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Animal models for highly pathogenic emerging viruses

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Exotic and emerging viral pathogens associated with high morbidity and mortality in humans are being identified annually with recent examples including Lujo virus in southern Africa, Severe Fever with Thrombocytopenia Syndrome virus in China and a SARS-like coronavirus in the Middle East. The sporadic nature of these infections hampers our understanding of these diseases and limits the opportunities to design appropriate medical countermeasures against them. Because of this, animal models are utilized to gain insight into the pathogenesis of disease with the overall goal of identifying potential targets for intervention and evaluating specific therapeutics and vaccines. For these reasons it is imperative that animal models of disease recapitulate the human condition as closely as possible in order to provide the best predictive data with respect to the potential efficacy in humans. In this article we review the current status of disease models for highly pathogenic and emerging viral pathogens.

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Introduction

Over the past several decades, sporadic and often isolated outbreaks of diseases associated with high lethality have led to the discovery of a diverse array of novel, highly pathogenic viruses belonging among others to the *Filoviridae*, *Arenaviridae*, *Bunyaviridae*, *Paramyxoviridae*, *Coronaviridae* and *Flaviviridae*. Recent examples include Lujo virus, which caused five known human infections in Southern Africa, four of which were fatal, and represents the first pathogenic arenavirus discovered in Africa in over forty years; Severe Fever with Thrombocytopenia Syndrome virus, a previously unidentified tick-borne bunyavirus which emerged in China with mortality rates as high as 30%; and a novel coronavirus which is responsible for severe acute respiratory distress in humans in the Middle East [1•,2,3]. Although individual agents often display a limited geographical distribution, which is usually dictated by the home range of specific zoonotic reservoirs or vectors, when combined they have worldwide distribution with representative high consequence viral pathogens present in most human populations.

The severe disease manifestations and frequently high mortality rates associated with these viruses, coupled with the risk of intentional release make the development of appropriate medical countermeasures a high priority. However, the unpredictable nature of these infections, the rare occasions of outbreaks, the usually small number of affected people, along with their predilection to occur in remote areas of developing countries, severely hamper the evaluation of therapeutic modalities against these agents directly in humans. In situations where evaluating the efficacy of medical countermeasures in humans is impractical, preclinical testing of therapeutics relies on the use of animal models of disease. Regulating this process, the United States Food and Drug Administration's (FDA) Animal Rule provides guidelines relating to study design and endpoints, pharmacokinetics and pharmacodynamics and the appropriateness of animal models which must be followed in order to utilize data generated from *in vivo* disease models for licensing purposes [4]. The purpose of this article is to review animal models of disease for emerging viral pathogens with specific emphasis on emerging viruses associated with high mortality rates and discuss their suitability in the context of the FDA's Animal Rule.

The nonhuman primate: a gold standard disease model

The intent of any disease model is to provide insight into the pathogenesis of disease for the purpose of designing and testing potential medical countermeasures to prevent the disease. To achieve this, an ideal disease model should faithfully reproduce all the hallmarks of the human condition as closely as possible in an immunocompetent animal following a realistic challenge dose via an appropriate exposure route. In addition to these conditions the Animal Rule stipulates that *in vivo* models must be based on a challenge virus that is a wild-type etiological agent of human disease. In general, nonhuman primates (NHPs, including Cynomolgus and Rhesus macaques, African Green Monkeys and Marmosets) fulfill the criteria of the FDA Animal Rule for most highly pathogenic viruses, making them the gold-standard for studying pathogenesis and evaluating potential medical countermeasures, although it should be noted that not all NHP species are equally susceptible to all agents.

Logistically working with NHPs is far more labor intensive than working with rodents, however frequently NHP models are the best characterized model for this group of pathogens, due largely to a wealth of commercially available reagents. In general, the pathogenesis of emerging viral diseases in humans is poorly defined and not well understood. Because of safety concerns, autopsies are rarely conducted on fatal human cases suspected to have died from a highly pathogenic viral agent. Further, the remote locations where cases typically occur limits the availability of sophisticated instruments required to thoroughly analyze specimens collected during the course of disease. In this regard, the disease course in NHPs is often better defined than the human condition which it is meant to model and therefore, much of our current understanding of pathogenesis for many high consequence viral pathogens has come from experimental infections of NHPs. On the basis of the similarity to humans, NHP models provide the best predicative value as to how a specific therapeutic or vaccine will work in humans. However, it should be noted that even when a vaccine or therapeutic is thoroughly evaluated in NHP models, there is still uncertainty regarding its efficacy in humans since for this group of agents no human vaccines or therapeutics have been thoroughly evaluated in humans (Table 1).

The limitations of small animal models of disease

For ethical, financial and safety reasons, NHPs are not frequently utilized as a first line model which necessitates the development, characterization and use of other, typically small, animal models. Commercially available rodents, predominantly mice and Guinea pigs, are commonly utilized as primary disease models for infectious agents, including emerging viral pathogens, due largely to their ease of use. While these models often provide important proof of concept data used to justify further evaluation of specific medical countermeasures, their use in licensing procedures is limited because many small animal disease models for emerging and highly pathogenic viruses often do not fulfill the FDA requirements. The main constraint for these models is the requirement of a model being developed on a wild-type etiological agent of human disease. Often rodent adaptation is needed to establish small animal disease models for high consequence viral pathogens.

Not only with a large variety of commercially available reagents but also a diverse array of genetic backgrounds including the on-going collaborative cross project, 'humanized' mice, and specific gene knockouts, mice offer an important tool for infectious diseases research and historically have been used as a first attempt in model development. While imperative to advancing our understanding of pathogenesis, knockout mice, especially those with impaired immunological functions, are not ideal for testing therapeutics and therefore will not be discussed further. Similarly, although suckling mice have commonly been used as an initial determination of virulence especially for arboviruses, they cannot be utilized in therapeutic or vaccine studies and therefore will not be further discussed. Often, wild-type high consequence viruses do not cause disease in immunocompetent adult mice; however, mouse models have been developed by serially passaging specific agents resulting in host-adapted viruses. Perhaps the most utilized mouse model for emerging viruses is the mouse-adapted Ebola model [5]. Following inoculation via the intraperitoneal route, mice infected with mouse-adapted Ebola virus develop lethal disease which appears similar to human Ebola virus infection with respect to high titer viremia, tissue tropism, lymphocyte apoptosis and cytokine production. However, mice infected with the adapted Ebola virus variant lack the characteristic coagulopathy which is an important hallmark of Ebola hemorrhagic fever. The overall impact of the lack of coagulopathy in mice is uncertain; however recent reviews of the published literature suggest the predictive power of testing therapeutic in the mouse model is rather low [5]. Historically, Guinea pigs have been utilized as secondary models following mice and before higher order models like NHPs. Similar to mouse models, the development of lethal disease models in

Table 1

Advantages and disadvantages of commonly used animals in disease models

Animal species	Ease of use	Cost	Genome available	Commercially available reagents		Additional comments
				Serological	Molecular	
Mice	Easy	Low	Yes	Yes	Yes	Availability of a variety of genetic backgrounds and specialized knock-out strains
Rats	Easy	Low	Yes	Yes	Yes	
Guinea pigs	Moderate	Low	No	Limited	Limited	Inbred guinea pigs are available from specialized breeding facilities
Hamsters	Easy	Low	No	Limited	Limited	
NHPs	Difficult	High	Yes	Yes	Yes	

Table 2

Summary of commonly used disease models for highly pathogenic members of the *Filoviridae*, *Arenaviridae*, *Bunyaviridae* and *Paramyoviridae*

	Mice (adult)	Guinea pig	Hamster	Nonhuman primates
Filoviruses				
Ebola virus (Ebola hemorrhagic fever)	Yes (adapted virus)	Yes (adapted virus)	Yes (adapted virus)	Cynomolgus and Rhesus macaques, Marmosets, Baboons, African Greens
Marburg virus (Marburg hemorrhagic fever)	Yes (adapted virus)	Yes (adapted virus)	N.D.	Cynomolgus and Rhesus macaques, Marmosets, Baboons, African Greens
Arenaviruses				
Lassa virus (Lassa fever)	Yes (IFNAR KO)	Yes (inbred)	N.D.	Cynomolgus and Rhesus macaques, Marmosets
Lujo virus	N.D.	Yes (inbred)	N.D.	N.D.
Junin virus (Argentine hemorrhagic fever)	N.D.	Yes	N.D.	Rhesus macaques, Marmosets
Guanarito virus (Venezuelan hemorrhagic fever)	N.D.	Yes	N.D.	N.D.
Machupo virus (Bolivian hemorrhagic Fever)	Yes (STAT-1 KO)	Yes	N.D.	Cynomolgus and Rhesus macaques, African Greens
Sabia virus (Brazilian hemorrhagic fever)	N.D.	N.D.	N.D.	N.D.
Chapare virus	N.D.	N.D.	N.D.	N.D.
Bunyaviruses				
CCHFV (Crimean Congo hemorrhagic fever)	Yes (IFNAR/STAT-1 KO)	N.D.	N.D.	N.D.
SFTSV (Severe Fever with Thromocytopenia Syndrome)	Yes	N.D.	N.D.	N.D.
Andes virus (Hantavirus pulmonary syndrome)	N.D.	N.D.	Yes	N.D.
Paramyxoviruses				
Nipah virus (Nipah disease)	N.D.	Yes	Yes	African Greens
Hendra virus (Hendra disease)	N.D.	Yes	Yes	African Greens

N.D. = none described.

Guinea pigs often require host adaption of pathogens, for example, Guinea pig-adapted Ebola. The use of Guinea pig disease models is further hampered by a lack of commercially available reagents which diminishes the ability to completely characterize disease in these animals. However, their use as model animals is imperative, especially for viral pathogens which are naturally harbored by mice or rats, for example arenaviruses. Lassa and Lujo viruses readily infect and cause disease in inbred strain 13 Guinea pigs without earlier host-adaption [6].

Hamsters offer an intriguing option for small animal disease models and their use in infectious diseases research is undergoing a renaissance, most notably in the field of emerging viral pathogens. In 2001, a hamster model of hantavirus pulmonary syndrome (HPS) was described in which hamsters challenged with the highly pathogenic South American Andes virus develop signs of disease which accurately mimic human HPS. To date the hamster model of HPS remains the only described disease model for this relatively rare, but frequently fatal disease of humans [7]. The highly pathogenic paramyxoviruses Nipah and Hendra lethally infect hamsters with disease manifestations similar to those observed in humans [8]. Importantly, depending on the challenge dose of Nipah or Hendra virus, hamsters develop either neurological or respiratory signs of disease, which are the predominant disease symptoms in humans [9]. The similarities in disease manifestations, coupled with the overall ease of handling of these animals makes the hamster model of Nipah and Hendra virus disease the preferred small animal model over other described options including ferrets and cats. A hamster model for Ebola virus hemorrhagic fever was also recently described, and although it is based on an adapted virus (mouse-adapted Ebola virus), the disease course in hamsters appears to more closely resemble the human condition, including coagulopathy [10]. Although to date a few studies have been published utilizing the recently described hamster model of Ebola hemorrhagic fever, the similarities in disease manifestations observed in hamsters and NHPs, suggest the results of efficacy testing of vaccines or therapeutics against Ebola virus infection in the hamsters will be more predicative of results in the NHP model and presumably, humans [11•].

It is interesting to note that compared with mice and Guinea pigs, the disease manifestations observed in hamster models appear to more closely recapitulate the human condition. The commercially available stocks of Syrian hamsters in North America were established over 60 years ago and are all offspring of three initial animals [11•]. Despite the initial genetic bottleneck, colonies of

Syrian hamsters are outbred though genetically and immunologically these animals are not well characterized. It is plausible that hamsters are deficient in specific host responses which enhance the virulence of human pathogens. For example, it has been previously demonstrated that hamsters have muted inducible nitric oxide response, which may increase susceptibility to specific pathogens [12]. The main detraction of hamster models is the extremely limited amount of commercially available reagents. Currently the impetus for reagent development falls on individual laboratories specializing in hamster models, however in order for these models to gain momentum and become thoroughly characterized, the commercial sector will need to become involved (Table 2).

Summary

Despite limitations of specific small animal models the data generated from them have served as an important benchmark and justification for further evaluation in higher order models. Currently there are no murine models described for highly pathogenic viruses which meet the Animal Rule requirements due largely to the need for adapting viruses to create disease models. Guinea pigs models are available for wild-type (nonhost adapted) Lassa and Lujo viruses, and although further characterization is necessary, these models should qualify as appropriate disease models. The use of hamsters in this line of research is gaining interest and in the past few years models for Andes virus (HPS), Nipah and Hendra viruses have been comprehensively characterized and should be considered as first line models for these agents. Although many small animal models do not conform to the Animal Rule their use in evaluating vaccines and model-appropriate therapeutics should not be discounted. For example, with a plethora of immunological reagents, the immunogenicity of vaccines can be readily evaluated in mice. Further, these models all provide a convenient method to evaluate and compare the specific effects of antiviral agents on virus replication in an *in vivo* setting.

For ethical reasons many believe that animal experimentation must follow a hierarchical approach with experiments in mouse models leading to work in secondary 'bridge' models including hamsters and Guinea pigs and if the data warrant it final testing in an apex NHP model. It is important to note that although preferable, it is not always necessary to demonstrate a beneficial result of a specific medical countermeasure in two disease models. The NHP will always be considered as the apex model for evaluating vaccines and therapeutics against highly pathogenic viruses, and in some situations the only appropriate model. For example, many agents discussed in this article are known to have immunomodulatory effects in humans. Although proof of concept experiments could be performed in small animal models, based on the vast differences in the immune systems of humans and rodents, any therapy aimed at reversing or minimizing deleterious immune responses associated with specific viral agents could only accurately be modeled in NHPs and possibly humanized mice. Under appropriate study conditions most if not all disease models, even those which do not completely meet the criteria set forth by the FDA, can be utilized to address specific scientific questions; therefore in the correct settings these models will provide valuable information regarding pathogenesis and/or therapeutic or vaccine efficacy. However, it is time to reconsider the tiered approach to research in laboratory animals and instead of focusing on proof of concept studies in lower order animals, consider the predictive power of specific models in order to generate useful data for the purpose of licensing compounds and vaccine for human use.

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- • of special interest
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