Type I interferon receptor knockout mice as models for infection of highly pathogenic viruses with outbreak potential

Gary Wong1,2,3,4*, Xiang-Guo Qiu3,4

1 Shenzhen Key Laboratory of Pathogen and Immunity, State Key Discipline of Infectious Disease, Shenzhen Third People's Hospital, Shenzhen Guangzhou 518020, China
2 Key Laboratory of Pathogenic Microbiology and Immunology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China
3 Special Pathogens Program, National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Manitoba R3E 3R2, Canada
4 Department of Medical Microbiology and Infectious Diseases, University of Manitoba, Winnipeg, Manitoba R3E 0J9, Canada

ABSTRACT

Due to their inability to generate a complete immune response, mice knockout for type I interferon (IFN) receptors (Ifnar−/−) are more susceptible to viral infections, and are thus commonly used for pathogenesis studies. This mouse model has been used to study many diseases caused by highly pathogenic viruses from many families, including the Flaviviridae, Filoviridae, Arenaviridae, Bunyaviridae, Henipaviridae, and Togaviridae. In this review, we summarize the findings from these animal studies, and discuss the pros and cons of using this model versus other known methods for studying pathogenesis in animals.

Keywords: Ifnar; Mice; Animal model; Flavivirus; Filovirus; Arenavirus; Bunyavirus; Henipavirus; Togavirus

INTRODUCTION

Outbreaks of infectious diseases amongst the human population have been documented for thousands of years. The earliest on record was the Plague of Athens between 429–426 B.C.. Caused by an unknown pathogen, the outbreak killed over 75 000 people (Littman, 2009). Epidemics that have occurred since are too numerous to list thoroughly in this review, but include multiple instances of plague (Yersinia pestis) that devastated populations of Europe, Asia and North Africa with hundreds of millions of deaths. The most infamous epidemic of plague was the “Black Death” during 1346–1350 (Haensch et al., 2010), in which an estimated 30%–60% of the population was wiped out. Viral outbreaks including those caused by smallpox, measles and viral hemorrhagic fevers in various locations worldwide have impacted tens of millions (CDC 2017; Moss & Griffin, 2012; Thèves et al., 2016). In the 20th century, three major influenza pandemics (H1N1 during 1918, H2N2 during 1957–1958 and H3N2 during 1968–1969) have killed over 75 million people combined (Johnson & Mueller, 2002; Kilbourne, 2006). As we enter the 21st century, the death toll from outbreaks of infectious diseases has decreased dramatically, and the highest numbers of fatalities were from the 2014–2016 Ebola virus outbreak in West Africa (over 11 000 deaths) (WHO, 2016a) and the 2009 H1N1 influenza pandemic (over 18 000 deaths) (WHO, 2010). Considerable advances and deployment of prophylactics, therapeutics, rapid point-of-care diagnostics and surveillance have limited the negative impacts from outbreaks in many parts of the world and saved many lives that would otherwise have been lost. However, outbreaks of re-emerging infectious diseases have been occurring with ever increasing frequency in recent years, and there is still much to do in the war against infectious diseases.

The use of animals to study pathogenesis, as well as test potential vaccines and drugs, have played a big role in accelerating the most promising compounds through the pre-clinical process before testing in clinical trials. Non-human primates (NHPs), the closest relative species to humans, are considered the gold standard animal model for many infectious diseases (Safronetz et al., 2013) because these animals recapitulate multiple aspects of human disease, and thus any
experimental results are expected to have high translatability and applicability to humans. However, NHPs are costly to acquire, difficult to handle, and require specialized facilities to house and provide husbandry (Coleman, 2011), and are unaffordable for many research laboratories. To address this, smaller animals have been used for many preliminary studies and screens of candidate vaccines and drugs. For instance, the domestic ferret (Mustela putorius furo) was used to study many member viruses belonging to the order Mononegavirales (Enkirch & von Messling, 2015).

Mice are an ideal species for studying human infectious diseases. The immune systems of mice and humans are often sufficiently similar that they can be infected with the same pathogens (Buer & Balling, 2003). For example, immunocompetent wild-type mice are susceptible to infections with a number of influenza virus subtypes (Belser et al., 2010; Driskell et al., 2010; Gubareva et al., 1998; Xu et al., 2013), severe acute respiratory syndrome coronavirus (SARS-CoV) (Channappanavar et al., 2016) and Rift Valley fever virus (RVFV) (Smith et al., 2010), and outbreaks with these pathogens can be rapidly and easily studied. Unfortunately, wild-type mice are not susceptible to many other pathogens with outbreak potential, and thus alternative strategies are needed. Mice lacking the type I interferon (IFN) receptor (Ifnar–/–) were generated in 1994 (Muller et al., 1994). While these transgenic mice do not show any overt abnormalities by six months of age and are fertile, the animals are entirely unresponsive to the effects of type I IFNs. Ablated immune responses in Ifnar–/– mice were observed after challenge with Vesicular stomatitis virus, Semliki Forest virus, vaccinia virus, or lymphocytic choriomeningitis virus, and the knockout animals showed enhanced susceptibility resulting in either higher viral organ titers, or death at lower doses compared to wild-type mice (Muller et al., 1994).

Since the type I IFN response plays such an important role in innate and adaptive immunity against viral infections (Mo et al., 2015), the Ifnar–/– mice, which are available in many backgrounds, have since been used to study many highly pathogenic viruses. In this review, we summarize the results of using Ifnar–/– mice to study selected pathogens from the Flaviviridae, Filoviridae, Arenaviridae, Bunyaviridae, Henipaviridae, and Togaviridae families, focusing on member viruses that either have, or may have the potential to cause large scale outbreaks in the future.

**FLAVIVIRIDAE**

The family Flaviviridae contains many member viruses which are highly pathogenic to humans and/or have high outbreak potential. West Nile virus, Dengue fever, Yellow fever, Japanese encephalitis, and Zika are all mosquito-borne diseases, caused by West Nile virus (WNV), Dengue virus (DENV), Yellow fever virus (YFV), Japanese encephalitis virus (JEV) and Zika virus (ZIKV), respectively.

**West Nile virus (WNV)**

Found in temperate and tropical regions, WNV is maintained in a mosquito-bird-mosquito cycle in nature, with humans as incidental hosts. WNV was firstly identified in Uganda in 1937, but the majority of infections (~80%) caused only mild disease or were asymptomatic (WHO, 2011). In case of symptomatic disease, fever, headache, fatigue, muscle pain, nausea, vomiting and rash is observed. Less than 1% of cases are neuroinvasive, in which patients present high fever, stupor, disorientation, coma, tremors, convulsions, muscle weakness, and paralysis (WHO, 2011). Large outbreaks of WNV occurred sporadically throughout the decades, but during 1996 WNV re-emerged in Romania and caused 393 confirmed infections, in which 352 patients had manifestations in the central nervous system (Tsai et al., 1998). Subsequent epidemics of WNV with high rates of neuroinvasive disease was then noted in Morocco in 1996, Tunisia in 1997, and large outbreaks in Italy and Israel in 1998 (Hubálek & Halouzka, 1999). At present, WNV is endemic in Africa, Asia, Europe, Australia, and has spread into Canada and the United States (Chen et al., 2013). An outbreak of WNV in 2012 in the United States claimed 286 lives (Murray et al., 2013).

Wild-type 129Sv/Ev mice infected subcutaneous (SC) with 10^2 plaque forming units (pfu) of WNV showed 62% mortality and died at a mean time to death of 11.6±1.9 days post infection (dpi), with no clinical signs observed until 8 dpi (Samuel & Diamond, 2005). In their study, Samuel et al. challenged 8–10 week old Ifnar–/– mice (129Sv/Ev background) with 10^0, 10^1 or 10^2 pfu or WNV strain 3000.0259 via footpad (SC) inoculation. Regardless of dose, all mice showed severe clinical symptoms by 3 dpi, including hunched posture, ruffled fur and reduced activities. Death occurred within 12–48 hours after the onset of symptoms, and the mean time to death was 3.8±0.5 dpi for Ifnar–/– mice in the 10^2 pfu group (Samuel & Diamond, 2005). Live infectious virus can be found in the muscle, heart, lung, kidney, liver, but not in the pancreas. Additionally, challenge of Ifnar–/– mice (C57BL/6 background) also showed 100% lethality and a mean time to death of 3.4±0.5 dpi (Samuel & Diamond, 2005).

**Dengue virus (DENV)**

DENV is widespread in the temperate and tropical regions of the world, and each year approximately 50–150 million people are infected (Bhatt et al., 2013), with over 10 000 deaths (Stanaway et al., 2016). Symptoms of Dengue fever include a high fever, headache, vomiting, muscle and joint pains, and skin rash. Severe cases of disease is usually associated with secondary infection with heterologous types of DENV (Halstead, 1988), and can develop into Dengue hemorrhagic fever (with hemorrhage, thrombocytopenia and blood plasma leakage), or into Dengue shock syndrome, both of which are potentially fatal (Kularatne, 2015).

Infection of 129Sv/Ev mice with 10^8 pfu DENV-2 via the intravenous (IV) route resulted in 87% survival (26 out of 30 mice), and inoculation with 4.4×10^4 pfu DENV-1 via IV resulted in 93% survival (40 out of 43 mice) (Shresta et al., 2004). In their study, Shresta et al. challenged 5–6 week old Ifnar–/– mice (129Sv/Ev background) with DENV-2 strain PL046 (n=12) and DENV-1 strain Mochizuki (n=16) at the same doses and inoculation routes. While no lethality was observed, sera and major organs harvested from infected mice at 3 and 7 dpi showed the...
presence of virus in all sera, liver, spleen, and lymph node samples, as well as some brain and spinal cord samples (Shresta et al., 2004). Interestingly, mice deficient for the type I and II IFN receptors (AG129) showed uniform death from DENV-2 infection with animals dying between 7–30 dpi, and DENV-1 as well with mice succumbing to disease between 7–14 dpi (Shresta et al., 2004).

Yellow fever virus (YFV)

YFV is endemic in tropical areas of Africa and South America (WHO, 2016b), when the virus was introduced via the slave trade during the 17th century. Many infections are asymptomatic, but if clinical symptoms appear, they include fever, chills, appetite loss, nausea, muscle pains, and headaches. A small percentage (~15%) of cases will go on to develop more severe disease including jaundice, dark urine, vomiting and abdominal pain. Hemorrhage from the mouth, nose, eyes or stomach may occur and 50% of patients with these symptoms succumb to disease (WHO, 2016b). YFV was responsible for ~127 000 severe infections and 45 000 deaths in 2013 (WHO, 2016b), with increased incidence over the past decades, and the risk of an outbreak in urban centers is a serious public health threat (Barrett & Higgs, 2007).

Inoculation of wild-type 129 mice SC in each rear footpad with 104 pfu of YFV did not result in any weight loss or death (Meier et al., 2009). In their study, Meier et al. (2009) challenged 3–4 week old Ifnar−/− mice (129 background) with YFV strains Asibi or Angola73 under the same conditions. The mice were shown to be susceptible to the challenge, with death occurring between 7–9 dpi. Additionally, the mice developed viscerotropic disease with virus dissemination to the visceral organs, spleen and liver, in which severe damage of the organs can be observed with gross pathological examination and hematoxylin/eosin staining. Elevated levels of MCP-1 and IL-6 in these organs are suggestive of a cytokine storm (Meier et al., 2009).

Japanese encephalitis virus (JEV)

Japanese encephalitis is an acute disease of the central nervous system in humans. Infected patients develop a febrile illness with headaches vomiting and diarrhea, as well as reduced levels of consciousness, seizures, and photophobia. Severe encephalitis occurs later in the disease course and is associated with a higher frequency of seizures, resulting in coma and death (Ghosh & Basu, 2009). Mental retardation may develop in the patient. JEV is endemic in large parts of Asia and the Pacific, and 30 000–50 000 infections (Solomon, 2006), including up to 15 000 deaths (Ghosh & Basu, 2009), are reported yearly. It is estimated that approximately 25%–30% of cases are fatal, but 50% result in permanent neurological sequelae (Ghosh & Basu, 2009).

Inoculation of wild-type 129 mice via the SC route with 10^4 pfu of YFV did not result in any weight loss or death (Meier et al., 2009). In their study, Meier et al. (2009) challenged 3–4 week old Ifnar−/− mice (129 background) with YFV strains Asibi or Angola73 under the same conditions. The mice were shown to be susceptible to the challenge, with death occurring between 7–9 dpi. Additionally, the mice developed viscerotropic disease with virus dissemination to the visceral organs, spleen and liver, in which severe damage of the organs can be observed with gross pathological examination and hematoxylin/eosin staining. Elevated levels of MCP-1 and IL-6 in these organs are suggestive of a cytokine storm (Meier et al., 2009).

Zika virus (ZIKV)

First isolated in 1947 from an infected monkey in Uganda and re-isolated from Aedes mosquitoes in the same area during 1948 (Dick et al., 1952), ZIKV infections in humans have sporadically occurred in Africa and Asia, but in 2007 the virus continued spreading, causing outbreaks in small island countries located in the Pacific Ocean, such as Yap Island (Duffy et al., 2009), French Polynesia (Cao-Lormeau et al., 2014) and Easter Island (Tognarelli et al., 2016). In early 2015, an epidemic of ZIKV infections, originating from Brazil, spread through most of North and South America and the Caribbean with tens of thousands of people over 80 countries infected (WHO, 2017), as well as thousands of imported cases from travelers returning to their home countries after visiting outbreak areas. The epidemic was declared over by the World Health Organization (WHO) on November 2016 (WHO, 2017), but many countries are still dealing with the long-term impact of ZIKV infections. Infections of ZIKV are typically asymptomatic, but if present they are mild in nature and includes fever, joint pain, maculopapular rash, and bloodshot eyes (Simpson, 1964). While no deaths have been reported from ZIKV infections, mother-to-child transmission during pregnancy may result in congenital Zika syndrome with abnormalities in the central nervous system (microcephaly, intellectual development, seizures and vision impairment) (Boeuf et al., 2016). ZIKV infections in adults is associated with Guillain–Barré syndrome (Frontera & da Silva, 2016). Distinct from other flavivirus infections, sexual transmission of ZIKV from male-to-male (Deckard et al., 2016), male-to-female (D’Ortenzio et al., 2016; Hills et al., 2016) and female-to-male (Davidson et al., 2016) have been documented.

Infection of wild-type 129Sv/Ev mice SC with 10^4 pfu of ZIKV MP1751 did not result in any observable clinical symptoms or histological changes, despite the virus being detected at low levels in the blood, spleen and ovaries (Dowall et al., 2016). In their study, Dowall et al. challenged 5–6 week old Ifnar−/− mice (129Sv/Ev background) under the same conditions as above, and showed that all animals succumbed to disease at 6 dpi with 20% body weight loss. High levels of virus could be detected by RT-qPCR at 3 and 7 dpi in the blood, spleen, brain, ovary and livers of these animals. Pathology studies show that inflammatory as well as degenerative changes could be seen in the brains of infected Ifnar−/− mice (Dowall et al., 2016). In another study, Lazear et al. (2016) inoculated 5–6 week old Ifnar−/− mice (C57BL/6 background) with 10^2 pfu of ZIKV strain H/PF/2013 or MR766 via the SC route in the footpad. The results show that Ifnar−/− mice all died within 8–10 dpi after challenge with H/PF/2013, and 80% death with MR766, with death between 9–13 dpi. Additionally, an SC challenge with 10^3 focus forming units (ffu) of ZIKV strain Dakar 41671, 41667 or...
41519 in Ifnar−/− mice results in uniform death by 6 dpi (Lazear et al., 2016). In a third study, Rossi et al. (2016) inoculated 3-, 5- and 11-week old Ifnar−/−mice (C57BL/6 background) with 1×10^6 pfu of ZIKV FSS13025 via the SC route. The results showed 100% lethality in 3-week old animals with death occurring at 6–7 dpi, but only 50% death in 5-week old animals and no deaths in 11-week old animals (Rossi et al., 2016), indicating that the disease caused by ZIKV infection in these animals is age-dependent.

**FILOVIRIDAE**

**Ebola virus (EBOV), Sudan virus (SUDV), Reston virus (RESTV), Tai Forest virus (TAFV), Marburg virus (MARV) and Ravn virus (RAVV)**

The family Filoviridae consists of many member viruses, including EBOV, SUDV, RESTV, TAFV and MARV, among others. With the exception of RESTV, all filoviruses are pathogenic in humans and infected patients initially present with fever, sore throat, muscular pain, headaches, vomiting, and diarrhea. As the infection develops, a rash is observed along with decreased organ function (especially liver and kidneys). Hemorrhage, shock and eventually multiple organ failure results in the death of the patient (Bradfute et al., 2012). Outbreaks of filovirus disease in humans are sporadic and unpredictable, and typically localized geographically to sub-Saharan Africa, but imported cases have occurred in the past to Europe and North America (CDC, 2014, 2017b). The case fatality rate (CFR) of EBOV and MARV can reach up to 90%, whereas SUDV is ~50%. Only one case of TAFV has been recorded, in which the patient fell ill but survived infection (Formenty et al., 1999). The CFR of RAVV cannot be estimated accurately since the only large scale outbreak during 1998–2000 in the Democratic Republic of the Congo (128 deaths out of 154 cases) was due to the simultaneous co-circulation of RAVV and MARV (Bausch et al., 2003, 2006).

Infection of wild-type adult immunocompetent 129 mice does not result in disease or death (Bray, 2001). Bray (2001) then inoculated 8–16 week old Ifnar−/− mice (129 background) with 1 000 pfu of EBOV, SUDV, RESTV, TAFV as well as MARV via intraperitoneal (IP) route. The results show that Ifnar−/− mice succumbed to infection with the Mayinga isolate of EBOV, with a mean time to death of 5.4 dpi, but resistant to the Kikwit isolate of EBOV. SUDV strain Boneface produced uniformly lethal infection with a mean time to death of 6.3 dpi, but RESTV and TAFV infections did not result in death of the Ifnar−/− mice. Infection with RAVV and MARV produced 100% and 67% lethal infections, with a mean time to death of 6.0 and 8.5 dpi, respectively. Additionally, a SC challenge of the Mayinga isolate of EBOV to Ifnar−/− mice was shown to be fully lethal with a mean time to death of 7.3 dpi (Bray, 2001). In another study, 6–9 week old Ifnar−/− mice (129 background) were challenged with an aerosol dose of MARV between 10^{2.8-5.8} 50% tissue culture infective doses (TCID_{50}), EBOV between 10^{0-2} TCID_{50} of EBOV, or SUDV at 10^{4.8} TCID_{50}. All animals challenged with MARV succumbed to disease at a mean time to death of 11–13 dpi, whereas EBOV-infected mice died at an average of 8 dpi. Clinical symptoms such as lethargy, weight loss and piloerection were observed prior to death. Although symptoms such as anorexia were observed in SUDV-infected mice from 7–11 dpi, all infected mice survived and returned to their pre-challenge weights by the conclusion of the experiment (Lever et al., 2012).

**ARENAVIRIDAE**

**Lassa virus (LASV)**

Lassa fever is prevalent in the West African countries of Nigeria, Liberia, Sierra, Leone, Mali, Ghana, and Guinea, in which 300 000–500 000 cases are reported yearly, including 5 000 deaths (CFR ~1%) (Ogbu et al., 2007); however, the CFR from nosocomial outbreaks can reach as high as 65% (Fisher-Hoch et al., 1995). Carried by the multimammate rat (Mastomys natalensis), most infected patients are asymptomatic, but if illness occurs the initial presentation includes fever, weakness, headaches, vomiting, and muscle pains. In advanced disease, haemorrhaging, encephalopathy, shock and organ failure is observed (Schmitz et al., 2002).

Wild-type 129S1/SvImJ mice are naturally resistant to infection with LASV (Yun et al., 2012). In their study, Rieger et al. infected 8–12 week old Ifnar−/− mice (129/Sv background) with 10^2 ffu of LASV strains Josiah, AV, BA366 and Nig04-10 via the IV route. No deaths were observed with the mice, but the peak of viremia (10^{4.5-6} ffu/mL of blood) was detected at 8 dpi and still not fully cleared by 21 dpi. Weight loss of approximately 15% by 8 dpi was observed, along with elevation of liver enzymes AST and ALT. Other findings include the presence of high levels (up to 10^7 ffu of tissue) of live LASV in the lung, kidney, heart, spleen, brain and liver infected animals at 9–10 dpi (Rieger et al., 2013). Thus, the results support the establishment of productive LASV infection in Ifnar−/− mice.

**BUNYAVIRIDAE**

**Crimean-Congo hemorrhagic fever virus (CCHFV)**

Crimean-Congo hemorrhagic fever is caused by infections with CCHFV, which was first reported in the 1940s, but a study suggests that the virus may have been present since 1500–1 100 B.C. (Carroll et al., 2010). Initial symptoms of CCHFV infection include fever (over 39.0 °C), muscle pains, fatigue, dizziness, vomiting, and diarrhea (Whitehouse, 2004). Advanced CCHFV infections are characterized by more severe symptoms including liver failure, petschiae as well as gastrointestinal and cerebral hemorrhage resulting in death (Whitehouse, 2004). The CFR can vary widely: it was reported to be 5% during an outbreak in Turkey (Kubar et al., 2011), but 60% during another outbreak in the UAE (Schwarz et al., 1996). Spread by *Hyalomma* ticks, cases of CCHFV infections in humans has been reported in western Asia, Eastern Europe, the Middle East, as well as South Africa, although the geographical distribution of the *Hyalomma* vector is widespread and encompasses all of Africa, as well as European and Asian regions south of the N50° latitude (WHO, 2008). Approximately 50 cases are reported per year worldwide, but over 200 cases were reported during 2003–2004 (Messina et al., 2015).
Infection of wild-type 129 Sv/Ew mice with CCHFV at high doses results in the establishment of an infection that is rapidly cleared from the kidney, brain, heart and blood within 3 dpi, and clearance from the liver and spleen by 11 dpi, but no clinical signs or mortality (Bereczky et al., 2010). In their study, Bereczky et al. challenged 7–10 week old Ifnar–/– mice (129 Sv/Ew background) IP with 1010–106 ffu per animal of CCHFV strain IbAr 2000. Symptoms including laboured breathing were observed between 42–70 hours after infection and uniform death was observed at all doses. The highest viral loads were observed between 42–70 hours after infection and uniform strain IbAr 2000. Symptoms including laboured breathing were observed between 42–70 hours after infection and uniform death was observed at all doses. The highest viral loads in Ifnar–/– mice were observed at 2 dpi in the spleen and liver (over 1010 viral RNA copies/g of tissue), but could also be detected in blood, as well as other major organs including the kidney, brain and heart (Bereczky et al., 2010). In another study, Zivcec et al. infected 6–12 week old Ifnar–/– mice (C57BL/6 background) with 104 TCID50 of CCHFV strain IbAr 2000 via the IP, intramuscular (IM), intranasal (IN) and SC routes, and showed that all animals died with an average time to death of 4±0.5, 5.2±0.6, 7±0 and 4.6±0.2 dpi, respectively (Zivcec et al., 2013). Thrombocytopenia, coagulopathy, strong pro-inflammatory responses were observed in these animals. CCHFV of up to 107 TCID50/mg of tissue could be detected in the blood, lymph node and various major organs (Zivcec et al., 2013). In contrast, infection of wild-type C57BL/6 mice with CCHFV did not result in any pathology (Zivcec et al., 2013).

Severe fever with thrombocytopenia syndrome virus (SFTSV)

Severe fever with thrombocytopenia is a newly recognized disease in rural areas of northeastern and central China, with several cases in Japan and South Korea (Promedmail, 2013). Caused by SFTSV, the transmission route of the virus is still unknown, but most likely involves arthropod vectors or animal hosts since the virus has been detected in ticks collected from domestic animals (Tian et al., 2017), and the animals (i.e., goats, cattle and dogs) also have high levels of SFTSV-specific antibodies (Jiao et al., 2012). Patients infected with SFTSV present with fever, vomiting, diarrhea, thrombocytopenia, leucopenia, and increased liver enzyme levels, in which severe cases of SFTSV eventually result in multiple organ failure resulting in death (Yu et al., 2011). The mortality rate amongst hospitalized patients can be up to 30%, and hundreds of cases are reported annually in China (Liu et al., 2015).

Infection of wild-type mice (BALB/c, C57BL/6) results in limited weight loss but the animals do not succumb to disease (Chen et al., 2012; Jin et al., 2012). In one study, Liu et al. (2014) infected 6–10 week old Ifnar–/– mice (129/Sv background) SC with 102 TCID50 of SFTSV strain SD4. The mice were highly susceptible to challenge, with all mice appearing ill by 3 dpi, resulting in death between 3–4 dpi. Blood and major organs (brain, heart, kidney, intestine, liver, lung and spleen) were collected from infected Ifnar–/– mice daily, and results showed high levels of virus replication with systemic spread to all organs. In particular, the spleen and intestine had the highest peak virus titers at death (Liu et al., 2014). In another study, Matsuno et al. infected 6–12 week old Ifnar–/– mice (C57BL/6 background) with either a high dose (104 TCID50 per animal) or a low dose (102 TCID50 per animal) of SFTSV strain SD4 via the intradermal (ID), IP, IM or SC routes. The results showed that the Ifnar–/– mice were susceptible to infection via all routes, with animals succumbing to death at 4 and 6 dpi in the high and low dose groups, respectively (Matsuno et al., 2017).

HENIPAVIRIDAE

Hendra virus (HeV) and Nipah virus (NIV)

HeV was discovered in 1994 as the etiologic agent that caused an acute respiratory disease in horses in Australia with sporadic but lethal transmission to humans, with one fatal case developing pneumonitis, respiratory and renal failure, arterial thrombosis, and eventually cardiac arrest seven days after admission (Selvey et al., 1995). HeV currently still poses a threat to Australian livestock, and the CFR is estimated to be 60% for humans and 75% for horses (Field et al., 2011). NIV was discovered in 1999 in Malaysia with spread to neighbouring Singapore, resulting in 100 deaths from 257 human cases (CDC, 1999a). Patients typically present with respiratory problems and fever, as well as encephalitis with symptoms of headache, drowsiness, disorientation and confusion, rapidly progressing to coma. Since then, outbreaks of NIV have caused severe encephalitis in Bangladesh and India, with a CFR of ~75% (Lo & Rota, 2008). Pigs are susceptible to infection and act as amplifying hosts to humans (CDC, 1999b). Fruit bats are the natural reservoir for both viruses (Halpin et al., 2011).

Wild-type mice are only susceptible to HeV or NIV infection if the virus is administered via the intracranial (IC) route, but not through any other types of inoculations (Dhondt et al., 2013). In their study, Dhondt et al. infected 3–18 week old Ifnar–/– mice (C57BL/6 background) IP with 106 pfu of HeV. It was observed that while the infection was fully lethal in 3-week old mice, the susceptibility decreased with increasing age and the same dose of HeV in 18-week old mice only resulted in 50% mortality. The moribund mice died between 7–13 dpi. For NIV strain UMMC1, 4–12 week old Ifnar–/– mice (C57BL/6 background) were infected IP with increasing dosages from 100–106 pfu. The mice were found to be uniformly susceptible with deaths between 6–9 dpi in the 106 pfu group, and the LD50 was calculated to be 8×103 pfu in Ifnar–/– mice. Infected mice with both viruses first showed behavioural changes including agitation, edginess and no grooming. Neurological symptoms were observed with advanced disease including tilted head and paralysis. A weight loss of approximately 15%–25% was observed 1–2 days before death and found to be a good predictor of mortality (Dhondt et al., 2013).

TOGAVIRIDAE

Venezuelan equine encephalitis virus (VEEV)

Venezuelan equine encephalitis was first identified in Venezuela in 1938, and outbreaks of the causative agent, VEEV, have occurred mostly in Central and South America, but the United States have also reported cases (Weaver et al., 2004). A mosquito-borne virus (Beam & Turell, 1991), VEEV can infect and amplify in equine species, resulting in encephalitis as well as progressive disorders in the central nervous system.
Transmission of the virus to humans via the mosquito vector can result in the patient presenting with malaise, fever, headache and encephalitis (Weaver et al., 2004). The CFR is estimated to be 0.7%–1% (Weaver et al., 1996), but permanent neurological damage have been noted with survivors of VEEV-induced encephalitis (León et al., 1975).

Infection of wild-type mice with a virulent VEEV strain (V3000) results in death at approximately 8.3±0.5 dpi, but infection with an attenuated VEEV strain (V3032) does not result in mortality (Schoneboom et al., 2000). In their study, Schoneboom et al. infected 8–12 week old Ifnar–/– mice (129Sw/Ev background) with 1×10^3 pfu of VEEV either a virulent (V3000) or attenuated strain (V3032) SC into the left rear footpad. Within 18–20 hours of infection with either virus, the mice displayed hunching, ruffled fur and appeared lethargic. Advanced disease includes convulsions and prostration resulting in death. The mean time to death was 1 dpi for both VEEV strains, and high levels of live VEEV could be detected in the sera and brains of moribund Ifnar–/– mice (Schoneboom et al., 2000).

Chikungunya virus (CHIKV)
First isolated in Tanzania in 1952 and carried by Aedes mosquitoes, CHIKV infections in humans result in Chikungunya fever, which is a severe illness in humans characterized by fever, headache, myalgia, rash, and acute as well as persistent arthralgia (Burt et al., 2012). Despite considerable morbidity, the CFR is estimated to be 0.1%, with those older than 65 and/or underlying medical problems to be most at risk of death (Caglioti et al., 2013). CHIKV outbreaks occurred mostly in central/southern Africa and southeast Asia during the 1960s–2000s (Powers & Logue, 2007), but in recent years large-scale outbreaks have been reported on the island of Reunion (Roth et al., 2014), India (Pialoux et al., 2007) and also the Americas (Staples & Fischer, 2014), in which millions of infections were reported.

Infection of wild-type adult mice with 10^6 pfu of CHIKV does not result in mortality (Couderc et al., 2008). In their study, Couderc et al. infected adult Ifnar–/– mice (129SvEv) via ID with 20 pfu of CHIKV (Couderc et al., 2008) and found that the mice died within an average of 3±0.2 dpi, with an LD_{50} of 3 pfu. Infectious virus could be detected in the liver within 16 hours after infection and abundantly detected in the muscles, joints, skin, brain, liver, spleen and sera by 3 dpi. Another study by Pal et al. infected 6–8 week old Ifnar–/– mice (C57BL/6 background) SC in the footpad with 20 pfu of CHIKV, and found that all mice died within 4 dpi, but that these animals did not develop the arthritis observed in humans (Pal et al., 2013).

SUMMARY

Immunocompromised Ifnar–/– mice have been shown to be a very good alternative small animal model for highly virulent pathogens that do not cause disease in immunocompetent mice. In this review, we described the different parameters and results from experimental infection of Ifnar–/– mice with various pathogens (Table 1). It is obvious that the advent of Ifnar1–/– mice undoubtedly constituted a major step forwards in allowing researchers to easily and rapidly study the pathogenesis of clinical isolates during a potential outbreak situation, as these animals are more susceptible to viral infections (Table 2).

However, Ifnar–/– mice as a model do have some weaknesses. Since these mice have defective innate immune responses which lead to impaired adaptive immunity, they are not good animal models for studying antiviral compounds, particularly vaccines (Züst et al., 2014). Additionally, it appears that the age of the Ifnar–/– mice plays a role in host susceptibility to some viruses, as ZIKV loses the ability to cause disease in mice older than six weeks, whereas only partial lethality could be achieved with HeV infection in mice older than six weeks (Table 2). With respect to viruses from the Coronaviridae family, the Ifnar–/– mutation was found instead to prevent the lethal pneumonia observed in SARS-CoV mice (Channappanavar et al., 2016), whereas the absence of the human CD26 (a.k.a. DPP4) receptor for Middle East respiratory syndrome coronavirus (MERS-CoV) means that Ifnar–/– mice must be first transduced with a human adenovirus serotype 5-vector expressing human CD26 in order to become transiently susceptible to MERS-CoV infection via the IN route (Zhao et al., 2014). The transduced Ifnar–/– mice were shown to experience ~20% body weight loss and delayed virus clearance by approximately 3 days compared to transduced wild-type mice, but do not die from the infection (Zhao et al., 2014).

Aside from the transduction strategy, a popular method is to generate host-adapted viruses by sequential passaging in the livers and spleens of rodents (i.e., mice and guinea pigs) in vivo, in order to generate increasingly pathogenic virus variants that cause lethal disease to the immunocompetent host. This method has been widely used in the Filoviridae field to generate adapted viruses for EBOV (Bray et al., 1998; Volchkov et al., 2000), MARV (Qiu et al., 2014), RAVV (Warfield et al., 2009) and SUDV (Wong et al., 2016) in wild-type mice or guinea pigs. In many cases, these viruses harbour very few mutations compared with the original clinical isolates, and the ability of the adapted virus to evade the host Type I IFN response (via mutations in the viral antigen responsible for this function) is positively correlated with its virulence in the host (Ebihara et al., 2006).

While animal models for studying virus pathogenesis leading to severe disease or lethality should always be the primary priority, an important future aim would be to also establish small animal models for studying pathogen transmission (initial work includes a guinea pig-based model of EBOV transmission (Wong et al., 2015), as well as developing small animal models to study various important phenomena of disease, such as the persistence of ZIKV in the testes of immunocompromised and immunocompetent mice (Govero et al., 2016; Ma et al., 2016), ZIKV infections leading to birth defects in wild-type mice (Cugola et al., 2016), ZIKV infections leading to microcephaly in neonatal mice (Li et al., 2016), or the adulthood sequelae of mice who survived congenital ZIKV infections (Cui et al., 2017). These studies in small animals will set the stage and provide important directives in subsequent investigations of similar disease phenomenon/sequelae in larger animal models, and ultimately, humans.
Table 1 Summary of experimental parameters and results of Ifnar\(-/-\) mice challenged with various outbreak viruses

| Pathogen | Strain          | Age (weeks) and background | Challenge dose | Challenge route | Death rate | Mean time to death, or range | References                  |
|----------|-----------------|----------------------------|----------------|----------------|------------|-----------------------------|-----------------------------|
| WNV      | 3000.0259       | 8–10, 129Sv/Ev             | 10^6 pfu       | SC             | 100%       | Not provided                | Samuel & Diamond, 2005      |
|          |                 | 8–10, C57BL/6              | 10^1 pfu       | SC             | 100%       | 3.4±0.5 days                |                             |
|          |                 |                            | 10^2 pfu       | SC             | 100%       | 3.4±0.5 days                |                             |
| DENV-2   | PL046           | 5–6, 129Sv/Ev              | 10^6 pfu       | IV             | 0%         | N/A                         | Shresta et al., 2004        |
| DENV-1   | Mochizuki       | 5–6, 129Sv/Ev              | 4.4×10^4 pfu   | N/A            | 7–9 days | Meier et al., 2009          |
| YFV      | Asibi           | 3–4, 129                   | 10^4 pfu       | SC             | 100%       | 7–8 days                    |                             |
| JEV      | JEV JaOArS982   | 5–6, 129                   | 10^5 pfu       | SC             | 90%        | 120 hours                   | Aoki et al., 2014           |
| ZIKV     | MP1751          | 5–6, 129Sv/Ev              | 10^5 pfu       | SC             | 100%       | 6 days                      | Dowall et al., 2016         |
|          | MR766           | 5–6, C57BL/6               | 10^5 pfu       | SC             | 100%       | 8–10 days                   | Lazear et al., 2016         |
|          | Dakar 41671     | 5–6, C57BL/6               | 10^5 pfu       | SC             | 100%       | 6–7 days                    | Rossi et al., 2016          |
|          | Dakar 41519     | 3, C57BL/6                 | 1×10^5 pfu     | SC             | 100%       | 6 days                      |                             |
| ZIKV     | FSS13025        | 5–6, C57BL/6               | 1×10^5 pfu     | SC             | 100%       | 8–9 days                    |                             |
|          |                 | 3, C57BL/6                 | 1×10^5 pfu     | SC             | 50%        | 6–7 days                    |                             |
| EBOV     | Mayinga         | 8–16, 129                  | 10^3 pfu       | SC             | 100%       | 7.3 days                    | Bray, 2001                  |
|          |                 |                            | 10^3 pfu       | IP             | 100%       | 5.4 days                    |                             |
|          |                 |                            | 10^3 pfu       | IP             | 0%         | N/A                         |                             |
| SUDV     | Boneface        | 5, C57BL/6                 | 10^4 pfu       | SC             | 100%       | 6.3 days                    |                             |
| RESTV    | 10%             |                            |                |                | 6.3 days |                             |                             |
| TAFV     | 5%              |                            |                |                | 6.3 days |                             |                             |
| MARV     | Musoke          | 67%                        | 8.5 days       |                |            | 6.0 days                    |                             |
| RAVV     | Popp            | 6–9, 129                   | 10^{2.8} TCID_{50} | Aerosol      | 100%       | 13.0 days                   | Lever et al., 2012          |
|          |                 |                            | 10^{2.8} TCID_{50} | Aerosol      | 100%       | 12.0 days                   |                             |
|          |                 |                            | 10^{2.8} TCID_{50} | Aerosol      | 100%       | 11.0 days                   |                             |
| EBOV     | E719            | 10^6 pfu                   | 8.0 days       |                |            | 8.0 days                    |                             |
|          |                 |                            | 10^6 pfu       | IP             | 0%         | N/A                         | Rieger et al., 2013         |
| SUDV     | Boneface        | 10^{4.8} TCID_{50}         | 0%             | N/A            |            |                             |                             |
| LASV     | Josiah          | 8–12, 129/Sv               | 10^3 pfu       | IV             | 0%         | N/A                         | Rieger et al., 2013         |
| AV       |                |                            | 10^3 pfu       | IV             | 0%         | N/A                         |                             |
| BA366    | Nig04-10        |                            | 10^3 pfu       | IV             | 0%         | N/A                         |                             |
| CCHFV    | IbAr2000        | 7–10, 129 Sv/Ev            | 10^1 pfu       | IP             | 100%       | 4 days                      | Bereczky et al., 2010       |
|          |                 |                            | 10^1 pfu       | IP             | 100%       | 3 days                      |                             |
|          |                 |                            | 10^1 pfu       | IP             | 100%       | 2 days                      |                             |
|          |                 |                            | 10^1 pfu       | IP             | 100%       | 2 days                      |                             |
|          |                 |                            | 10^1 pfu       | IP             | 100%       | 4±0 days                    | Zivcsec et al., 2013        |
|          |                 |                            | 10^1 pfu       | IP             | 100%       | 4±0 days                    |                             |
|          |                 |                            | 10^1 pfu       | IP             | 100%       | 4±0 days                    |                             |
| Pathogen | Strain | Age (weeks) and background | Challenge dose | Challenge route | Death rate | Mean time to death, or range | References |
|----------|--------|----------------------------|----------------|----------------|------------|-----------------------------|------------|
| CCHFV    | IbAr2000 | 6–12, C57BL/6 | $10^4$ TCID$_{50}$ | IM | 100% | 5.2±0.6 days | Zivcec et al., 2013 |
|          |         |               |                | IN |                | 7±0 days |                         |
|          |         |               |                | SC |                | 4.6±0.2 days |                         |
| SFTSV    | YL-1   | 6–10, 129/Sv | $10^6$ ffu | SC | 100% | 3–4 days | Liu et al., 2014 |
|          | SD4    | 6–12, C57BL/6 | $10^2$ TCID$_{50}$ | IP | 100% | 5 days | Matsuno et al., 2017 |
|          |         |               |                | IM |                | 5–6 days |                         |
|          |         |               |                | SC |                | 5 days |                         |
|          |         |               |                | ID |                | 6 days |                         |
|          |         |               |                | $10^5$ TCID$_{50}$ | IP | 3–4 days |                         |
|          |         |               |                | IM |                | 4 days |                         |
|          |         |               |                | SC |                | 4 days |                         |
|          |         |               |                | ID |                | 6 days |                         |
| HeV      | SD4    | 3, C57BL/6 | $10^6$ pfu | IP | 100% | 11 days | Dhondt et al., 2013 |
|          |         | 6, C57BL/6 |                |      | 83% | 11–13 days |                         |
|          |         | 18, C57BL/6 |                |      | 50% | 7 days |                         |
| NiV      | UMMC1  | 4–12, C57BL/6 | $10^2$ pfu | IP | 0% | N/A |                         |
|          |         |               | $10^3$ pfu |      | 17% | 10 days |                         |
|          |         |               | $10^4$ pfu |      | 67% | 10 days |                         |
|          |         |               | $10^5$ pfu |      | 83% | 8–10 days |                         |
|          |         |               | $10^6$ pfu |      | 100% | 6–9 days |                         |
| VEEV     | V3000  | 8–12, 129Sv/129Sv/2  | $1×10^3$ pfu | SC | 100% | 1 day | Schoneboom et al., 2000 |
|          | V3032  |               |                |      |      | 1 day |                         |
| CHIKV    | 21     | Adult (age not given), 129Sv/2 | 20 pfu | ID | 100% | 3±0.2 days | Coudrec et al., 2008 |
| LR       | 6–8, C57BL/6 | 20 ffu | SC | 100% | 3–4 days | Pal et al., 2013 |

IM: Intramuscular; IN: Intranasal; SC: Subcutaneous; IP: Intraperitoneal; ID: Intradermal; IV: Intravenous.

Table 2 Advantages and disadvantages of using Ifnar−/− mice for studying human infectious diseases, compared to other strategies

| Strategy in small animal models | Advantages | Disadvantages |
|--------------------------------|------------|---------------|
| Knockout mice                  | Susceptible to a wide range of clinical isolates of viruses | Cannot study immune responses properly due to abnormal innate immunity |
|                                | Can study pathogenesis of a new pathogen rapidly | Some viruses may not cause disease in knockout mice |
|                                | Cannot test drugs and vaccines effectively | |
|                                | Can be age-sensitive: older mice may lose their susceptibility to the pathogen | |
| Virus adaptation to host via sequential passaging | Can cause uniform lethality | Not always successful in creating a lethal variant |
|                                | Good for screening drugs and vaccines | Can be time consuming to create a lethal variant |
|                                | Wild-type mice are widely available | Not clinical isolate of virus and thus may harbour important differences in pathogenesis |
| Transduction with adenoviral vectors encoding the entry receptor to confer sensitivity | Useful when no other known small animal models exist (i.e., MERS) | Need to know the identity of the receptor |
|                                | Can test with clinical isolate of virus | Time consuming to create the recombinant adenovirus |

MERS: Middle East respiratory syndrome.
COMPETING INTERESTS
The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS
G.W. wrote the manuscript. X.Q. revised the manuscript. All authors read and approved the final manuscript.

REFERENCES
Aoki K, Shimada S, Simantini D, Tun MM, Buerano CC, Morita K, Hayasaka D. 2014. Type-I interferon response affects an inoculation dose-independent mortality in mice following Japanese encephalitis virus infection. Virology Journal, 11: 105.

Barrett ADT, Higgs S. 2007. Yellow fever: a disease that has yet to be conquered. Annual Review of Entomology, 52(1): 209–229.

Bausch DG, Borchert M, Grein T, Roth C, Swanepoel R, Libande ML, Talarmin A, Berthier E, Muyembe-Tamfum JJ, Tugume B, Colebunders R, Kondé KM, Pirard P, Olinda LL, Rodier GR, Campbell P, Tomori O, Ksiazeck TG, Rollin PE. 2003. Risk factors for Marburg hemorrhagic fever, Democratic Republic of the Congo. Emerging Infectious Diseases, 9(12): 1531–1537.

Bausch DG, Nichol ST, Muyembe-Tamfum JJ, Borchert M, Rollin PE, Steurs H, Campbell P, Tshikoku FK, Roth C, Colebunders R, Pirard P, Mandel S, Olinda LA, Zeller H, Tshomb A, Kuliadi L, Libande ML, Mulangu S, Fromenty P, Grein T, Leirs H, Braack L, Ksiazeck T, Zaki S, Bowen MD, Smit SB, Lema PA, Burt FJ, Kemp A, Swanepoel R. 2006. Marburg hemorrhagic fever associated with multiple genetic lineages of virus. The New England Journal of Medicine, 355(9): 909–919.

Beaman JR, Turell MJ. 1991. Transmission of Venezuelan equine encephalomyelitis virus by strains of Aedes albopictus (Diptera: Culicidae) collected in North and South America. Journal of Medical Entomology, 28(1): 161–164.

Belser JA, Watford DA, Pappas C, Gustin KM, Maines TR, Pearce MB, Zeng H, Swayne DE, Panint-Jackwood M, Katz JM, Tumpey TM. 2010. Pathogenesis of pandemic influenza A (H1N1) and triple-reassortant swine influenza A (H1) viruses in mice. Journal of Virology, 84(9): 4194–4203.

Bereczky S, Lindegren G, Karberg H, Akerström S, Klingström J, Mirazimi A. 2010. Crimean-Congo hemorrhagic fever virus infection is lethal for adult type I interferon receptor-knockout mice. Journal of General Virology, 91(6): 1473–1477.

Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, Drake JM, Brownstein JS, Hoera AG, Sankoh O, Myers MF, George DB, Jaenisch T, Wint GRW, Simmons CP, Scott TW, Farrar JJ, Hay Si. 2013. The global distribution and burden of dengue. Nature, 496(7446): 504–507.

Boeuf P, Drummer HE, Richards JS, Scourll MUL, Beeson JG. 2016. The global threat of Zika virus to pregnancy: epidemiology, clinical perspectives, mechanisms, and impact. BMC Medicine, 14: 112.

Bradfute SB, Warfield KL, Bray M. 2012. Mouse models for filovirus infections. Viruses, 4(9): 1477–1408.

Bray M, Davis K, Geisbirt T, Schmaljohn C, Huggins J. 1998. A mouse model for evaluation of prophylaxis and therapy of Ebola hemorrhagic fever. The Journal of Infectious Diseases, 178(3): 651–661.
Cui LY, Zou P, Chen E, Yao H, Zheng H, Wang Q, Zhu JN, Jiang SB, Lu L, Zhang JY. 2017. Visual and motor deficits in grown-up mice with congenital Zika virus infection. *EBioMedicine*, 20: 193–201.

Davidson A, Slavinski S, Komoto K, Rakeman J, Weiss D. 2016. Suspected female-to-male sexual transmission of Zika virus-New York City, 2016. *Morbidity and Mortality Weekly Report*, 65(28): 716–717.

Deckard DT, Chung WM, Brooks JT, Smith JC, Woldai S, Hennessey M, Kwit N, Mead P. 2016. Male-to-male sexual transmission of Zika virus-texas, January 2016. *Morbidity and Mortality Weekly Report*, 65(14): 372–374.

Dhondt KP, Mathieu C, Chalons M, Reynaud JM, Vallve A, Raoul H, Horvat B. 2013. Type I interferon signaling protects mice from lethal henipavirus infection. *The Journal of Infectious Diseases*, 207(1): 142–151.

Dick GW, Kitchen SF, Haddow AJ. 1952. Zika virus (I). Isolations and serological specificity. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 46(5): 509–520.

D’Ortenzio E, Matheron S, de Lamballerie X, Hubert B, Plorkowski G, Maquart M, Descamps D, Damond F, Yazdanpanah Y, Leparc-Goffart I. 2016. Evidence of sexual transmission of Zika virus. *The New England Journal of Medicine*, 374(22): 2195–2198.

Dowall SD, Graham VA, Rayner E, Atkinson B, Hall G, Watson RJ, Bosworth A, Bonney LC, Kitchen S, Hewson R. 2016. A susceptible mouse model for Zika virus infection. *PLoS Neglected Tropical Diseases*, 10(5): e0004658.

Driskell EA, Jones CA, Stallknecht DE, Howerth EW, Tompkins SM. 2010. Avian influenza virus isolates from wild birds replicate and cause disease in a mouse model of infection. *Virology*, 399(2): 280–289.

Duffy MR, Chen TH, Hancock WT, Powers AM, Kool JL, Lanciotti RS, Pretrick M, Marfel M, Holzbaumer S, Dubray C, Guillamont L, Griggs A, Bel M, Lambert AJ, Laven J, Kosoy O, Panella A, Biggerstaff BJ, Fischer M, Hayes EB. 2009. Zika virus outbreak on Yap Island, Federated States of Micronesia. *The New England Journal of Medicine*, 360(24): 2536–2543.

Ebihara H, Takada A, Kobasa D, Jones S, Neumann G, Theriault S, Bray M, Feldmann H, Kawaoka Y. 2006. Molecular determinants of Ebola virus virulence in mice. *PLoS Pathogens*, 2(7): e73.

Enkrich T, von Messling V. 2015. Ferret models of viral pathogenesis. *Virology*, 479–480: 259–270.

Field H, de Jong C, Melville D, Smith C, Smith I, Broos A, Kung YH, McLaughlin A, Benard V, Zuckerman B, Widmer A. 2011. Hendra virus infection dynamics in Australian fruit bats. *PLoS One*, 6(12): e28678.

Fisher-Hoch SP, Tomori O, Nasidi A, Perez-Onorozzi GI, Fakile Y, Hutwagner L, McCormick JB. 1995. Review of cases of nosocomial Lassa fever in Sierra Leone: the high price of poor medical practice. *BMJ*, 311(7009): 857–859.

Formenty P, Hatz C, Le Guenno B, Stoll A, Rogenmoser P, Widmer A. 1999. Human infection due to Ebola virus, subtype Côte d’Ivoire: clinical and biologic presentation. *The Journal of Infectious Diseases*, 179(Suppl. 1): S48-S53.

Frontera JA, da Silva IRF. 2016. Zika getting on your nerves? The biologic presentation. *The Lancet Neurology*, 15(8): S48-S53.

Gubareva LV, McCullers JA, Bethell RC, Webster RG. 1998. Characterization of influenza A/HongKong/156/97 (H5N1) virus in a mouse model and protective effect of zanamivir on H5N1 infection in mice. *The Journal of Infectious Diseases*, 178(6): 1592–1596.

Haensch S, Bianucci Raffaella, Signoli Michel, Rajerison Minaroisa, Schultz Michael, Kacki Sacha, Vermunt Marco, Weston DA, Hurst D, Achtmann M, Carmiel E, Bramanti B, Besansky NJ. 2010. Distinct clones of Yersinia pestis caused the black death. *PLoS Pathogens*, 6(10): e1001134.

Halpin K, Field HE, Hyatt AD, Smith C, Halpin K, Epstein JH, Middleton D, Fogarty R, Hughes T, Bingham J, Rahman SA. 2011. Perez-Oronoz GI, Fakile Y, Hutwagner L, McCormick JB. 1995. Review of cases of nosocomial Lassa fever in Sierra Leone: the high price of poor medical practice. *BMJ*, 311(7009): 857–859.

Jiao YJ, Zeng XY, Guo XL, Qi X, Zhang X, Shi YZ, Zhou MH, Bao CJ, Zhang WS, Xu Y, Wang H. 2012. Preparation and evaluation of recombinant severe fever with thrombocytopenia syndrome virus nucleocapsid protein for detection of total antibodies in human and animal sera by double-antigen sandwich enzyme-linked immunosorbent assay. *Journal of Clinical Microbiology*, 50(2): 372–377.

Johnson NPAS, Mueller J. 2002. Updating the accounts: global mortality of the 1918–1920 “Spanish” influenza pandemic. *Tropical Medicine & Parasitology*, 53(1): 1–12.

Kilbourne ED. 2006. Influenza pandemics of the 20th century. *Emerging Infectious Diseases*, 12(1): 9–14.

Kumar A, Haciomeroglu M, Ozkul A, Bagriacik U, Akinci E, Sener K, Bodur H. 2011. Prompt administration of Crimean-Congo hemorrhagic fever (CCHF) virus hyperimmunoglobulin in patients diagnosed with CCHF and viral load monitoring by reverse transcriptase-PCR. *Japanese Journal of Infectious Diseases*, 64(5): 439–443.

Kularatne SAM. 2015. Dengue fever. *The BMJ*, 351: h4661.

Lazar HM, Govero J, Smith AM, Platt DJ, Fernandez E, Miner JJ, Diamond MS. 2016. A mouse model of zika virus pathogenesis. *Cell Host & Microbe*, 19(5): 720–730.

León CA, Jaramillo R, Martinez S, Fernandez F, Téllez H, Lasso B, De Guzmán R. 1975. Sequence of Venezuelan equine encephalitis in humans: a four year follow-up. *International Journal of Epidemiology*, 4(2): 131–141.

Lever MS, Piercy TJ, Steward JA, Eastaugh L, Smithers SJ, Taylor C, Salguero FJ, Phillpotts RJ. 2012. Lethality and pathogenesis of airborne Hendra virus in bats. *Virology*, 449–450: 17–20.

McLaughlin A, Zeddeman A, Fooks AR. 2011. Hendra virus infection in Australian fruit bats. *PLoS Neglected Tropical Diseases*, 5(5): e73.

McKinney RM, Field H. 2001. Hendra virus: discovery, pathogenesis and vaccine development. *Emerging Infectious Diseases*, 7(5): 692–695.

Moriarity JA, Jr., Long TE, Jr., Hrelia AS, Jr. 2005. Hendra virus, a highly pathogenic equine paramyxovirus. *Virology*, 336(1): 1–12.

O’Brien DP, Dobos G, Davis T, Palese P. 2002. Construction of a recombinant Hendra virus expressing the E protein from Nipah virus. *Virology*, 305(2): 273–286.

Okafor C, Hanington B, Togari C, Bello F, Jekiri S. 2004. Hendra virus causes severe illness in ferrets. *Clinical Microbiology and Infection*, 10(Suppl. 1): h4661.

Pimentel DA, McQuiston JH, Jernigan DB, Meltzer MI, MacIntyre CR, et al. 2015. Projected 2050 burden of severe disease associated with influenza A(H1N1)pdm09 and influenza A(H3N2) in the United States under the optimistic and pessimistic scenarios. *Emerging Infectious Diseases*, 21(9): 1619–1628.

Postupalsky V, Eastaugh L, Lever M, Steward J. 2013. Hendra virus infection in ferrets: a new model for rapid evaluation of therapeutic strategies. *Virus Research*, 169(1): 134–140.

Pulch IM, White PA, Pritt JH, Wiltshire AA. 2007. Experimental studies of Hendra virus. *Australian Veterinary Journal*, 85(5): 235–239.
infection with filoviruses in A129 α/β interferon receptor-deficient mice. *Journal of Medical Microbiology*, **61**(1): 8–15.

Li C, Xu D, Ye Q, Hong S, Jiang YS, Liu XY, Zhang NN, Shi L, Qin CF, Xu ZL. 2016. Zika virus disrupts neural progenitor development and leads to microcephaly in mice. *Cell Stem Cell*, **19**(1): 120–126.

Littman RJ. 2009. The plague of Athens: epidemiology and paleopathology. *Mount Sinai Journal of Medicine: A Journal of Translational and Personalized Medicine*, **76**(5): 456–467.

Liu K, Zhou H, Sun RX, Yao HW, Li Y, Wang LP, Mu D, Li XL, Yang Y, Gray GC, Cui N, Yin WW, Fang LQ, Yu HJ, Cao WC. 2015. A national assessment of the epidemiology of severe fever with thrombocytopenia syndrome. China. *Scientific Reports*, **5**: 9679.

Liu Y, Wu B, Paeslers S, Walker DH, Tesh RB, Yu XJ. 2014. The pathogenesis of severe fever with thrombocytopenia syndrome virus infection in alpha/beta interferon knockout mice: insights into the pathologic mechanisms of a new viral hemorrhagic fever. *Journal of Virology*, **88**(3): 1781–1786.

Lo MK, Rota PA. 2008. The emergence of Nipah virus, a highly pathogenic paramyxovirus. *Journal of Clinical Virology*, **43**(4): 396–400.

Ma WQ, Li SH, Ma SQ, Jia LN, Zhang FC, Zhang Y, Zhang JY, Wong G, Zhang SS, Lu XC, Liu M, Yan JH, Li W, Qin C, Han DS, Qin CF, Wang N, Li XD, Gao GF. 2016. Zika virus causes testis damage and leads to male infertility in mice. *Cell*, **167**(6): 1511–1524.e10.

Matsuno K, Orba Y, Maede-White K, Scott D, Feldmann F, Liang MF, Schmitz H, Köhler B, Laue T, Drosten C, Veldkamp PJ, Günther S, Nitschko H, Ameen A, Jager G, Nsanze H. 1996. Polymerase chain reaction assessment of the epidemiology of severe fever with thrombocytopenia syndrome, China. *Medical Microbiology*, **61**(6): 456–467.

McNab F, Mayer-Barber K, Sher A, Wack A, O’Garra A. 2015. Type I interferons in infectious disease. *Nature Reviews Immunology*, **15**(2): 87–103.

Meier KC, Gardner CL, Khoretonenko MV, Klimstra WB, Ryman KD. 2009. A mouse model for studying viscerotropic disease caused by yellow fever virus infection. *PLoS Pathogens*, **5**(10): e1000614.

Messina JP, Pigott DM, Duda KA, Brownstein JS, Myers MF, George DB, Hay SI. 2015. A global compendium of human Crimean-Congo haemorrhagic fever virus occurrence. *Scientific Data*, **2**(1): 150016.

Moss WJ, Griffin DE. 2012. Measles. *The Lancet*, **379**(9811): 153–164.

Muller U, Steinhoff U, Reis L, Hemmi S, Pavlovic J, Zinkernagel R, Aguet M. 1994. Functional role of type I and type II interferons in antiviral defense. *Science*, **264**(5167): 1918–1921.

Murray KD, Ruktanonchai D, Hesalroad D, Fonken E, Nolan MS. 2013. West Nile virus, Texas, USA, 2012. *Emerging Infectious Diseases*, **19**(11): 1836–1838.

Ogbu O, Ajuluchukwu E, Uneke CJ. 2007. Lassa fever in West African sub-region: an overview. *Journal of Vector Borne Diseases*, **44**(1): 1–11.

Pal P, Dowd KA, Brien JD, Edeling MA, Gortatov S, Johnson S, Lee I, Akahata W, Nabel GJ, Richter MK, Smit JM, Fremont DH, Pierson TC, Heise MT, Dondi MS. 2016. Development of a highly protective combination monoclonal antibody therapy against Chikungunya virus. *PLoS Pathogens*, **9**(4): e1003312.

Pialoux G, Gaüzère BA, Jaureguyberrny S, Strobel M. 2007. Chikungunya, an epidemic arbovirus. *The Lancet Infectious Diseases*, **7**(5): 319–327.

Powers AM, Logue CH. 2007. Changing patterns of chikungunya virus: re-emergence of a zoonotic arbovirus. *Journal of General Virology*, **88**(9): 2363–2377.

Promedmail. 2013. Severe fever with thrombocytopenia syndrome-Japan, South Korea: Update. [cited 2017 July 30]. Available from: http://www.promedmail.org/direct.php?id=20130526.1738022.

Qiu XG, Wong G, Audet J, Cutts T, Niu YL, Booth S, Kobinger GP. 2014. Establishment and characterization of a lethal mouse model for the Angola strain of Marburg virus. *Journal of Virology*, **88**(21): 12703–12714.

Rieger T, Merkler D, Gunther S. 2013. Infection of type I interferon receptor-deficient mice with various old world arenaviruses: a model for studying virulence and host species barriers. *PLoS One*, **8**(8): e72290.

Rossi SL, Tesh RB, Vasilakis N, Paessler S, Rossi SL, Langsjoen RM, Auguste AJ, Weaver SC, Muruto AE, Hanley KA. 2016. Characterization of a novel murine model to study zika virus. *The American Journal of Tropical Medicine and Hygiene*, **94**(6): 1362–1369.

Roth A, Hoy D, Horwood PF, Ropa B, Hancock T, Guillamot L, Rickart K, Frison P, Pavlin B, Souares Y. 2014. Preparedness for threat of chikungunya in the pacific. *Emerging Infectious Diseases*, **20**(8), doi: 10.3201/eid2008.130696.

Safronetz D, Geisbert TW, Feldmann H. 2013. Animal models for highly pathogenic emerging viruses. *Current Opinion in Virology*, **3**(2): 205–209.

Samuel MA, Diamond MS. 2005. Alpha/beta interferon protects against lethal West Nile virus infection by restricting cellular tropism and enhancing neuronal survival. *Journal of Virology*, **79**(2): 13350–13361.

Schmitz H, Köhler B, Laue T, Drosten C, Veldkamp PJ, Günther S, Emmerich P, Geisen HP, Fleischer K, Behrens MFC, Hoerauf A. 2002. Monitoring of clinical and laboratory data in two cases of imported Lassa fever. *Microbes and Infection*, **4**(1): 43–50.

Schoneboom BA, Lee JS, Grieder FB. 2000. Early expression of IFN-alpha/beta and iNOS in the brains of Venezuelan equine encephalitis virus-infected mice. *Journal of Interferon & Cytokine Research*, **20**(2): 205–216.

Schwarz TF, Shurie H, Acharya UG, Schwarz TF, Gilch S, Zahir ARM, Nitschko H, Ameen A, Jager G, Nsanze H. 1996. Polymerase chain reaction for diagnosis and identification of distinct variants of Crimean-Congo hemorrhagic fever virus in the United Arab Emirates. *The American Journal of Tropical Medicine and Hygiene*, **55**(2): 190–196.

Selvey LA, Wells RM, McCormack JG, Ansford AJ, Murray K, Rogers RJ, Lavercombe PS, Selleck P, Sheridan JW. 1995. Infection of humans and horses by a newly described morbillivirus. *The Medical Journal of Australia*, **162**(12): 642–645.

Shresta S, Kyle JL, Snider HM, Basavapatna M, Beatty PR, Harris E. 2004. Interferon-dependent immunity is essential for resistance to primary dengue virus infection in mice, whereas T- and B-cell-dependent immunity are less critical. *Journal of Virology*, **78**(6): 2701–2710.

Simpson DI. 1964. Zika virus infection in man. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **58**(4): 335–338.

Smith DR, Steele KE, Shamblin J, Honko A, Johnson JJ, Reed C, Kennedy M, Chapman JL, Hensley LE. 2010. The pathogenesis of Rift Valley fever virus in the mouse model. *Virology*, **407**(2): 256–267.

Solomon T. 2006. Control of Japanese encephalitis—within our grasp? *Journal of the New England Journal of Medicine*, **355**(9): 869–871.

Stanaway JD, Shepard Donald S, Undurraga Eduardo A, Halasa Yara A, Coffeng Luc E, Brady Oliver J, Hay Simon I, Bedi N, Bensenor IM, Castañeda-Orjuela CA, Chuang TW, Gibney KB, Memish ZA, Rafay A, Ukwaja KN, Yonemoto N, Murray CJL. 2016. The global burden of dengue
an analysis from the Global Burden of Disease Study 2013. The Lancet Infectious Diseases, 16(6): 712–723.

Staples JE, Fischer M. 2014. Chikungunya virus in the Americas—what a vectorborne pathogen can do. The New England Journal of Medicine, 371(10): 887–889.

Thèves C, Crubézy E, Biagini P. 2016. History of smallpox and its spread in human populations. Microbiology Spectrum, 4(4), doi: 10.1128/microbiolspec.PoH-0004–2014.

Tian HY, Yu PB, Chowell G, Li S, Wei J, Tian H, Lv W, Han ZQ, Yang J, Huang SQ, Zhou S, Brownstein JS, Wang JJ, Xu B. 2017. Severe fever with thrombocytopenia syndrome virus in humans, domesticated animals, ticks, and mosquitoes, Shaanxi Province, China. The American Journal of Tropical Medicine and Hygiene, 96(6): 1346–1349.

Tognarelli J, Ulloa S, Villagra E, Lagos J, Aguayo C, Fasce R, Parra B, Mora J, Becerra N, Lagos N, Vera L, Olivares B, Vilches M, Fernández J. 2016. A report on the outbreak of Zika virus on Easter Island, South Pacific, PLoS-0004–2014.

WHO. 2016a. Ebola virus disease—Situation report, 10 June 2016. Available from: http://apps.who.int/iris/bitstream/10665/208883/1/ebolaSitrep_10Jun2016_eng.pdf?ua=1.

WHO. 2016b. Yellow fever—Fact sheet, Updated May 2016. Available from: http://www.who.int/mediacentre/factsheets/fs100/en/.

WHO. 2017. Zika virus and complications: 2016 public health emergency of international concern. [cited 2017 May 22]. Available from: http://www.who.int/emergencies/zika-virus/en/.

Wong G, Qiu XG, Richardson JS, Cutts T, Collignon B, Gren J, Aviles J, Embury-Hyatt C, Kobinger GP. 2015. Ebola virus transmission in guinea pigs. Journal of Virology, 89(2): 1314–1323.

Wong G, He SH, Wei HY, Kroecker A, Audet J, Leung A, Cutts T, Graham J, Kobasa D, Embury-Hyatt C, Kobinger GP, Qiu XG. 2016. Development and characterization of a guinea pig-adapted sudan virus. Journal of Virology, 90(1): 392–399.

Xu L, Bao LL, Deng W, Zhu H, Chen T, Lv Q, Li FD, Yuan J, Xiang ZG, Gao K, Xu Y, Huang L, Li YH, Liu JN, Yao YF, Yu P, Yong WD, Wei Q, Zhang LF, Qin Y. 2013. The mouse and ferret models for studying the novel avian-origin human influenza A (H7N9) virus. Virology Journal, 10: 253.

Yun NE, Poussard AL, Seregin AV, Walker AG, Smith JK, Aronson JF, Smith JN, Soong L, Paessler S. 2012. Functional interferon system is required for clearance of lassa virus. Journal of Virology, 86(6): 3389–3392.

Züst R, Toh YX, Valdés I, Cerny D, Heinrich J, Hermida L, Marcos E, Guillén G, Kalinke U, Shi PY, Fink K. 2014. Type I interferon signals in macrophages and dendritic cells control dengue virus infection: implications for a new mouse model to test dengue vaccines. Journal of Virology, 88(13): 7276–7285.

Zhou J, Li K, Wohlford-Lenane C, Agnihotram SS, Fett C, Zhao JX, Gale MJ, Baric RS, Enjuanes L, Gallacher T, McCray PB, Perlman S. 2014. Rapid generation of a mouse model for Middle East respiratory syndrome. Proceedings of the National Academy of Sciences of the United States of America, 111(13): 4970–4975.

Zivic L, SaffromeTz D, Scott D, Robertson S, Ebiha H, Feldmann H. 2013. Lethal Crimean-Congo hemorrhagic fever virus infection in interferon α/β receptor knockout mice is associated with high viral loads, proinflammatory responses, and coagulopathy. The Journal of Infectious Diseases, 207(12): 1909–1921.