FILOVIRUSES: AFRICAN AND RESTON SPECIES

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SUMMARY

Etiology

- Filoviruses are enveloped, pleomorphic RNA viruses that belong to the family *Filoviridae*. The genus *Ebolavirus* contains five species, commonly named Ebola virus, Sudan virus, Tai Forest virus, Reston virus and Bundibugyo virus.

Cleaning and Disinfection

- Filoviruses are sensitive to many ordinary disinfectants, including sodium or calcium hypochlorite. However, no commercial disinfectants sold at present claim efficacy specifically against filoviruses. It is recommended that higher potency agents (those that are also effective against a non-enveloped virus) be used as an additional safety measure.
- Physical control methods that can be used to inactivate filoviruses or reduce infectivity include heat (e.g., heating at 60°C for one hour; incineration; autoclaving), UV light, gamma irradiation and filtration.

Epidemiology

- Filoviruses occur mainly in regions of Africa. Bats are thought to be the primary reservoir hosts.
- Filoviruses are zoonotic, and in humans Ebola virus infections are life-threatening.
- Pigs are not traditionally considered to be among the species affected by filoviruses. However, Reston virus has been detected in some porcine reproductive and respiratory syndrome (PRRS) virus-infected swine in China and the Philippines, and experimentally, infection of pigs with both Reston virus and Ebola virus has been successfully achieved.
- Natural Ebola infections have not been reported in pigs; the prevalence of Reston virus in pigs is currently unknown.

Transmission

- In experimental studies, transmission of Ebola virus in pigs has been achieved after intranasal, oral, and conjunctival transmission. Piglets 3–6 weeks old inoculated with Ebola virus shed this virus consistently in oral and nasal secretions (with live virus recovered between days 3 and 7), but virus shedding from the gut was sporadic and inconsistent, and viremia was documented occasionally, but not in all animals.
- Pigs inoculated with Reston virus shed the virus in nasopharyngeal secretions (with live virus found between day 4 and day 8), and sometimes in rectal swabs and/or blood, but not in urine. It is not known how pigs acquired Reston virus in China or the Philippines.

Infection in Swine / Pathogenesis

- In experimentally infected 5–6 week-old piglets, Ebola virus caused mainly respiratory signs with no hematologic abnormalities. Infections in 3–4 week-old piglets were less severe, characterized only by transient/delayed fever and increased respiratory rate, with no change in hematologic parameters.
- In pigs co-infected with Reston virus and PRRS virus, clinical signs were consistent with severe, atypical PRRS in both the Philippines and China. Post-mortem lesions also seemed to be consistent with atypical PRRS. Experimentally, five-week-old piglets inoculated with Reston virus subcutaneously or oronasally remained asymptomatic.

Diagnosis

- Tests used in pigs experimentally infected with Ebola virus included real-time reverse transcriptase polymerase chain reaction (RT-PCR), virus isolation and immunohistochemistry. Antibodies to Ebola virus in experimentally infected pigs were detected by IgM and IgG enzyme linked immunosorbent assays (ELISAs) and virus neutralization.
- Diagnostic tests used to detect Reston virus infections in the Philippines included RT-PCR, a panviral microarray assay, an ELISA to detect viral antigens, immunohistochemistry (IHC) and virus isolation. Real time RT-PCR assays were used to detect Reston virus in the spleen of infected pigs in China (with no confirmatory test). Assays used during laboratory experiments on Reston virus-infected pigs included real-time RT-PCR, virus isolation and IHC. In the Philippines, serological tests used to detect antibodies to Reston virus in pigs included the immunofluorescent antibody (IFA) test, virus neutralization and an IgG ELISA.

Immunity

- There is no available information about post-infection immunity in pigs.
- There is no vaccine for filoviruses in pigs, nor is there an indication that vaccination is necessary at this time. Human vaccines are in development.

Prevention and Control

- Keeping pigs indoors can reduce exposure to bats, the suspected reservoir species for Ebola virus and Reston virus.
- Biosecurity plans should consider contact with other hosts, such as infected humans and fomites.
- Filoviruses may be found in semen for months after recovery in humans; this may also occur in pigs, potentially affecting breeding or artificial insemination procedures.

Gaps in Preparedness

- More information is needed about filovirus infection in swine.
- There are no diagnostic tests validated for filoviruses in swine.
- There is no filovirus vaccine approved for use in humans or pigs.

OVERVIEW

Filoviruses are enveloped, pleomorphic RNA viruses in the family *Filoviridae* that affect humans and animals. Five species are recognized in the genus *Ebolavirus*: *Zaire ebolavirus*, *Sudan ebolavirus*, *Taï Forest ebolavirus* (formerly *Cote d'Ivoire ebolavirus*), *Reston ebolavirus* and *Bundibugyo ebolavirus*. The common name for the single virus in each of these species is Ebola virus, Sudan virus, Tai Forest virus, Reston virus and Bundibugyo virus, respectively.

Filoviruses occur mainly in regions of Africa and bats are thought to be the primary reservoir hosts. In humans, Ebola virus infections are life-threatening. Pigs are not traditionally considered to be among the species affected by filoviruses. However, Reston virus has been detected in some porcine reproductive and respiratory syndrome (PRRS) virus-infected swine in China and the Philippines, and experimentally, infection of pigs with both Reston virus and Ebola virus has been successfully achieved. Natural Ebola virus infections have not been reported in pigs; the prevalence of Reston virus in pigs is currently unknown.

In experimental studies, transmission of Ebola virus in pigs has been achieved after intranasal, oral, and conjunctival transmission. Piglets 3–6 weeks old inoculated with Ebola virus shed this virus consistently in oral and nasal secretions (with live virus recovered between days 3 and 7), but virus shedding from the gut was sporadic and inconsistent, and viremia was documented occasionally, but not in all animals. Pigs inoculated with Reston virus shed the virus in nasopharyngeal secretions (with live virus found between day 4 and day 8), and sometimes in rectal swabs and/or blood, but not in urine. It is not known how pigs acquired Reston virus in China or the Philippines.

Piglets (5–6 weeks old) experimentally infected with Ebola virus developed respiratory signs similar to those caused by other respiratory diseases of swine. No evidence of multiorgan failure or hematologic abnormalities was noted. Infections in 3–4 week-old piglets were less severe, characterized only by transient/delayed fever and increased respiratory rate, again with no change in hematologic parameters. Clinical signs in pigs co-infected with Reston virus and PRRS virus were consistent with severe, atypical PRRS in both the Philippines and China. Because all affected pigs were co-infected with both PRRS virus and Reston virus, and some of the pigs in the Philippines were also infected with porcine circovirus type 2, it is difficult to determine the contribution of Reston virus (if any) to these outbreaks. Post-mortem lesions also seemed to be consistent with atypical PRRS. Experimentally, five-week-old piglets inoculated with Reston virus subcutaneously or oronasally remained asymptomatic.

Various diagnostic tests have been used to detect filoviruses in naturally or experimentally infected pigs. Assays used to diagnose Reston virus infections in the Philippines included real-time reverse transcriptase polymerase chain reaction (RT-PCR), a panviral microarray assay, an enzyme linked immunosorbent assay (ELISA) to detect viral antigens, immunohistochemistry (IHC) and virus isolation. Real time RT-PCR assays were used to detect Reston virus in the spleen of infected pigs in China (with no confirmatory test). Assays used during laboratory experiments on Reston virus-infected pigs included real-time RT-PCR, virus isolation and IHC. Tests used in pigs experimentally infected with Ebola virus included real-time RT-PCR, virus isolation and IHC.

In the Philippines, serological tests used to detect antibodies to Reston virus in pigs included the immunofluorescent antibody (IFA) test, virus neutralization and an IgG ELISA. An ELISA was also used in experimentally infected pigs, which all seroconverted by day 10. Antibodies to Ebola virus in experimentally infected pigs were detected by IgM and IgG ELISAs and virus neutralization. IgG titers and neutralizing antibodies to Ebola virus appeared to develop relatively late.

Blood (or serum) is considered to be the most reliable sample for diagnosis in acutely ill human patients, although other samples (e.g., oral swabs) can contain live virus, antigens or nucleic acids, and may be used. After death, filoviruses have been detected in almost every organ examined, including skin. The spleen and liver are important target tissues for filoviruses, and tissue samples collected from animal carcasses for filoviruses surveillance in Africa have included liver, spleen, muscle and skin, if the carcass is in good condition. PCR techniques may be able to detect nucleic acids in the bones or bone marrow of older carcasses.

Filoviruses are sensitive to many ordinary disinfectants, including sodium or calcium hypochlorite, although small amounts of residual infectivity may remain, especially when viruses are protected by substances such as proteinaceous material. In laboratory studies, some older commercial disinfectants completely destroyed the virus, while others did not. No commercial disinfectants sold at present claim efficacy specifically against filoviruses. Although the Centers for Disease Control and Prevention (CDC) notes that these viruses are enveloped and thus likely to be susceptible to a broad range of hospital disinfectants, it currently recommends that higher potency agents (those that are also effective against a non-enveloped virus) be used as an additional safety measure. Physical control methods that can be used to inactivate filoviruses or reduce infectivity include heat (e.g., heating at 60°C for one hour; incineration; autoclaving), UV light, gamma irradiation and filtration. No published controlled studies have examined filovirus inactivation in carcasses after either burial or other procedures (e.g., composting).

Factors that would need to be considered in protecting pigs include fomites, as well as contact with reservoir hosts or other species that may be infected. Whether rodents or other small animals can act as vectors (mechanical or biological) for filoviruses is uncertain. At present, there is little or no evidence to suggest that filoviruses would be shed by pigs after the acute stage of the illness, except possibly in semen. Nevertheless, information about infections in pigs is still very limited, and the potential for prolonged persistence in animals that may be immunosuppressed should also be considered. No diagnostic tests have been validated yet for routine diagnostic testing of imported pigs, but some assays used to detect Reston viruses during outbreaks or filoviruses in experimentally infected animals could be adapted if necessary. No vaccines are licensed for use in humans or pigs, but vaccine research is ongoing.

LITERATURE REVIEW

1. Etiology

1.1 Key Characteristics

Filoviruses are filamentous, enveloped, pleomorphic RNA viruses, which can appear in multiple forms including rod- or ring-like, crook-shaped (or shaped like a "6") or branched under the electron microscope.[1] Filoviruses have a single-stranded, negative-sense RNA genome.[2-4]

1.2 Strain Variability

As of 2014, the International Committee on Taxonomy of Viruses (ICTV) recognized five species in the genus *Ebolavirus*: *Zaire ebolavirus*, *Sudan ebolavirus*, *Taï Forest ebolavirus* (formerly *Cote d'Ivoire ebolavirus*), *Reston ebolavirus* and *Bundibugyo ebolavirus*.[2,5] The common name for the single virus in each of these species is Ebola virus, Sudan virus, Tai Forest virus, Reston virus and Bundibugyo virus, respectively. Collectively, Ebola virus, Sudan virus, Taï Forest virus and Bundibugyo virus are sometimes called the African ebolaviruses. While this grouping is based on geography, it also reflects their zoonotic potential: all four of the African ebolaviruses cause severe illness in people, while Reston virus affects animals but is not known to be pathogenic for humans.

The genomes of Ebola virus, Sudan virus, Tai Forest virus and Reston virus differ from each other by 37–42%, while Bundibugyo virus diverges from the other four viruses by 32–42%.[2] The Reston virus detected among pigs in China had 96–99% nucleotide similarity to the isolates from pigs and macaques in the Philippines (based on the viral L gene) but formed a separate branch on the phylogenetic tree.[6]

2. Cleaning and Disinfection

2.1 Survival

Filoviruses appear to be relatively stable when they are suspended in liquid media, and may retain infectivity for long periods, even at room temperature. Several studies have suggested that filoviruses may remain infectious for a time after drying, although survival may differ with the fomite, the temperature, and any biological material (e.g., blood) protecting the virus. Collectively, these experiments suggest that filoviruses could remain infectious on fomites for long enough to infect susceptible species, especially if the initial amount of virus is high.[7]

One group examining wildlife carcasses for ebolaviruses in Africa observed that, under tropical rain forest conditions, no infectious virus could be recovered from carcasses after 3–4 days.[8] A later study, which measured the decay rate for viruses aerosolized in tissue culture medium and kept in the dark, reported that the estimated total decay rate was 4.81% min^{-1} for Marburg virus (Popp strain), 4.29% min^{-1} for Ebola virus and 2.72% min^{-1} for Reston virus.[7] All three viruses could still be found after 90 minutes in this system, but Reston virus decayed significantly more slowly than the other two viruses. Unlike the previous study, infectivity was evaluated in tissue culture.

Refrigeration and freezing are likely to prolong the survival of filoviruses in meat or other tissues.[7,9,10] These viruses also survived freezing and thawing.[11] There is no data on the effects of salting, drying or smoking, although drying or salting would be expected to decrease viral loads in meat, at least to some extent.[12] Thorough cooking to 100°C is expected to rapidly destroy filoviruses.[12]

2.2 Disinfection

Filoviruses are sensitive to many ordinary disinfectants, although small amounts of residual infectivity may remain, especially when viruses are protected by substances such as proteinaceous material.[13,14]

Sodium hypochlorite (bleach) can destroy the infectivity of filoviruses and is commonly recommended as a disinfectant.[9,15-17] World Health Organization (WHO) guidelines for outbreaks in Africa have recommended household bleach diluted at 1:100 for ordinary disinfection (e.g., gloved hands, boots, equipment such as thermometers, and spills), or at 1:10 for disinfecting items such as patient urine and feces.[17] During field sampling of wild animal carcasses in Africa, a 2% chlorine spray was used to disinfect reusable equipment, the autopsy site and carcass remnants.[15] Calcium hypochlorite(bleach powder), at concentrations of 0.02% to 2%, is also considered acceptable for disinfection.[18]

No commercial disinfectants sold at present claim efficacy specifically against ebolaviruses.[19] Although the CDC notes that filoviruses are enveloped—and thus likely to be susceptible to a broad range of hospital disinfectants—it currently recommends that higher potency agents be used as an additional safety measure.[19,20] Specifically, it recommends that an EPA-registered hospital disinfectant with a label claiming efficacy against a non-enveloped virus (e.g., norovirus, rotavirus, adenovirus, poliovirus) be used in potentially contaminated areas of hospitals, as well as for routine use in diagnostic laboratories and to handle spills. Any cleaning product may be used to clean surfaces before disinfection.[19,20]

In the United States, the CDC currently recommends that ebolavirus-contaminated disposable materials to be discarded, including laboratory wastes, be placed in leak-proof containment, followed by incineration or autoclaving.[19,20] In endemic areas of Africa, boiling heat resistant items for 20 minutes has been recommended to kill filoviruses if autoclaving is not available.[17]

Filoviruses were inactivated by UV light in several experiments, which used different types and intensities of light, but the efficacy of this treatment varied.[9,21,22] Gamma irradiation from a cobalt-60 source can be used to inactivate filoviruses for assays that detect antigens and antibodies.[23] This form of irradiation is also useful for the preparation of immunological reagents, as it inactivates filoviruses without significant changes in the biological activity of their proteins.[21,24] Filtration can remove infectivity, provided the pore size is small enough; however, this method is not suitable for all purposes.[25]

No published controlled studies have examined filovirus inactivation in carcasses after either burial or other procedures (e.g., composting). Anecdotal evidence indicates that, under tropical rain forest conditions in Africa, infectious virus was not found in unburied carcasses after 3–4 days,[8] but whether similar times would be sufficient for buried carcasses or in colder temperatures is not known. Recommended procedures for human remains are cremation or burial in a sealed casket, with minimal handling.[16]

3. Epidemiology

3.1 Species Affected

3.1.1 African ebolaviruses

Current evidence suggests that bats are the primary reservoir hosts for ebolaviruses. Nested RT-PCR techniques have detected low levels of Ebola virus nucleic acids in at least three bat species (*Hypsignathus monstrosus*, *Epomops franqueti* and *Myonycteris torquata*) in areas where Ebola virus infected nonhuman primate carcasses had recently been discovered.[26] Bats are also suspected to be the reservoir hosts for Sudan virus, Tai Forest virus and Bundibugyo virus, although there are currently no studies to support this hypothesis.[27]

Pigs can be infected experimentally with Ebola virus, shed the virus, and develop clinical signs.[28-30] The initial studies in pigs were limited to animals 3–6 weeks of age. While no experiments with Sudan virus, Tai Forest virus or Bundibugyo virus have been published in pigs, they might be susceptible to these viruses as well.

3.1.2 Reston virus

Rousettus amplexicaudatus fruit bats are suspected to be a reservoir host for Reston virus, as one study found antibodies to this virus in 5 of 16 bats collected in the Philippines.[31] However, the virus has not yet been isolated or detected by PCR in these bats. Antibodies to filoviruses were found in several bat species in China, particularly *Rousettus leschenaulti*, *Pipistrellus pipistrellus* and *Myotis* species.[32] Sera from these bats reacted to both Reston virus and Ebola virus. In nature, Reston virus has been detected only in cynomolgus macaques, which become ill, and domesticated pigs.[6,33-36] Infections have been confirmed virologically, as well as by testing for antibodies, in both species. To date, Reston virus has been found only in pigs that were co-infected with porcine reproductive and respiratory syndrome (PRRS) virus.[6,33] Five-week-old pigs shed virus but remained asymptomatic after oronasal or subcutaneous inoculation with a Philippines isolate of Reston virus.[34]

3.2 Zoonotic Potential

3.2.1 African ebolaviruses

Human infections with African filoviruses are regarded as serious, life-threatening illnesses. In Africa, case fatality rates have ranged from 33% to 90%.[1,37-40]

3.2.2 Reston virus

The evidence at present suggests that Reston virus does not cause any illness in humans. Antibodies have been reported in a small number of people who worked with infected nonhuman primates in the United States or the Philippines, or with pigs in the Philippines; however, none of these people had any apparent illness.[13,14,33,35] Seroconversion does not seem to be common. In the Philippines, investigations in the 1990s found that 1% of animal handlers, trappers and administrative personnel tested at nonhuman primate export facilities were seropositive,[41] while 4% of serum samples from humans contained antibodies during the outbreak among pigs in 2008.[33] One assessment noted that, of more than 800 humans potentially exposed to Reston virus in the various outbreaks, approximately 2% were seropositive.[10] Antibodies have only been detected in people who were directly exposed to infected animals (i.e., in primate quarantine facilities and an infected primate export facility) but not other primate export facilities, or on infected pig farms or in slaughterhouses.[33,41]

3.2.3 Potential for filoviruses to exist in pork meat and other edible tissues

Whether filoviruses occur at sufficient levels to infect humans who eat undercooked tissues from naturally infected pigs, and whether such viruses can survive long enough to reach consumers, are both uncertain.

Studies in experimentally infected pigs have documented the presence of filoviruses in tissues that may be eaten by consumers. Meat (skeletal muscle), blood, heart, liver, kidneys and intestines (e.g., sausage casings), have been shown to contain Reston virus nucleic acids in some experimentally infected pigs, and virus could be isolated from some tissues including skeletal muscle.[34] Live Ebola virus was also recovered occasionally from porcine blood and heart, and viral RNA was detected at times in skeletal muscle, liver and gut.[28-30] Epidemiological evidence from Africa suggests that direct exposure to infected animal carcasses (i.e., bushmeat such as nonhuman primates) is a significant risk factor for infection, although there are no reports of infections in people who were exposed to bushmeat but did not participate in butchering.[10]

3.3 Geographic Distribution

3.3.1 African ebolaviruses

As of 2015, human outbreaks caused by Ebola virus had originated in the Democratic Republic of the Congo (DRC), the Republic of the Congo, Gabon and Guinea, although the movements of infected people sometimes spread the virus to additional regions.[37,42] The 2013–2015 ongoing outbreak originated in Guinea, but was subsequently transmitted to other countries, particularly Liberia and Sierra Leone.[37,42]

More than 15,000 Ebola cases were laboratory confirmed and more than 11,000 deaths were reported. These numbers are likely underreported however, with the number of suspected cases being more than 28,000.[43]

Bat species shown to be infected with Ebola virus in the wild[26,44] have broad geographic ranges that include the entire tropical forest regions of equatorial central Africa.[45] Human outbreaks caused by Sudan virus have been reported in Sudan and Uganda.[37] All known outbreaks have occurred within 400 miles of each other, and the range of this virus may be limited.[42] Tai Forest virus has been reported from West Africa. An outbreak in the Taï National Park in Côte d'Ivoire in 1994 mainly affected chimpanzees, although either one[37] or two[46] human cases were documented in people who had close contact with infected animals. Human outbreaks caused by Bundibugyo virus were reported in Uganda in 2007 and in the DRC in 2012.[37,42]

3.3.2 Reston virus

In 2008, Reston virus was found in domesticated pigs in the Philippines during an investigation of a severe PRRS outbreak caused by an atypical PRRS virus.[33] Pigs on farms in the outbreak area had antibodies to Reston virus, but 98 pigs from an unaffected region (Tarlac) were seronegative.[36] Whether pigs in other parts of the Philippines are free of Reston virus remains to be determined by additional surveys.[36] Reston virus nucleic acids were later detected in pigs on three farms in Shanghai, China that had experienced a severe PRRS outbreak in 2008.[6] There was no link between the Chinese farms and the Philippines, suggesting that the virus had been acquired locally.

3.4 Morbidity and Mortality

Natural Ebola infections have not been reported in pigs, although they can be experimentally infected. The current prevalence of Reston virus in pigs is not known.

4. Transmission

4.1 African ebolaviruses

At present, the only information about pigs infected with African filoviruses comes from a series of experiments in pigs 3–6 weeks old, which developed clinical signs and shed virus after combined intranasal, oral and conjunctival inoculation with 1 x 10^6 PFU Ebola virus per animal.[28-30] Infectious virus and viral RNA were found in oral and nasal secretions, but viral shedding from the gut was sporadic and inconsistent, and viremia (infectious virus and/or viral nucleic acids) was documented occasionally, but not in all animals. Moderate levels of infectious virus were found in the bladder of one pig 5–6 weeks old with viremia, but urine was not tested directly in these studies.[28] Ebola virus shedding in the semen or milk of pigs also has not been tested.

In a study of 5–6 week old pigs, infectious virus was isolated from nasal washes, oral swabs and/or rectal swabs on days 3 and/or 5 post-inoculation (dpi), although the titers were relatively low (approximately 100 to < 1000 $TCID_{50}$/mL per site).[28] High levels of infectious virus (up to 3.2 x 10^7 $TCID_{50}$/mL) were found in the lungs between days 3 and 7.

Study of pigs 3–4 weeks old showed that infectious virus in nasal secretions peaked on day 5 at < 1000 $TCID_{50}$/ml, with lower average virus titers (≤ 100 $TCID_{50}$/ml) shed on days 3 and 7, and no virus isolated on day 10 or later.[28] Virus was also isolated from oral secretions (average titers ≤ 100 $TCID_{50}$/ml) from day 3 to day 7. Despite the low titers, these animals shed sufficient Ebola virus to infect pigs of the same age that were placed in the same pen the day after inoculation. Contact animals shed low amounts of infectious virus (mean titers ≤ 100 $TCID_{50}$/ml) in nasal secretions on days 7 and 10 of the experiment, and in oral secretions on day 10.

Viral RNA was detected on days 1–7 in oral secretions and days 1–14 in nasal secretions of inoculated pigs, and on days 3–14 in oral secretions and days 3–10 in nasal secretions of contact pigs. Because the inoculated and contact pigs shared the same pen, and RT-PCR is very sensitive, it appears that positive samples at some time points might have been caused by environmental contamination from other animals. In a later experiment in 4-week-old pigs, Ebola virus nucleic acids were detected in nasal and oral swabs between days 1 and 7 after inoculation, and in rectal swabs on day 1 and 5 but not days 7 or 12.[30] In contact pigs, Ebola virus nucleic acids were found in some organs, including the intestines and tonsil, up to day 28/ 29 of the experiment (i.e., 18–19 days after the last detection of infectious virus in secretions from these animals).[28]

The dynamics of virus recovery and nucleic acid detection in the transmission study suggest a replication and transmission cycle of approximately five days.[28] While these experiments demonstrate that pig-to-pig transmission of Ebola virus is possible, it is still not known whether ebolavirus transmission can be sustained in a swine population.[47] Transmission from pigs to primates was demonstrated when six 4-week-old pigs inoculated oronasally with 10^6 $TCID_{50}$ Ebola virus transmitted this virus to 4 cynomolgus macaques.[30] The lung lesions and pattern of viral antigens in the lungs of the nonhuman primates suggested that viruses had reached this organ by inhalation as well as by transmission from the blood.[30] How bats transmit filoviruses to each other, or to other animals, is uncertain. Virus titers normally appear to be very low in infected bats tested in the wild: nucleic acids have only been detected by nested RT-PCR.[26,48]

4.2 Reston virus

While Reston virus-infected pigs were detected during PRRS outbreaks in the Philippines and China, nothing is known about how they acquired the virus.[6,33] Viruses isolated from pigs in the Philippines differed by approximately 4% in nucleotide sequence, suggesting that either there was more than one distinct spillover event from another reservoir host, or that pigs have maintained these viruses for many years.[33,34] At present, these two possibilities cannot be distinguished, and both are possible.

A Reston virus isolated from pigs in the Philippines replicated in 5-week-old pigs inoculated subcutaneously or oronasally with 10^6 $TCID_{50}$ of this virus, but did not cause clinical signs.[34] Most oronasally inoculated pigs shed the virus in nasopharyngeal secretions; virus isolation was successful on days 4 to 8, and nucleic acids were found by RT-PCR on days 2 to 8. While specific virus titers are not provided, peak titers occurred on day 6. For unknown reasons, Reston virus was only detected in the nasopharyngeal secretions of subcutaneously inoculated pigs in the second of 2 trials (although oronasally inoculated pigs shed the virus in these secretions in both trials). In addition, nucleic acids were found in rectal swabs and/or blood from oronasally or subcutaneously inoculated pigs in the second trial, but not the first. The authors speculated that coinfection with other pathogens might have accounted for these discrepancies. No virus was found in urine collected from the floor of the pen by either RT-PCR tests or virus isolation. During the acute stage of the illness, nucleic acids were widely distributed in the organs and tissues of pigs inoculated by either route, including the lungs, kidneys and ileum, and the virus was isolated from the lungs. These samples were collected during the second trial. Shedding in semen or milk has not been tested in pigs.

In these pigs, virus clearance appeared to take place around days 10–12, coinciding with the appearance of antibodies.[34] No nucleic acids were found in tissue samples collected during necropsy on day 28 in the first trial, suggesting that persistent infections do not occur. Transmission studies with Reston virus in pigs have not been published.

At present, there is no evidence that arthropods are involved in transmission to humans or other species.[49] Nevertheless, viruses have been found in the blood of bats and other hosts, and some authors have

speculated that blood-sucking arthropods might be able to act as mechanical or biological vectors. There is no information about potential routes of Reston virus transmission in bats.

5. Infection in Swine/Pathogenesis

5.1 Clinical Signs

5.1.1 African ebolaviruses

In one study, five to six week-old pigs infected with Ebola virus developed respiratory signs that resemble other respiratory diseases of swine.[28] A fever was initially detected on the 4th day after inoculation, and lasted until day 7, the final day of the experiment. Additional clinical signs were anorexia, lethargy and an increased respiratory rate, which progressed to labored breathing. Coughing was not noted. The disease in pigs appeared to be limited to the respiratory system: no skin rash, gastrointestinal signs, coagulopathies, hemorrhagic tendencies, shock or evidence of multiorgan failure were noted. Blood cell counts and blood chemistry profiles also did not reveal any abnormalities, although increased fibrinogen levels on days 5 and 7 were suggestive of an inflammatory response.

The illness was less severe in 3–4 week-old pigs, which had a transient and/or delayed fever and an increased respiratory rate.[28,30] In one study, these animals apparently recovered by day 9.[30] Hematology and coagulation parameters were normal.

5.1.2 Reston virus

Clinical signs in pigs co-infected with Reston virus and PRRS virus were consistent with severe, atypical PRRS in both the Philippines and China.[6,33] Detailed clinical descriptions were not provided in the articles describing these outbreaks, but high fever and blue ears were mentioned in the report from China.[6] While "typical" PRRS viruses mainly cause reproductive problems in sows, with stillborn or weak piglets that die soon after birth, atypical viruses cause more severe illness. High fever, respiratory signs, diarrhea, lameness, "blue ears," petechiae, significantly elevated mortality in gilts and sows, and other signs have been caused by certain PPRS virus isolates found in China since 2006.[50] These isolates appeared to be responsible for the outbreaks in both China and the Philippines.[6,33] Because all affected pigs were co-infected with both PRRS virus and Reston virus, and some of the pigs in the Philippines were also infected with porcine circovirus type 2, it is difficult to determine the contribution of Reston virus (if any) to these outbreaks.[34]

Five-week-old pigs inoculated subcutaneously or oronasally with Reston virus (Philippines pig isolate) remained asymptomatic, although lymphadenomegaly affecting the submandibular lymph nodes and mild acute rhinitis were noted at necropsy.[34] Fever was not detected. Despite the absence of respiratory signs, some pigs had areas of consolidation in the lungs, which may or may not have been caused by Reston virus.

5.2 Postmortem Lesions

5.2.1 African ebolaviruses

At present, there are no published reports that describe naturally occurring Ebola virus infections in domesticated or wild pigs.

Five to six week-old, experimentally infected pigs had signs of bronchointerstitial pneumonia, with progressive and sometimes extensive consolidation of the lungs, mainly in the dorsocaudal lobes.[28,29] Some pigs had hemorrhages in the lungs and/or inflammatory exudates in the trachea.[29] The lung-associated lymph nodes were enlarged, and occasionally mildly hemorrhagic.[28] A hemorrhagic right atrium of the heart was observed in 2 pigs euthanized 7 days after inoculation, but whether this lesion was related to the infection was uncertain.[28] No other organs had any lesions.

Histopathologic lesions in the lungs were described as bronchointerstitial pneumonia with the accumulation of neutrophils, macrophages and necrotic debris in the lumen of alveoli and bronchioli, and peribronchiolar/perivascular infiltration of inflammatory cells.[28,29] Numerous multinucleated cells were found in the alveoli of pigs euthanized on day 5/6.[29]

Three to four week-old pigs infected with the same virus were less severely affected.[28,30] One animal analyzed 7 days after inoculation was reported to have macroscopic and microscopic lung lesions similar to those in older pigs, but not as severe.[28] In a second study of 3–4 week old pigs, no significant gross lesions were noted, and microscopic lesions were limited to focal (not extensive) bronchointerstitial pneumonia with a lobular pattern.[30]

5.2.2 Reston virus

Lesions in pigs co-infected with Reston virus and PRRS virus were incompletely described, but they were reported to be consistent with atypical PRRS.[6,33] Reston virus antigens were detected focally in areas of minimal necrosis in lymphoid tissues and lymph node capsule tissues of the pigs in the Philippines.[33] This contrasted with the localization of PRRSV antigens, which were present in the germinal centers of lymphoid follicles with germinal cell hyperplasia and focal necrosis. Antigens of both viruses were found in areas of interstitial pneumonia in the lungs.

Most experimentally infected pigs euthanized 6 to 8 days after inoculation had lymphadenomegaly affecting the submandibular, retropharyngeal, and bronchial lymph nodes.[34] Lymph node lesions were confirmed as reactive hyperplasia by histopathology. In the lungs, one oronasally inoculated pig and 3 of 4 pigs inoculated subcutaneously had areas of gross consolidation in one or more apical and cardiac lobes and/or the hilus, identified by histopathology as acute bronchopneumonia. Pigs also had mild acute rhinitis at necropsy and focal necrosis of tonsillar epithelium associated with neutrophil infiltrates (without evidence of Reston virus antigens in the tonsillar lesions).[34] No gross or microscopic lesions were found in the spleen, liver, kidney, heart, intestines or brain.[34]

6. Diagnosis

6.1 Tests to Detect Nucleic Acids, Virus, or Antigens

6.1.1 Tests for viral nucleic acids

PCR assays used to detect filovirus nucleic acids include reverse transcriptase (RT)-PCR, quantitative real time RT-PCR (RRT-PCR) and the reverse transcription loop–mediated isothermal amplification method.[38] RT–PCR and RRT–PCR are used most often for diagnosis in humans, but the loop–mediated isothermal amplification method is simpler and costs less, and may eventually replace these assays. Advantages to RT-PCR tests include their ease of use; speed, sensitivity and specificity; and the ability to conduct these tests without BSL-4 biocontainment once the sample has been properly inactivated.[38,46,51] However, RT-PCR may miss novel viruses (variants or new species), and cross-contamination can cause false-positives.[46,51] While integrated filovirus-like genetic sequences found in some animal species might theoretically affect detection by RT-PCR,[6] the sequence identity with currently circulating viruses is low,[52,53] and this is not known to be a concern at present.

Cross-contamination is a particular concern with the index case or single cases, which should not be diagnosed solely by RT-PCR.[51] In human diagnostics, independent assays recommended to confirm results from RT-PCR include antigen capture ELISA or virus isolation. If these techniques are unavailable, the minimum recommended confirmation is RT-PCR on an independent target gene and/or independent sample. Consideration should be given to confirming the results in another reference laboratory. During filovirus surveillance of animal carcasses in Africa, three tests (RT-PCR,

immunohistochemical staining and antigen capture ELISA) were used for diagnosis, and positive results in two tests were required to consider the diagnosis definitive.[8,15]

Microarray assays that can detect filovirus nucleic acids have also been developed. Although these tests are not used routinely for diagnosis in humans,[38] a panviral microarray assay was used to detect Reston virus during the initial outbreak among pigs in the Philippines.[33]

Ebola virus nucleic acids were detected in experimentally infected pigs with real-time RT-PCR assays targeting either the Ebola virus L gene or glycoprotein gene.[28-30] RRT-PCR targeting the nucleoprotein gene was also reported to detect this virus, and assays for these 3 genes were reported to have comparative results between laboratories where applicable (e.g. between the National Microbiology Laboratory [NML] and the National Centre for Foreign Animal Disease [NCFAD]).[47]
In situ hybridization can also detect filovirus nucleic acids; however, this test has been used mainly in research.[54]

6.1.2 Virus isolation

Isolation of filoviruses can only be performed at a few laboratories that have biocontainment facilities capable of handling dangerous human pathogens.[38] Vero cells (usually clone E6) and MA-104 cells are used most often, but other cell types such as SW-16 have also been employed.[14,51] Some cell types may give better results than others in some circumstances,[14] and Reston virus has been reported to replicate less efficiently in Vero cells than Ebola virus.[55] Most filoviruses do not cause extensive cytopathic effect (CPE) during primary isolation.[14,51] Primary isolation can also be accomplished in guinea pigs, if a filovirus grows poorly in tissue culture, but universally fatal illness should not be expected before several passages in these animals.[13,51] While virus isolation may be used for other purposes, one use in human diagnosis is as a confirmatory test for a positive result from RT-PCR or antigen detection ELISA.[38]

Virus isolation in E6 Vero cells was one of the confirmatory tests during the PRRS/ Reston virus outbreak among pigs in the Philippines.[33] Vero cells were also used to detect Reston virus in experimentally infected pigs.[34] A single blind passage was used in this experiment, and virus was detected in cell cultures by staining with antibodies to the viral NP protein.

E6 Vero cells were also used to reisolate Ebola virus from experimentally infected pigs.[28,29,47] The viruses from these animals replicated but did not cause CPE until the second or third passage, and the authors note that blind passages may be necessary when attempting to isolate viruses from field samples.[47] A swine kidney cell line (PK15) is being evaluated as a possible alternative to E6 cells for virus isolation from pigs.[47]

6.1.3 Tests to detect antigens

Antigen detection ELISAs are one of the primary tests (with RT-PCR) used to diagnose clinical cases in humans.[51] They are also used as an independent confirmatory test for a positive RT-PCR assay. Antigen detection ELISAs in humans use either hyperimmune serum or antibodies specific to a filoviral protein such as the NP.[38] Filovirus antigens can also be detected in tissues by immunofluorescence or immunohistochemistry.[13]

Two of the confirmatory tests used to identify Reston viruses in naturally infected pigs were an antigen detection ELISA and immunohistochemistry.[33] The reagents for immunohistochemical staining were polyclonal mouse or rabbit antibodies. Immunohistochemistry on fixed tissues, using rabbit polyclonal antibodies to the NP protein, also detected Reston virus antigens in experimentally infected pigs.[34]

Ebola virus antigens in experimentally infected pigs were also detected by immunohistochemistry, with rabbit polyclonal antibody targeting the Ebola virus VP40 protein.[28,29] Immunohistochemistry on fixed

tissues, especially lungs and lymph nodes, has been suggested as a potential diagnostic test for filovirus infections in pigs; however, monoclonal antibodies might need to be developed for diagnostic purposes.[47] There is no information about the suitability of rapid tests in development for humans[51,56] being used in pigs.

6.1.4 Electron microscopy

Electron microscopy can detect filovirus particles, which are distinctive in appearance, in tissues or blood.[13,38,51] However, factors such as equipment availability and the inability to reliably distinguish different filoviruses limit its use. Because viremia is relatively high in humans, this test has sometimes been used as confirmatory test for other assays.[13,38] Electron microscopy was described during cell culture of Reston virus from naturally infected pigs,[33] but there are no reports of its use for direct examination of porcine tissues or blood. Immunoelectron microscopy for filoviruses has also been described, and was used during some outbreaks in humans, as well as in nonhuman primates.[13]

6.2 Tests to Detect Antibody

6.2.1 African ebolaviruses

Antibodies to Ebola virus were detected in experimentally infected pigs with an IgM capture ELISA test that used cell lysate antigen[29,47] or an IgG ELISA that employed gamma irradiated, sucrose gradient purified whole Ebola virus.[28] Neutralizing antibodies to Ebola viruses in pigs were detected by the classical CPE-reduction virus neutralization assays in Vero E6 cells.[47]

In an initial experiment in 5–6 week old, experimentally infected pigs, neither IgG antibody titers by ELISA nor neutralizing antibodies were detected by day 7, when the pigs were euthanized.[28] IgM titers to Ebola virus could be found by ELISA on day 5/6 in a second study that used pigs of the same age.[29]

In 3–4 week old pigs, IgG antibody titers (by ELISA) and neutralizing antibodies were measured during a contact transmission experiment.[28] Neutralizing antibodies and/or ELISA IgG titers were found in inoculated and contact pigs on days 21 and 28/29 after the start of the experiment, but were not reported to be present on day 10.

6.2.2 Reston virus

Tests used to detect antibodies to Reston virus among pigs in the Philippines included an IFA test based on HeLa cells expressing recombinant Reston virus GP or NP; virus neutralization; and an IgG ELISAs based on recombinant viral GP or NP.[36] Virus neutralization was conducted in Vero (E6) cells; however, VSV-pseudotype bearing REBOV-GP was used as an alternative to live virus, which requires high containment facilities. The IFA and virus neutralization tests detected a high seroprevalence rate among pigs in infected regions, but not in an uninfected area or outside the Philippines (i.e., in Japan).

Antibodies to Reston virus were found in 71% to 79% of pigs on infected farms by IFA in this study. In addition, 72% of these pigs had neutralizing antibodies, with titers ranging from 100 to 12,800, an average of titer of 790, and median titer of 400. The ELISA tests also demonstrated high seroprevalence among pigs in infected areas (67–90%), although a small number of false positives were evident in this test (approximately 1% of sera collected from pigs in an uninfected region of the Philippines, and 2% of pigs in Japan). In an earlier study, no antibodies to Reston virus were found in pigs that were acutely co-infected with this virus and the PRRS virus, despite the detection of antibodies to PRRS virus.[33]

During a study of experimental Reston virus infections in pigs, antibodies were detected with an indirect in-house ELISA targeting the viral NP (based on a recombinant NP expressed in *Escherichia coli*).[34] All of these pigs seroconverted to Reston virus after challenge by either oronasal or subcutaneous inoculation, with antibody first detected in most pigs between days 6 and 8, and all pigs seroconverting by day 10. Pigs inoculated subcutaneously had higher antibody titers.

While serological reactions to marburgviruses can be distinguished from reactions to ebolaviruses, antibodies to ebolaviruses are cross-reactive.[2] The most likely causative agent can be distinguished with comparative serological assays against a panel of different viruses.[57,58]

Tests used to detect Reston virus and Ebola virus in pigs have been developed mainly for research purposes.[6,28-30,33,34] While these assays may be useful for routine diagnosis in pigs, they have not yet been validated for this purpose. In some cases, improved reagents may be needed for diagnostic assays (e.g., monoclonal antibodies in antigen detection tests).[47]

6.3 Secretions, excretions and tissues that may contain filoviruses

6.3.1 African ebolaviruses

Ebola virus nucleic acids and/or infectious virus were detected regularly in nasal and oral swabs from experimentally infected young pigs (though not on all days from all animals), but only sporadically and inconsistently from rectal swabs, and occasionally in blood.[28-30]

Ebola virus nucleic acids were detected most consistently in the lungs and submandibular and bronchial lymph nodes of experimentally infected pigs, but could sometimes be found in other tissues and organs including the liver, spleen, mesenteric lymph nodes, heart, muscle and gut.[28-30] Infectious virus could be isolated from the lung and sometimes from the lung-associated lymph nodes between days 3 and 7, and lower levels of infectious virus were sometimes found in other organs.[28,30] Within the lungs, Ebola virus antigens were abundant in affected lobules, but could be absent from adjacent, unaffected lobules.[28-30]

6.3.2 Reston virus

During an outbreak in the Philippines, Reston virus RNA was detected in samples from the lung, spleen and lymph node, but not liver, by RT-PCR.[33] Virus was isolated from the lungs and lymph nodes, and viral antigens were found in the lungs, lymphoid tissues and lymph nodes of pigs. In China, Reston virus nucleic acids were detected in the spleen, but whether any other samples were collected or tested is unclear.[6]

In oronasally inoculated young pigs, Reston virus nucleic acids were detected by RT-PCR in nasopharyngeal secretions on days 2 to 8 after exposure, and virus isolation was successful on days 4 to 8, with levels peaking around day 6.[34] Nucleic acids and infectious virus could be found in both deep nasal and oral (throat) swabs, but from limited data, it appears that nasal samples were positive more often. No evidence of virus was found in urine collected from the floor of the pen. For unknown reasons, Reston virus was detected in the nasopharyngeal secretions of subcutaneously inoculated pigs in the second of 2 trials, but not the first. These trials were identical, but conducted during different seasons. Oronasally inoculated pigs shed virus in nasopharyngeal secretions during both trials. The detection of nucleic acids in the blood and rectal swabs of oronasally and subcutaneously inoculated pigs was also inconsistent; evidence of infection was found only in the second trial.

Tissue and organ samples were evaluated during the acute stage in only the second trial. In addition to blood, nucleic acids occurred in numerous organ and tissue samples collected on day 6 or 8 from pigs inoculated by either route.[34] Tissue samples positive by RT-PCR included lung, heart, liver, kidney, spleen, ileum, superficial lymph nodes (submandibular), nasal turbinates, tonsil and skeletal muscle.

Virus was also isolated from some tissues including the lung, superficial lymph nodes (submandibular, axillary, inguinal), internal lymph nodes (bronchial, mesenteric), nasal turbinates and skeletal muscle. In the lungs, the highest concentrations of virus occurred in regions of pneumonia, but virus was also found in healthy lungs. Reston virus antigens were detected in the lymphoid tissues of pigs inoculated SC, the spleen of one of these pigs, and the lungs of oronasally or subcutaneously inoculated pigs that had lung

lesions. Virus appeared to be present only during the acute stage of disease; in the first trial, no nucleic acids were found in tissue samples collected from pigs necropsied 28 days after inoculation.

The use of oral fluids as a diagnostic specimen has not been evaluated for any *Ebolavirus.*

6.4 Safety considerations during diagnostic testing

6.4.1 Virus inactivation for diagnostic tests

The use of tests that minimize human exposure should be considered when selecting diagnostic assays.[51] Filovirus isolation requires high containment (BSL 4 biocontainment), but most other diagnostic assays can be conducted on samples containing inactivated virus.[46] Some procedures, such as RT-PCR, require minimal sample handling.[51] A number of techniques have been used to inactivate viruses in diagnostic specimens, including heat, treatment with chaotropic buffers or betapropionolactone, irradiation (e.g., from a cobalt-60 source) or a combination of heat and detergent, as well as other physical or chemical methods (see also section 12.2).[16,21,23,25,38,46,51,59] Small amounts of residual infectivity may remain after some forms of inactivation, especially when viruses are protected by substances such as proteinaceous material.[14] As an example, infected cells fixed on slides with acetone or alcohol can retain some infectivity if they are eluted from the slide.[14]

- Samples to be used in molecular assays (e.g., PCR) are commonly inactivated with guanidinium isothiocyanate, which is a major component in most commercial RNA extraction buffers.[23,38,51] This agent inactivates filoviruses by denaturing the viral proteins.[23]
- Gamma irradiation from a cobalt-60 source or heat inactivation can be used to inactivate filoviruses for assays that detect antigens and antibodies.[23] One group reported that gamma irradiation was preferable to UV irradiation, as UV treatment did not completely inactivate viruses under some conditions, and decreased antigenicity at longer exposures.[21] Gamma irradiation levels that inactivate filoviruses are not suitable for all purposes, as this process has been reported to alter some clinical chemistry panel values, including some enzyme levels, prothrombin time and partial thromboplastin time.[25] It was reported not to affect the leukocyte (WBC) count in clinical samples.[25]
- One group reported that heating serum spiked with high titers of Ebola virus or a marburgvirus for one hour at 60°C eliminated viral infectivity in cell culture, but did not change values in clinical chemistry assays for thermostable blood components (serum glucose, blood urea nitrogen or electrolytes).[25]
- Heat in the presence of appropriate ionic or non-ionic detergents has been used to inactivate viruses in serum for filovirus diagnostic assays.[51] Procedures that have been used by the National Microbiology Laboratory, Public Health Agency of Canada, include a combination of sodium dodecyl sulphate (SDS)/Tween 20 and heat treatment of 60° C for 15 minutes, for samples that will be used for ELISA based assays; and heat and SDS for immunofiltration based antigen detection assays.[51] CDC guidelines published in 1995 recommended that serum used in laboratory tests be pretreated with polyethylene glycol p-tert-octylphenyl ether (Triton(R) X-100), noting that 10 uL of 10% Triton X/ ml of serum for 1 hour reduces virus titers but should not be assumed to be completely effective.[16]
- Fixation of tissues in 10% formalin is reported to inactivate filoviruses within a week for procedures such as immunohistochemistry.[14]
- Fixation of blood smears in solvents destroys most viruses present.[16]
- Diluting blood 1:100 in 3% acetic acid (15 minutes at room temperature) to determine the leukocyte (WBC) count was reported to inactivate high concentrations of Ebola virus and a Marburgvirus.[25]

6.4.2 Considerations for personnel

Standard, contact and droplet precautions have been recommended during contact with human filovirus-infected patients.[20] Standard personal protective equipment (PPE) during human outbreaks in endemic areas has included two pairs of gloves that reach well above the wrist, (4–6 inches); a scrub suit or other dedicated inner layer of clothing; a gown or outer layer of clothing that closes around the wrists; an apron impervious to liquids (e.g., plastic); head covering; mask containing a HEPA filter or other biosafety mask (surgical mask if these are unavailable); and eye protection such as non-fogging goggles.[17] Rubber boots or overshoes are also recommended if the environment is contaminated. Standard medical gloves (latex, vinyl or surgical) are considered suitable for patient care, but the outer gloves should be thick neoprene or rubber (approx. 12 inches above the wrist) when handling spills, disinfecting excreta, laundering linens and conducting autopsies and burials. To prevent personal contamination, removal of protective gear should follow a specific order described in guidelines published by the WHO and CDC.

The CDC currently recommends that laboratory staff testing diagnostic specimens that may be contaminated wear gloves, a water-resistant gown, a full face shield or goggles, and a mask that covers the entire nose and mouth.[20] The CDC also recommends that samples be handled in a certified class II Biosafety cabinet or Plexiglas splash guard with PPE to protect skin and mucous membranes, and that all manufacturer-installed safety features for laboratory instruments be used. For the collection of human diagnostic samples from suspected Ebola virus patients, the recommendations at present include at least gloves, a water-resistant gown, full face shield or goggles, and a mask that covers the entire nose and mouth, with a provision for additional PPE in some situations.[20]

The suitability of these precautions for use with livestock, and any modifications that might be advisable in this environment, would need to be considered by policy-makers. During outbreaks among nonhuman primates, recommended PPE may also include non-slip, steel-toed shoes/boots, and stainless-steel or Kevlar meshed gloves may be recommended for use in some procedures where deep punctures and lacerations could occur.[13] Face shields should be worn during procedures that have a high potential to generate aerosols.[13] Necropsies are considered to be high risk procedures, and specific advice should be obtained from the health departments and the CDC.[16] During a field sampling program of wild animal carcasses in Africa, high level precautions used during the necropsy included watertight clothes equipped with air filtration equipment and face shields, and the lancets and forceps used were disposable.[15]

6.4.3 Sample handling

Field diagnostic laboratories have been used during some outbreaks in humans.[51] Minimizing exposure for workers in these investigations is critical. Ideally, this would include a portable class III biosafety cabinet, which allows samples to be handled safely until infectious agents are inactivated or packaged for shipping.[51] A constraint is that these units must often be shipped as cargo or by charter, as they are often too large to travel as checked baggage on commercial flights. An alternative is to use personal protective equipment (PPE) similar to that used by medical staff who must handle infectious material in filovirus isolation wards (see section 12.4).[51]

7. Immunity

7.1 Post-exposure

There is no information about immunity post-infection in pigs. Studies of human survivors of Ebola virus indicate that serum-neutralizing antibodies can be detected 10 to 12 years after infection.[60,61]

7.2 Vaccines

Vaccination of pigs does not appear to be necessary at present, but this could change if testing reveals that filovirus infections occur with some frequency in these animals.[46]

A number of Ebola virus vaccines, based on a wide variety of platforms, have been tested in laboratory rodents and/or nonhuman primates.[62] Standard inactivated vaccines were assessed as human vaccines in the past, and some appeared to be successful in laboratory rodents (e.g., guinea pigs); however, they were not developed further when experiments in nonhuman primates were disappointing.[13] Classical subunit vaccines (e.g., recombinant expressed viral proteins) were only partially protective in rodent models, and have not been developed further.[63] Improvements in immunogenicity would be necessary to justify testing these vaccines in nonhuman primates,[62] and presumably other species. A variety of other vaccines have shown some promise. They include 1) virus-like particles consisting of the Ebola virus VP40, glycoprotein and sometimes the NP, together with an adjuvant; 2) viral vectored vaccines that express genes encoding Ebola virus proteins; and 3) DNA vaccines combined with viral vectored vaccines.[62] One human adenovirus 5-vectored vaccine has advanced to human phase I clinical trials, as did one DNA vaccine. While most of these vaccines are intended for use in humans, a recombinant murine cytomegalovirus-vectored vaccine expressing an NP epitope is being developed with the goal of immunizing African wildlife such as gorillas and chimpanzees.[62] The initial vaccine was partially protective in mice. An advantage for use in wildlife is that this vector is replicating and can spread from animal to animal, but it is also highly species-specific and would be unlikely to infect non-target species including humans.

The WHO maintains a list of vaccines being tested here: http://www.who.int/medicines/emp_ebola_q_as/en/. Vaccine types in development for humans may not be the optimal approach in livestock, and if vaccines are developed in the future, consideration should be given to using vaccine vector systems that have had good safety and efficacy profiles in livestock.[46]

7.3 Cross-protection

There is some evidence that Ebola vaccines capable of eliciting cellular immunity provide cross-protection between strains; in one study, cynomolgus macaques immunized with DNA/rAd5 vaccine expressing Ebola virus (Zaire and Sudan strains) glycoprotein were protected against challenge with Bundibugyo virus.[64]

8. Prevention and Control

Current evidence suggests that bats are most likely to be the reservoir hosts for filoviruses,[26,27,31,37,44,65-71] although other susceptible hosts, including people, can transmit these viruses once infected.[8,37,49,72] Indoor housing is probably the most effective measure for protecting pigs from exposure, but methods that have been used to protect animals from other bat-associated viruses in endemic regions (e.g., wire screens to prevent entry into open-sided pig sheds, and the removal of fruit trees that may attract bats) may also have some benefit. Biosecurity plans should also consider the possibility of contact with other hosts, such as infected humans, and fomites. Whether rodents or other small animals can act as vectors (mechanical or biological) for filoviruses is uncertain. There was no evidence of infection in commensal rodents trapped in one ebolavirus ward in Africa,[73] or in rodents at one Reston-virus infected primate export facility in the Philippines.[41] Laboratory rodents including mice are also resistant to inoculation with wild type filoviruses.[74,75] Nevertheless, investigations of wild species have been limited, and mechanical transmission remains a possibility in all species.

At present, there is little or no evidence to suggest that filoviruses would be shed by pigs after the acute stage of the illness, except possibly in semen. Semen appears to be the only secretion that contains filoviruses in humans or nonhuman primates during convalescence, although viruses were reported to persist in milk to day 15.[76-80] Likewise, Reston virus in pigs seems to disappear from tissues by 1 month.[34] Nevertheless, information about infections in pigs is still very limited, and the potential for prolonged persistence in animals that may be immunosuppressed should also be considered. Filoviruses in semen could be a concern both in live animals and during artificial insemination. Infectious viruses have been

found in human semen for as long as 3 months after recovery,[77-80] and filoviruses are reported to be relatively stable in some biological samples during cold storage or freezing.[7,9]

In the Philippines, the main control measure for swine herds infected with Reston virus was depopulation.

9. World Organization for Animal Health (OIE) Terrestrial Animal Health Code

Filoviruses are not covered in the 2015 OIE Terrestrial Animal Health Code.

10. Gaps in Preparedness

Little is known about filovirus infections in pigs. No diagnostic tests have been validated yet for routine diagnostic testing of imported pigs, but some assays used to detect Reston virus during outbreaks, or Reston or Ebola virus in experimentally infected animals could be adapted if necessary in an outbreak situation. No vaccines are licensed for use in humans or pigs, but vaccine research is ongoing.

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