques, which show all hallmarks of human EHF and are considered to have the best predictive value for efficacy in humans due to their close genetic relationship.

Efforts to develop EBOV vaccines go back to the early '80s of the last century when killed virus vaccines were produced, however, with low efficacy in protecting nonhuman primates [2, 14, 15] (see Table 2). This was followed by DNA vaccination and the use of subunit vaccines based on either purified EBOV proteins or vector-expressed EBOV proteins (i.e. vaccinia virus, baculovirus), mainly the sole surface glycoprotein (GP), which is the target for neutralizing antibodies. Despite promising results with some approaches in rodent models, efficacy testing in nonhuman primates largely failed. Funding of countermeasures against biothreat pathogens has changed the concept of vaccine development in the field of rare emerging/re-emerging pathogens. Emergency vaccines are of higher priority with features such as single vaccination, short-time to immunity, long-lasting immunity and ease

of application being important criteria. More recent approaches include attenuated replication-deficient and -competent viral vectors expressing GP as the sole immunogen or in combination with certain other viral structural proteins. Today, the leading platforms with good efficacy in nonhuman primate models are replication-deficient adenovirus (Ad5) and replication-competent vesicular stomatitis virus (VSV) vectors expressing the EBOV GP (different vectors for the distinct EBOV species). These vectors have resulted in 100% protection of macaques against lethal homologous (same species) challenge following a single-shot immunization approach. Unfortunately, cross-species protective efficacy is rather low, but can be overcome by blending of vectors expressing GPs from different EBOV species. In case of VSV, the vaccine has also shown complete or partial protective efficacy (species-dependent) when used up to 48 hours after lethal EBOV challenge [16]. In addition, virus-like particles (VLPs), minimally consisting of the EBOV matrix protein VP40 and GP, have shown promising efficacy in macaque models of EHF [17]. The biggest advantage of VLPs over the most promising vector-based platforms is their safety record (potential problem with VSV-based vaccines) and the lack of pre-existing immunity (problem with Ad5-based vaccines), but in contrast to the vector-based platforms, VLP-based vaccines require booster immunization, which limits their use in emergency situations. Currently, only the adenovirus-based vectors and DNA-based vaccines are in clinical trials.

conclusions

Given the more recent development with evidence of *Reston ebolavirus* in pigs in certain Asian countries, EBOV may be considered a potential livestock pathogen with transboundary threat potential. Testing for *Reston ebolavirus* or EBOV-like viruses in pigs may be considered for certain countries as well as for countries that import from areas that have reported infections. In general, it should not be a problem to adapt the existing diagnostic tests for use in livestock species such as pigs, but a commercial test would certainly improve the use in the veterinary field and provide better quality diagnostic results.

In order to provide better guidance for authorities in public and animal health we need more surveillance data on pig populations in Asia and other parts of the world. Further experimental studies have to address the virulence of *Reston ebolavirus* or other EBOVs in pigs and perhaps other livestock species. We also need to study the transmission potential among pigs and the potential of cross-species transmission into other mammalian species including humans. Interestingly, transmission from pigs to nonhuman primates has recently been observed during experimental infection of pigs with *Zaire ebolavirus* [18].

Vaccination of pigs against EBOV, however, seems premature at this time and currently not justified. Nevertheless, experimental vaccine approaches could be developed and tested in pig models as a measure for preparedness, should the situation change with more evidence for infection in expanded pig populations. The current vaccine approaches developed for human applications may not all be best suited for livestock applications and new approaches should be evaluated using vaccine vector systems with good safety and efficacy profiles in livestock species.

Overall, the potential threat posed to livestock and humans through EBOV or EBOV-like viruses in pigs should not be underestimated and certainly needs further attention and careful follow-up.

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Table 1**Ebola Diagnostics**

Test System	Target	Specimen (preferred)	Comments
Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)*	viral genome	blood** / tissue	primary assay
Loop-Mediated Isothermal PCR (LAMP)	viral genome	blood** / tissue	primary assay
Microarray	viral genome	blood** / tissue	alternative assay
In situ-hybridization	viral genome	tissue	alternative assay
Antigen ELISA	viral protein	blood** / tissue	primary assay / confirmatory assay
Fluorescence assay (FA)	viral protein	tissue	alternative assay
Immunohistochemistry (IHC)	viral protein	tissue	alternative assay
Electron microscopy (EM)	viral particle	blood** / tissue	alternative assay
Virus isolation	viral particle	blood** / tissue	alternative assay / confirmatory assay
IgM-capture ELISA	virus-specific antibody	serum	primary assay
IgM direct ELISA	virus-specific antibody	serum	alternative assay
IgG direct ELISA	virus-specific antibody	serum	primary assay
Neutralization assay	virus-specific antibody	serum	alternative assay / confirmatory assay
Immunofluorescence assay (IFA)	virus-specific antibody	serum	alternative assay
Immunoblot	virus-specific antibody	serum	alternative assay

* multiple assays are available and evaluated ranging from RT-PCR/nested PCR to real-time RT-PCR;

** whole blood, serum or plasma, (whole blood preferred); ELISA = enzyme-linked immunosorbent assay; primary assay = first choice for diagnostics; alternative assay = not commonly used as a diagnostic assay; confirmatory = used for confirmatory purposes.

Table 2**Ebola Vaccines**

Vaccine Platform	Characteristic	Efficacy	Status
Adenovirus-based (Ad5 & other serotypes) (GP)	replication-deficient	nonhuman primates (pre-exposure; single shot)	clinical trial
Vesicular stomatitis virus (VSV)-based (GP)	replication-competent	nonhuman primates (pre- & post-exposure; single shot)	experimental
Virus-like particle (GP & VP40 & NP)	replication-incompetent	nonhuman primates (pre-exposure; boosts)	experimental
Paramyxovirus-based (HPiV) (GP)	replication-competent	nonhuman primates (pre-exposure; single shot)	experimental
Virus-like replicon particle (VRP) (GP)	replication-deficient	nonhuman primates (pre-exposure; boost)	experimental
Subunit (GP)	replication-incompetent	rodents (pre-exposure; boost)	experimental
DNA (GP & N)	replication-incompetent	rodents (pre-exposure; boost)	clinical trial

Ad5 = adenovirus serotype 5; GP = Ebola glycoprotein; HPiV = human parainfluenzavirus; N = Ebola nucleoprotein; VP40 = Ebola matrix protein; VSV = vesicular stomatitis virus, serotype Indiana