EBOLAVIRUSES:
AFRICAN SPECIES AND RESTON VIRUS

Swine Health Information Center

The mission of the Swine Health Information Center is to protect and enhance the health of the United States swine herd through coordinated global disease monitoring, targeted research investments that minimize the impact of future disease threats, and analysis of swine health data.

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SUMMARY

IMPORTANCE

- Reston virus is the only known ebolavirus that occurs in Asia. It has been detected in the Philippines and China in pigs co-infected with porcine reproductive and respiratory syndrome virus (PRRSV).
- Reston virus is not known to cause disease in humans; however, concern about spillover events and changes in pathogenicity remain.

TAXONOMY

- There are six ebolavirus species. They are known by species name (common name) as follows: *Bombali ebolavirus* (Bombali virus), *Bundibugyo ebolavirus* (Bundibugyo virus), *Reston ebolavirus* (Reston virus), *Sudan ebolavirus* (Sudan virus), *Taï Forest ebolavirus* (Taï Forest virus), and *Zaire ebolavirus* (Ebola virus). Herein, "Ebola virus" refers specifically to the species *Zaire ebolavirus*.

PUBLIC HEALTH

- Three of six ebolavirus species (Ebola virus, Sudan virus, and Bundibugyo virus) cause Ebola virus disease (EVD), a severe and life-threatening illness in humans.
- Antibodies to Reston virus have been detected in humans, but there are no reports of clinical illness.

INFECTION IN SWINE

- Natural infection with Ebola virus has not been described. Young pigs experimentally infected with Ebola virus develop non-specific respiratory disease.
- Clinical signs and lesions in pigs co-infected with Reston virus and PRRSV were consistent with severe, atypical PRRS (i.e., fever, respiratory distress, diarrhea, lameness, blue ears, petechiae, and elevated mortality).
- In one experimental study, 5-week-old pigs inoculated with Reston virus remained asymptomatic. However, more recently, it was shown that piglets aged three, five, or seven weeks developed respiratory distress following oronasal inoculation with Reston virus.

TREATMENT

- There is no treatment for pigs infected with Reston virus.

CLEANING AND DISINFECTION

- Filoviruses are sensitive to many ordinary disinfectants, including sodium hypochlorite.
- Physical disinfection methods include heat, UV light, and gamma irradiation.

PREVENTION AND CONTROL

- Keeping pigs indoors can reduce exposure to bats, the suspected reservoir species.
- 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.

TRANSMISSION

- In experimental studies, inoculated pigs shed Ebola virus consistently in oral and nasal secretions but shedding from the gut was sporadic. Viremia was documented occasionally.
- Inoculated pigs shed Reston virus in nasopharyngeal secretions and sometimes in rectal swabs and/or blood, but not in urine. It is not known how natural infections are acquired.

PATHOGENESIS

- In humans, Ebola virus enters through mucous membranes, breaks in the skin, or parenterally. Virus migrates to regional lymph nodes and then other organs. Hepatocellular necrosis results in coagulopathy, and combined with a release of pro-inflammatory cytokines, it leads to multiorgan failure and shock.

DIAGNOSIS

- Tests used in experimentally infected pigs (Ebola virus) include reverse transcriptase polymerase chain reaction (RT-PCR), virus isolation, immunohistochemistry, virus neutralization, and IgM and IgG enzyme-linked immunosorbent assays (ELISAs).
- Tests used for Reston virus in pigs in the Philippines include RT-PCR, panviral microarray, antigen ELISA, immunohistochemistry, and virus isolation. Quantitative RT-PCR assays were used to detect Reston virus in the spleen of infected pigs in China (with no confirmatory test).

EPIDEMIOLOGY

- Ebolaviruses occur mainly in regions of Africa. Bats are thought to be the primary reservoir hosts.
- Clinical illness has not been reported in pigs; however, serosurveys indicate pigs in some regions have been exposed to Ebola virus. The prevalence of Reston virus in pigs is unknown.

ETIOLOGY

- Ebolaviruses are single-stranded RNA viruses belonging to the family *Filoviridae*.

HISTORY IN SWINE

- Reston virus infection occurred in pigs co-infected with PRRSV in the Philippines in 2008.
- Reston virus nucleic acids were later detected in pigs on three farms in Shanghai, China, that had experienced a severe PRRS outbreak in 2008.

IMMUNITY

- There is no available information on post-infection immunity in pigs; there are no Reston virus vaccines.

GAPS IN PREPAREDNESS

- Natural swine infection has been documented only for Reston virus, and current prevalence is unknown.
- Humans can be asymptomatically infected with Reston virus; genetic changes and interspecies transmission could lead to increased viral pathogenicity in the future.
- Recent evidence suggests that Reston virus should be considered a livestock pathogen with unknown zoonotic potential. Additionally, potential routes of transboundary spread should be explored.

LITERATURE REVIEW: EBOLAVIRUSES

IMPORTANCE

There are six known ebolaviruses; four cause Ebola virus disease (EVD) in people and nonhuman primates and occur mainly in Sub-Saharan Africa. They are collectively known as African ebolaviruses. Reston virus is the only known ebolavirus that occurs in Asia. In the Philippines, outbreaks have been documented in nonhuman primates (1998-1990, 1992-1993, 1996,[1] and 2015[2]) and pigs (2008-2009).[1] Reston virus has also been detected in pigs in China.[3] Reston virus is not known to cause disease in humans; however, concern about spillover events and changes in pathogenicity remain.[4]

TAXONOMY

Ebolaviruses (the collective term for species in the genus *Ebolavirus*) are single-stranded RNA viruses in the family *Filoviridae*. As of 2020, there are six species in the genus *Ebolavirus*.[5] Species names and common names, in parentheses, are as follows: *Bombali ebolavirus* (Bombali virus), *Bundibugyo ebolavirus* (Bundibugyo virus), *Reston ebolavirus* (Reston virus), *Sudan ebolavirus* (Sudan virus), *Taï Forest ebolavirus* (Taï Forest virus, formerly *Cote d'Ivoire ebolavirus*), and *Zaire ebolavirus* (Ebola virus). Herein, "Ebola virus" refers specifically to the species *Zaire ebolavirus*.

PUBLIC HEALTH

African Ebolaviruses

Three of six ebolavirus species (Ebola virus, Sudan virus, and Bundibugyo virus) cause EVD, a severe and life-threatening illness in humans. Signs of EVD include fever, headache, muscle and joint pain, fatigue, sore throat, loss of appetite, abdominal pain, diarrhea, vomiting, and unexplained hemorrhaging, bleeding, or bruising.[6] Case fatality rates can reach up to 90%.[7] Patients who survive can develop post-Ebola virus disease syndrome, presenting with a number of chronic conditions that affect the quality of life.[8]

Ebola virus, discovered in 1976, is the cause of most human outbreaks. The 2013–2016 West African epidemic was the largest in recent history, resulting in more than 11,000 deaths.[7, 9] Eleven people were treated for EVD in the United States. A nurse who cared for a sick patient contracted EVD, the first known transmission on U.S. soil.[10] Sporadic EVD outbreaks continue to occur in Africa. Taï Forest virus has caused only one case of human infection.[7, 11] It is unclear whether Bombali virus causes disease humans.[12, 13]

Whether ebolaviruses are a food safety risk is uncertain. Ebola virus has been occasionally recovered from porcine blood and heart, and viral RNA has been detected in skeletal muscle, liver, and intestines.[14-16] Epidemiological evidence suggests that direct exposure to infected animal carcasses (i.e., bushmeat) is a significant risk factor.[17]

Reston Virus

Reston virus does not cause illness in humans. Antibodies have been detected in a few people who worked with infected nonhuman primates in the United States or the Philippines or with pigs in the Philippines; however, none developed signs of disease.[18-21] Seropositivity estimates include:

- 1% of animal handlers, trappers, and administrative personnel working at nonhuman primate export facilities in the Philippines (1990s)[1]
- 4% of people during the outbreak among pigs in 2008[20]
- 2% of people potentially exposed to Reston virus in the various outbreaks[17]

In experimentally infected pigs, skeletal muscle, blood, heart, liver, kidneys, and intestines (e.g., sausage casings) have been shown to contain Reston virus nucleic acids, and the virus has been isolated from tissues, including skeletal muscle.[22]

A risk assessment found that human Reston virus infection remains likely in the Philippines since the virus has been detected in both nonhuman primates and pigs and contact with these animals is common.[4] As long as Reston virus remains non-pathogenic in humans, the consequences of infection are minor; however, genetic changes and interspecies transmission could result in a more virulent virus.[4, 23]

A study of ebolaviruses adapted to guinea pigs and mice found that very few mutations were required for Ebola virus to become pathogenic in a novel host.[24] In particular, mutations of VP24, which plays a role in nucleocapsid assembly, virus release, and immune response suppression,[8] seem to be vital for adaptation.[24] Reston virus isolates from different swine farms in the Philippines demonstrated up to a 4.5% divergence in one year, and they had a higher evolution rate (nucleotide substitutions/site/year) than Sudan virus.[23]

INFECTION IN SWINE

African Ebolaviruses

At present, no published reports describe illness attributed to natural Ebola virus infection in pigs. However, several recent serosurveys have found evidence of exposure to African ebolaviruses in swine (see *Morbidity and Mortality*). Several Ebola virus infection studies in pigs have been described.

- In a study of 5–6-week-old pigs inoculated with Ebola virus, animals developed non-specific respiratory disease (fever, anorexia, lethargy, increased respiratory rate, and labored breathing).[14] Bronchointerstitial pneumonia was observed, with progressive and sometimes extensive consolidation of the lungs, mainly in the dorsocaudal lobes.[14, 15] Some pigs had hemorrhages in the lungs and/or inflammatory exudates in the trachea.[15] 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.[14, 15] The lung-associated lymph nodes were enlarged and occasionally mildly hemorrhagic.[14]

- In two studies of 3–4-week-old pigs inoculated with Ebola virus, animals developed transient or delayed fever and an increased respiratory rate.[14, 16] In one study, animals recovered by nine days post-infection (dpi).[16] A pig analyzed seven days after inoculation was reported to have macroscopic and microscopic lung lesions similarto those in older pigs but not as severe.[14] In the second study, no significant gross lesions were noted, and microscopic lesions were limited to focal (not extensive) bronchointerstitial pneumonia with a lobular pattern.[16]

Reston Virus

Clinical signs and lesions in pigs co-infected with Reston virus and PRRS virus (PRRSV) were consistent with severe, atypical PRRS in both the Philippines and China.[3, 20] The mains signs associated with atypical PRRS are high fever, respiratory distress, diarrhea, lameness, blue ears, petechiae, and significantly elevated mortality in gilts and sows.[25]

In all reported cases, pigs have been co-infected with Reston virus and either PRRSV or porcine circovirus type 2, making it difficult to determine the contribution of Reston virus (if any) to these outbreaks.[22] In the Philippines, both Reston virus and PRRSV antigens were found in areas of interstitial pneumonia in the lungs; however, Reston virus antigens were detected in lymphoid tissues with minimal necrosis, while PRRSV antigens occurred in lymphoid follicles with focal necrosis.[20]

In one experimental study, 5-week-old pigs inoculated subcutaneously or oronasally with Reston virus (Philippine pig isolate) remained asymptomatic, although lymphadenomegaly of the submandibular lymph nodes and mild acute rhinitis were noted at necropsy.[22] Despite the absence of respiratory signs, some pigs had areas of consolidation in the lungs (apical and cardiac lobes or hilus), which may or may not have been caused by Reston virus. Lymph node lesions were confirmed as reactive hyperplasia by histopathology. In addition to rhinitis, pigs developed focal necrosis of the tonsillar epithelium associated with neutrophil infiltrates (without evidence of

Reston virus antigens in the tonsillar lesions).[22] No gross or microscopic lesions were found in the spleen, liver, kidney, heart, intestines, or brain.[22]

More recently, the pathogenicity of Reston virus has been confirmed in domestic piglets. Haddock and colleagues[26] inoculated three groups of pigs (aged three, five, or seven weeks) with a Reston virus isolated from a Philippine pig in 2008. Following oropharyngeal and nasal exposure, anorexia began at three dpi. By six dpi, all animals developed respiratory distress (tachypnea, dyspnea with abdominal pumping, central cyanosis). Serous nasal discharge and cough were also noted in some animals. Five animals were euthanized for significant breathing difficulties at seven dpi; those that survived recovered quickly. Organ tropism, pathology, and pathophysiology were similar to a previous study.[22] Reston virus-infected pigs developed interstitial pneumonia and enlarged and edematous mediastinal lymph nodes.[26]

TREATMENT

There is no treatment for pigs infected with Reston virus.

CLEANING AND DISINFECTION

SURVIVAL

Filoviruses are relatively stable when suspended in liquid media, even at room temperature. Filoviruses may also remain infectious for a time after drying. Collectively, data suggest that filoviruses could remain infectious on fomites long enough to infect susceptible species, especially if the initial amount of virus is high.[27]

No infectious virus could be recovered from wildlife carcasses in Africa after 3–4 days in tropical forest conditions.[28] In aerosolized tissue culture medium kept in the dark, Reston virus appears to decay significantly more slowly than Ebola virus or Marburg virus.[27]

Refrigeration and freezing are likely to prolong the survival of ebolaviruses in meat or other tissues.[17, 27, 29] These viruses also survived freezing and thawing.[30] 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.[31] Thorough cooking to 100°C is expected to destroy ebolaviruses rapidly.[31]

DISINFECTION

Filoviruses are sensitive to many ordinary disinfectants.[18, 19] Sodium hypochlorite is commonly recommended. According to the World Health Organization (WHO), household bleach diluted at 1:100 is adequate for ordinary disinfection (e.g., gloved hands, boots, equipment such as thermometers, and spills), but 1:10 is needed for disinfecting urine and feces.[32]

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.[33] Calcium hypochlorite (bleach powder), at concentrations of 0.02% to 2%, is another acceptable disinfectant.[34] Experimentally, filoviruses are also inactivated by UV light[29,35] and Gamma irradiation.[36, 37]

A list of EPA-registered antimicrobial products that meet the Centers for Disease Control and Prevention (CDC) criteria for use against Ebola virus is maintained online.[38] Ebolavirus-contaminated disposable materials should be discarded, placed in leak-proof containers, and incinerated or autoclaved. In endemic areas of Africa, boiling heat-resistant items for 20 minutes has been recommended to kill filoviruses if autoclaving is not available.[32] Human remains should be cremated or buried in a sealed casket, with minimal handling.[39]

PREVENTION AND CONTROL

DISEASE REPORTING

Ebolavirus infection is not an OIE-listed disease. However, infections should be reported to the OIE as an

emerging disease if detected (under Article 1.1.4 of the *Terrestrial Animal Health Code*). There are no restrictions for the importation of animals from countries or zones affected by ebolaviruses. Similarly, Ebola virus and Reston virus are not notifiable to the U.S. Animal and Plant Health Inspection Service (APHIS). However, any suspicious clinical or necropsy findings should always be reported to the USDA and your State Animal Health Official.

DISEASE PREVENTION

Bats are the most likely reservoir hosts for filoviruses,[40-52] although other susceptible hosts, including people, can transmit these viruses once infected. Indoor housing is probably the most effective measure for protecting pigs, though other methods to prevent bat contact (e.g., wire screens to prevent entry into open-sided pig sheds, removal of fruit trees that may attract bats) may also have some benefit. Biosecurity plans should consider transmission via infected humans and fomites. Limited evidence suggests that rodents do not play a role in filovirus transmission.[1, 53-55]

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, where infectious virus has been found in humans up to three months post-recovery.[56-60] Reston virus in pigs seems to disappear from tissues by one month.[22] Nevertheless, information about infections in pigs is still limited, and the potential for prolonged persistence should be considered.

DISEASE CONTROL

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

TRANSMISSION

African Ebolaviruses

In humans, transmission of ebolaviruses probably occurs through direct or indirect contact with infected animals or bushmeat consumption.[61, 62] Secondary human-to-human transmission is due to contact with infectious blood, secretions, or other body fluids.[61] How bats transmit filoviruses to each other or other animals is uncertain.[63] Virus titers usually appear to be very low in wild infected bats, and nucleic acids have only been detected by nested RT-PCR.[40, 64]

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.[14-16]

- Infectious virus and viral RNA were found in oral and nasal secretions, but viral shedding from the gut was sporadic and inconsistent, and viremia 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.[14] Ebola virus shedding in the semen or milk of pigs was not tested.

The dynamics of virus recovery and nucleic acid detection suggest a replication and transmission cycle of approximately five days.[14] While these experiments demonstrate that pig-to-pig transmission of Ebola virus is possible, it is not known whether ebolavirus transmission can be sustained in swine.[65]

Transmission from pigs to primates was demonstrated when six 4-week-old pigs inoculated oronasally with Ebola virus transmitted the virus to four cynomolgus macaques.[16] Lesions and patterns of viral antigens in the lungs of macaques suggested that the virus was transmitted by inhalation and blood.[16]

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.[3, 20] Viruses isolated from pigs in the Philippines differed by approximately 4% in nucleotide sequence, suggesting that either there was more than one spillover event from

another reservoir host or that pigs have maintained these viruses for many years.[20, 22]

A Reston virus isolated from pigs in the Philippines replicated in 5-week-old pigs inoculated subcutaneously or oronasally but did not cause clinical signs.[22]

- Most oronasally inoculated pigs shed the virus in nasopharyngeal secretions, with peak titers occurring at six dpi. Reston virus was only detected in the nasopharyngeal secretions of subcutaneously inoculated pigs in the second of two trials.
- Nucleic acids were found in rectal swabs and blood from oronasally or subcutaneously inoculated pigs in the second trial, but not the first.
- No virus was found in urine collected from the floor of the pen.
- During the acute stage of illness, nucleic acids were widely distributed in the organs and tissues of pigs inoculated by either route (lungs, kidneys, and ileum). No nucleic acids were found in tissue samples collected during necropsy (28 dpi) in the first trial, suggesting that persistent infections do not occur.
- Shedding in semen or milk was not tested.

PATHOGENESIS

According to the CDC, Ebola virus can be acquired through mucous membranes, breaks in the skin, or parenterally and infects many cell types, including monocytes, macrophages, dendritic cells, endothelial cells, fibroblasts, hepatocytes, adrenal cortical cells, and epithelial cells.[6]

The incubation period ranges from 6–10 days, depending on the exposure route. From the initial infection site, Ebola virus migrates to regional lymph nodes and subsequently the liver, spleen, and adrenal gland. Hepatocellular necrosis impacts clotting factor regulation and results in subsequent coagulopathy. Adrenal necrosis is associated with hypotension and impaired steroid production. A release of pro-inflammatory cytokines causes vascular leakage, and along with impaired clotting, leads to multiorgan failure and shock.[6]

DIAGNOSIS

Although filovirus isolation requires high biocontainment (BSL-4), most diagnostic assays can be conducted on samples containing inactivated virus.[66] Field diagnostic laboratories have been used during some outbreaks in humans. The CDC provides guidance for U.S. laboratories when there is a concern about EVD in human clinical specimens.[6]

Standard, contact, and droplet precautions have been recommended during contact with human filovirus-infected patients.[67] Precautions needed for collecting samples from livestock are not clear. Necropsies are considered high-risk procedures, and specific advice should be obtained from health departments and the CDC.[39]

Tests used to detect Reston virus and Ebola virus in pigs have been developed mainly for research purposes.[3, 14-16, 20, 22] While these assays may be helpful for routine diagnosis in pigs, they have not been validated for this purpose. In some cases, improved reagents may be needed for diagnostic assays (e.g., monoclonal antibodies in antigen detection tests).[65]

TESTS TO DETECT NUCLEIC ACIDS, VIRUS, OR ANTIGENS

African Ebolaviruses

Isolation of filoviruses can only be performed at a few laboratories that have biocontainment facilities capable of handling dangerous human pathogens.[43] Virus is most often isolated in Vero or MA-104 cells.[19, 68] In humans, virus isolation is used as a confirmatory test for a positive result from reverse transcriptase polymerase chain reaction (RT-PCR) assays or antigen detection enzyme-linked immunosorbent assays (ELISA).[43]

RT-PCR, quantitative RT-PCR (qRT-PCR), and reverse transcriptase loop–mediated isothermal amplification (RT-LAMP) have been used to detect viral RNA in humans. Microarray assays that can detect filovirus nucleic

acids have also been developed, but they are not used routinely for diagnosis in humans.[43]

Antigen detection ELISAs are one of the primary tests (with RT-PCR) used to diagnose clinical cases in humans.[68] They are also used as an independent confirmatory test for a positive RT-PCR assay. Tests use either hyperimmune serum or antibodies specific to a filoviral protein such as the NP.[43]

Electron microscopy can detect filovirus particles, which are distinctive in appearance, in tissues or blood.[18, 43, 68] However, factors such as equipment availability and the inability to reliably distinguish different filoviruses limit its use. Filovirus antigens can also be detected in tissues by immunofluorescence or immunohistochemistry.[18] Ebola virus antigens in experimentally infected pigs were detected by immunohistochemistry, with rabbit polyclonal antibody targeting the Ebola virus VP40 protein. [14, 15]

Immunoelectron microscopy was used during some outbreaks in humans, as well as in nonhuman primates.[18] In situ hybridization can also detect filovirus nucleic acids; however, this test has been used mainly in research.[69]

Reston Virus

Reston virus has been reported to replicate less efficiently in Vero cells than Ebola virus.[70] However, virus isolation in Vero cells was one of the confirmatory tests during the PRRS/Reston virus outbreak among pigs in the Philippines.[20] Vero cells have also been used to detect Reston virus in experimentally infected pigs[22] and to reisolate Ebola virus from experimentally infected pigs.[14, 15]A swine kidney cell line (PK-15) is being evaluated as a possible alternative to Vero cells for virus isolation from pigs.[65]

Ebola virus nucleic acids have been detected in experimentally infected pigs with qRT-PCR assays targeting either the Ebola virus *L* gene, *GP* gene, or *NP* gene.[14-16, 65]A panviral microarray assay was used to detect Reston virus during the initial outbreak among pigs in the Philippines.[20]

Two of the confirmatory tests used to identify Reston viruses in naturally infected pigs were antigen detection ELISA and immunohistochemistry.[20] 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.[22]

Electron microscopy was described during cell culture of Reston virus from naturally infected pigs,[20] but there are no reports of its use for direct examination of porcine tissues or blood. There is no information about the suitability of rapid tests in development for humans[68, 71] being used in pigs.

TESTS TO DETECT ANTIBODY

Antibodies to ebolaviruses are cross-reactive.[72] The most likely causative agent can be distinguished with comparative serological assays against a panel of different ebolaviruses.[73, 74]

African Ebolaviruses

In humans, IgM and IgG ELISAs may be used for some clinical specimens and to monitor the immune response in confirmed EVD-patients.[6]

In pigs experimentally infected with Ebola virus, tests to detect antibodies have been described, including IgM capture ELISA using cell lysate antigen,[15, 65] IgG ELISA using gamma-irradiated, sucrose gradient purified whole Ebola virus.[14] and CPE-reduction virus neutralization assays (Vero E6 cells).[65] Furthermore, Pickering and colleagues validated three assays for detection of Ebola virus antibodies in experimentally infected swine, including a microtitre immunostained plaque reduction neutralization test (miPRNT) and an indirect IgG ELISA (antigen Ebola virus NP).[75] An immunoblot assay was used to confirm indirect ELISA results.[75]

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 ELISA based on recombinant viral GP or NP.[76] A high seroprevalence rate was detected among pigs in infected regions, but not in an uninfected area or outside the Philippines (i.e., Japan).

During a study of experimental Reston virus infections in pigs, antibodies were detected with an indirect ELISA targeting viral NP (based on a recombinant NP expressed in *Escherichia coli*).[22] 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 six and eight, and all pigs seroconverting by day 10. Pigs inoculated subcutaneously had higher antibody titers.

SAMPLES

African Ebolaviruses

In experimentally infected pigs, Ebola virus has been detected regularly in nasal and oral swabs, sporadically from rectal swabs, and occasionally in blood.[14-16] Tissues that contain viral RNA included lungs and submandibular and bronchial lymph nodes, and sometimes the liver, spleen, mesenteric lymph nodes, heart, muscle, and gut.[14-16]

Reston Virus

During the outbreak in the Philippines, Reston virus RNA was detected in samples from the lung, spleen, and lymph node, but not the liver, by RT-PCR.[20] Virus was isolated from the lungs and lymph nodes, and viral antigens were found in the lungs, lymphoid tissues and lymph nodes of pigs. Reston virus nucleic acids were detected in the spleen in China, but whether any other samples were collected or tested is unclear.[3]

Experimentally, Reston virus nucleic acids have been detected in nasopharyngeal secretions, nasal and oral swabs, and—inconsistently—blood and rectal swabs. Additionally, tissues/organs containing viral RNA included lung, heart, liver, kidney, spleen, ileum, superficial lymph nodes (submandibular), nasal turbinates, tonsil, and skeletal muscle. The use of oral fluids as a diagnostic specimen has not been evaluated for any ebolavirus.

EPIDEMIOLOGY

SPECIES AFFECTED

African Ebolaviruses

Current evidence suggests that bats are the primary reservoir hosts for ebolaviruses.[40-52] African ebolaviruses cause illness in humans and nonhuman primates. Pigs can be infected experimentally with Ebola virus, shed the virus, and develop clinical signs.[14-16] While no experiments with Sudan virus, Taï Forest virus, or Bundibugyo virus have been published in pigs, they might be susceptible to these viruses as well.

The susceptibility and exposure of other potential ebolavirus hosts have been investigated (invertebrates, birds, monkeys, rodents, other small mammals) but have revealed surprisingly little (as described by Caron and colleagues).[63] Ebola virus antibodies have been detected in dogs.[77-79] Although the role of pigs in ebolavirus epidemiology remains unclear, factors that could facilitate zoonotic transmission have been suggested in Uganda,[80] including:

- Lack of serological evidence for presumed reservoir species (bats)
- Number of human cases unable to account for their source of infection
- Domestic pig habitat overlap with potential ebolavirus host environments
- Interactions at the human-pig-wildlife interface (e.g., food competition at fruit trees)
- High incidence of reported fever in pigs (i.e., possibility of undetected infections)
- Temporal correlation between ebolavirus outbreaks and peak pork consumption periods (including increased handling, butchering, and transport of pigs)

Reston Virus

Fruit bats are suspected to be a reservoir host for Reston virus in the Philippines. Molecular and serologic evidence of Reston virus infection has been confirmed in multiple bat species.[81] Interestingly, a recent study failed to detect antibodies to Reston virus in *Rousettus amplexicaudatus*,[81] which had previously been described.[42]

In nature, Reston virus has been detected only in cynomolgus macaques, which become ill, and domesticated pigs co-infected with PRRSV.[3, 20-22, 76] Antibodies have been detected in humans, but they do not become ill (see *Public Health*). Experimentally, ferrets have been used as a small animal model to assess Reston virus pathogenicity.[82]

GEOGRAPHIC DISTRIBUTION

African Ebolaviruses

Human outbreaks of EVD have originated in the Democratic Republic of the Congo (DRC), the Republic of the Congo, Gabon, and Guinea, although the movements of infected people can disperse the virus to additional regions.[44, 83] During the 2013–2016 West African epidemic, disease spread from Guinea to Liberia and Sierra Leone. Travel also resulted in cases in Mali, Nigeria, Senegal, Italy, the United Kingdom, and the United States.[6]

Human outbreaks caused by Sudan virus have been reported in Sudan and Uganda.[44] All known outbreaks have occurred within 400 miles ofeach other, and the range of this virus may be limited.[83] Taï 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[44] or two[66] 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.[44, 83]

Bat species shown to be infected with Ebola virus in the wild[40, 45] have broad geographic ranges that include the entire tropical forest regions of equatorial central Africa.[84]

A swine serosurvey from Uganda found that pigs sampled in June were more likely to have antibodies to ebolaviruses compared to pigs sampled in October.[85] This time period corresponded to the beginning of the dry period in Uganda, and other studies have suggested that spillover to humans[86, 87] and great apes[28] is also more likely to occur at this time.

Reston Virus

In 2008, Reston virus was found in domesticated pigs in the Philippines.[20] Reston virus nucleic acids were later detected in pigs on three farms in Shanghai, China, in 2008 (see *History in Swine*).[3]

MORBIDITY AND MORTALITY

Illness or death due to natural Ebola virus infection has not been reported in pigs. Ebolavirus exposure has been assessed in several studies; however, the current prevalence of Reston virus in pigs is not known.

- During the Philippines outbreak, antibodies to Reston virus were found in 71% to 79% of pigs on infected farms using an immunofluorescence assay (IFA). Prevalence was estimated to be 67–90% via ELISA. An earlier study found no antibodies to Reston virus in pigs that were acutely co-infected with this virus and PRRSV, despite the detection of antibodies to PRRSV.[20]
- In Uganda, 7% of pigs (46/658) at slaughter had antibodies to the NP from either Sudan virus, Ebola virus, or both when tested at two different institutions.[85] Additionally, sera from four of the ELISA-positive pigs reacted in Western blot, and one sample had fully neutralizing antibody to Sudan virus via virus neutralization.[85]
- A study of domestic pigs in Sierra Leone found that only 0.75% (3/400) were clearly positive using an indirect ELISA detecting antibodies to Ebola virus NP.[88] These samples were confirmed as positive via

immunoblot, reacting with Ebola virus NP; however, none of the samples reacted with Ebola virus GP or contained neutralizing antibodies. The authors speculated that pigs may have had contact with an antigenically related ebolavirus and that serological cross-reactivity occured.[88] A similar phenomenon is suspected in humans, where unknown filoviruses may account for serological cross-reactivity against known ebolaviruses in areas not affected by EVD (as described by Fischer et al.[89]).

- A related study of pigs in Guinea revealed that 4.5% (14/308) were seropositive (via Ebola virus NP ELISA) and also reacted with ebolavirus NP antigen using Western blot.[89] Again, cross-reactivity between ebolaviruses was suspected in serum.[89] Most reactive samples were from a rural site surrounded by mango trees, although no bats were observed during sampling.[89]

ETIOLOGY

CHARACTERISTICS OF FILOVIRUSES

Filoviruses are filamentous, enveloped, pleomorphic RNA viruses, which can be rod- or ring-like, crook-shaped (or shaped like a "6"), or branched.[5] Filoviruses have a single-stranded, negative-sense RNA genome. As of 2020, the family *Filoviridae* contains six genera (*Cuevavirus, Dianlovirus, Ebolavirus, Marburgvirus, Striavirus,* and *Thamnovirus*) and 11 species.[5]

CHARACTERISTICS OF EBOLAVIRUSES

There are currently six species belonging to the genus *Ebolavirus*: *Bombali ebolavirus* (Bombali virus), *Bundibugyo ebolavirus* (Bundibugyo virus), *Reston ebolavirus* (Reston virus), *Sudan ebolavirus* (Sudan virus), *Taï Forest ebolavirus* (Taï Forest virus, formerly *Cote d'Ivoire ebolavirus*), and *Zaire ebolavirus* (Ebola virus).[5]

Important ebolavirus proteins include the RNP complex, which consists of NP (nucleoprotein), VP24 (complex-associated protein), VP35 (polymerase cofactor), VP30 (transcriptional activator), and L (large protein). The RNP complex associates with VP40 (matrix protein), which lines the inner side of the virion and GP (glycoprotein), which forms spikes on the outer side of the virion. Three soluble glycoprotein peptides are also expressed from the *GP* gene: sGP, ssGP, and Δ-peptide.[5, 90] Key functions of ebolavirus proteins are described by Cantoni and colleagues.[91]

The genomes of different ebolavirus species can differ from each other by more than 23%.[5, 92] Reston virus most likely originated in Africa and diverged from Sudan virus about 1500 years ago.[91] Full-length Reston virus genomes from primates, pigs, and a human are generally quite similar.[90, 91] The highest degree of diversity occurs in the *GP* gene (< 10%). However, phylogenetic analysis shows that Reston virus isolates can be distributed into five distinct lineages. Swine isolates from three different farms in the 2008–2009 outbreak in the Philippines were assigned to three different lineages in a recent study.[90]

HISTORY IN SWINE

In 2008, Reston virus was found in domesticated pigs in the Philippines during an investigation of a disease outbreak caused by an atypical PRRSV.[20] Pigs on farms in the outbreak area had antibodies to Reston virus, but 98 pigs from an unaffected region were seronegative.[76] Whether pigs in other parts of the Philippines are free of Reston virus remains to be determined by additional surveys.[76] Reston virus nucleic acids were later detected in pigs on three farms in Shanghai, China, that had experienced a severe PRRS outbreak in 2008.[3] There was no link between the Chinese farms and the Philippines, suggesting that the virus had been acquired locally.

IMMUNITY

POST-EXPOSURE

In 5–6-week-old pigs experimentally infected with Ebola virus, neither IgG antibody titers (by ELISA) nor neutralizing antibodies were detected by day seven when the pigs were euthanized.[14] IgM titers toEbola virus could be found by ELISA on day 5/6 in a second study that used pigs of the same age.[15]

In 3–4-week-old pigs experimentally infected with Ebola virus, IgG antibody titers (by ELISA) and neutralizing antibodies were measured during acontact transmission experiment.[14] 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.

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 10–12 years after infection.[93, 94]

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.[66]A number of Ebola virus vaccines, based on a wide variety of platforms, have been tested in laboratory rodents and nonhuman primates,[94] including standard inactivated vaccines[18] and classical subunit vaccines,[95] which have not been developed further. Vaccines that have shown some promise include:

- Virus-like particles consisting of the Ebola virus VP40, glycoprotein, and sometimes the NP, together with an adjuvant,
- Viral vectored vaccines that express genes encoding Ebola virus proteins, and
- DNA vaccines combined with viral vectored vaccines.[94]

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.[94] 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.[66]

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) GP were protected against challenge with Bundibugyo virus.[96]

GAPS IN PREPAREDNESS

Overall, little is known about ebolavirus infection in pigs. Natural infection has been documented only for Reston virus, and the current prevalence is unknown. Humans can also be infected with Reston virus. Currently, illness does not seem to occur, but future viral mutations could impact pathogenicity. Recent evidence suggests that Reston virus should be considered a livestock pathogen with unknown zoonotic potential.[26] Additionally, potential routes of transboundary spread should be explored.[26]

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