EBOLA AND RESTON VIRUS

Swine Health Information Center

The Swine Health Information Center, launched in 2015 with Pork Checkoff funding, protects and enhances the health of the United States swine herd by minimizing the impact of emerging disease threats through preparedness, coordinated communications, global disease monitoring, analysis of swine health data, and targeted research investments.

September 2015 | Updated June 2024

SUMMARY

IMPORTANCE

- Ebola virus is one of three orthoebolaviruses that cause hemorrhagic fever in humans, mainly in Sub-Saharan Africa.
- Reston virus is the only known orthoebolavirus that causes clinical disease in pigs. It has been detected in the Philippines and China in pigs co-infected with porcine reproductive and respiratory syndrome (PRRS) virus. Reston virus does not cause disease in humans.

TAXONOMY

- Ebola virus (*Orthoebolavirus zairense)* and Reston virus (*Orthoebolavirus restonense)* are filoviruses in the genus *Orthoebolavirus*.
- There are four additional species in the genus: Bundibugyo virus (*Orthoebolavirus bundibugyoense*), Sudan virus (*Orthoebolavirus sudanese*), Taï Forest virus (*Orthoebolavirus taiense*), and Bombali virus (*Orthoebolavirus bombaliense)*.

PUBLIC HEALTH

- Ebola virus, which causes death in up to 90% of cases, is the cause of most human outbreaks. Sudan virus and Bundibugyo virus also cause hemorrhagic fever but with reduced mortality.
- Antibodies to Reston virus have been detected in humans, but there are no reports of clinical illness.
- Pigs can be naturally infected with Reston virus. Meat from sick or dead animals should not be consumed, but pork from healthy pigs is safe to eat when cooked properly.

INFECTION IN SWINE

- Natural Ebola virus infection has not been described in pigs. In experimental studies, respiratory disease varied from mild to severe.
- Pigs infected with Reston virus experimentally can remain asymptomatic or develop serious respiratory illness.
- Clinical signs and lesions in pigs co-infected with Reston virus and PRRS virus were consistent with severe, atypical PRRS.

TREATMENT

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

CLEANING AND DISINFECTION

- Filoviruses are sensitive to many ordinary disinfectants, including sodium hypochlorite.

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 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 to detect Ebola virus or antibodies in experimentally infected pigs include reverse transcriptase polymerase chain reaction (RT-PCR), virus isolation, immunohistochemistry, virus neutralization, and IgM and IgG enzyme-linked immunosorbent assays (ELISAs).
- Tests to detect Reston virus in pigs in the Philippines included 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 (no confirmatory test).

EPIDEMIOLOGY

- Ebola virus occurs in Sub-Saharan Africa. Bats are thought to be the primary reservoir hosts. Clinical illness has not been reported in pigs; however, serosurveys indicate pigs in some parts of Africa have been exposed to Ebola virus.
- Reston virus occurs in Asia. The prevalence of Reston virus in pigs can be very high in affected areas.

ETIOLOGY

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

HISTORY IN SWINE

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

IMMUNITY

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

GAPS IN PREPAREDNESS

- Overall, little is known about the possible role of pigs in Ebola virus and Reston virus transmission. Potential routes of transboundary spread should be explored.

- Reston virus should be considered a livestock pathogen with zoonotic potential. Genomic mutations could impact morbidity and mortality in pigs and the likelihood of human-to-human transmission in the future.

LITERATURE REVIEW: EBOLA AND RESTON VIRUS

IMPORTANCE

There are six known orthoebolaviruses. Three cause severe disease in people and nonhuman primates, mainly in Sub-Saharan Africa. Serology shows that pigs can mount an antibody response to Ebola virus, but clinical disease has not been detected in naturally infected swine.

Reston virus is the only known orthoebolavirus 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 does not cause disease in humans; however, concern about spillover events and changes in pathogenicity remain.[4]

TAXONOMY

Orthoebolaviruses are single-stranded RNA viruses in the family *Filoviridae*. As of 2024, there are six species in the genus *Orthoebolavirus*.[5] Those that cause illness in humans include *Orthoebolavirus zairense* (Ebola virus), *Orthoebolavirus bundibugyoense* (Bundibugyo virus), *Orthoebolavirus sudanese* (Sudan virus), and *Orthoebolavirus taiense* (Taï Forest virus). *Orthoebolavirus restonense* (Reston virus) infects nonhuman primates and pigs, and *Orthoebolavirus bombaliense* (Bombali virus) has been identified in bats.

Herein, "Ebola virus" refers specifically to the species *Orthoebolavirus zairense*.

PUBLIC HEALTH

EBOLA VIRUS

Ebola virus, discovered in 1976, is the cause of most human outbreaks. Signs 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 90%.[7] People who survive can develop post-Ebola virus disease syndrome, presenting with chronic conditions that affect the quality of life.[8] The 2013–2016 West African Ebola epidemic was the largest in recent history, resulting in more than 28,000 cases and 11,000 deaths.[7,9] Eleven people were treated for Ebola virus infection in the United States. A nurse who cared for a sick patient also contracted Ebola virus, the first known transmission on U.S. soil.[10] Sporadic Ebola outbreaks continue to occur in Africa.

Two additional orthoebolaviruses, Sudan virus and Bundibugyo virus, also cause hemorrhagic fever but with reduced mortality. Taï Forest virus has caused only one case of human infection.[7,11] It is unclear whether Bombali virus causes disease in humans.[12,13]

Whether orthoebolaviruses are a food safety risk is uncertain. Ebola virus has been 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 bushmeat is a significant risk factor.[17] Fear of Ebola has been linked to reduced consumption of pork and cost per kilogram in Africa.[102] Meat from sick or dead animals should not be consumed, but pork from healthy pigs is safe to eat when cooked properly.

RESTON VIRUS

Reston virus does not cause illness in humans, but antibodies have been detected in people who worked with infected nonhuman primates or pigs.[18-21] Seropositivity estimates include the following:

- 1% of animal handlers, trappers, and administrative personnel working at nonhuman primate export facilities in the Philippines in the 1990s[1]

- 4% of people that worked with pigs during the 2008 outbreak in the Philippines[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 have been shown to contain Reston virus RNA, and virus has been recovered from tissues including skeletal muscle.[22]

A risk assessment found that human Reston virus infections will likely continue in the Philippines, since the virus occurs in 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]

INFECTION IN SWINE

EBOLA VIRUS

At present, no published reports describe illness attributed to natural Ebola virus infection in pigs. However, recent serosurveys have found evidence of exposure to African orthoebolaviruses in swine (see *Morbidity and Mortality*). Experimental studies on infection in pigs include the following:

- Five to 6-week-old pigs inoculated with Ebola virus developed 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 inflammatory exudates in the trachea.[15] Histopathologic lesions in the lungs were 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 mildly hemorrhagic.[14]

- Three to 4-week-old pigs inoculated with Ebola virus developed transient or delayed fever and an increased respiratory rate.[14,16] In one study, animals recovered by 9 days post-infection (dpi).[16] At 7 dpi, one pig had macroscopic and microscopic lung lesions similar to those in older pigs but not as severe.[14] In another study, no significant gross lesions were noted, and microscopic lesions were limited to focal bronchointerstitial pneumonia with a lobular pattern.[16]

- Seven-week-old pigs oronasally inoculated with Bundibugyo virus did not become clinically ill, except for mild fever in two animals at 1 dpi.[24] Moderate interstitial pneumonia with generalized lymphadenopathy was observed grossly. Histopathological lesions in the lungs included perivasculitis, peribronchiolitis, and interlobular edema.[24]

RESTON VIRUS

Pigs are commonly co-infected with Reston virus and either PRRS virus or porcine circovirus type 2, making it difficult to determine the contribution of Reston virus to outbreaks.[22] In co-infected pigs, clinical signs and lesions are consistent with severe, atypical PRRS,[3,20] including fever, respiratory distress, diarrhea, lameness, blue ears, petechiae, and significantly elevated mortality in gilts and sows.[25] Both Reston virus and PRRS antigens have been found in areas of interstitial pneumonia in the lungs.[20]

Experimental studies on Reston virus infection in pigs include the following:

- Five-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). 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]

- Haddock and colleagues[26] inoculated three groups of pigs (aged three, five, or seven weeks) with a Reston virus isolated from a Philippine pig. Following oropharyngeal and nasal exposure, anorexia began at three dpi. By 6 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 7 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]

- Six-week-old pigs were inoculated oronasally with a nonhuman primate-adapted Reston virus isolate.[27] Most animals remained asymptomatic, while four became febrile, and one developed severe respiratory distress at 7 dpi. Grossly, interstitial pneumonia, pulmonary edema, and consolidation were seen in the lungs, as well as mild lymphadenopathy.[27] Histopathological lesions were similar to those previously described.

TREATMENT

Experimentally, prophylactic treatment with porcine type I IFN (IFNα) prevented clinical illness and viral shedding in pigs inoculated with Ebola virus. Tissue viral load was also reduced.[28]

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.[29]

No infectious virus could be recovered from wildlife carcasses in Africa after three to four days in tropical forest conditions.[30] In aerosolized tissue culture medium kept in the dark, Reston virus appears to decay significantly more slowly than Ebola virus or Marburg virus.[29]

Refrigeration and freezing are likely to prolong the survival of orthoebolaviruses in meat or other tissues.[17,29,31] These viruses also survived freezing and thawing.[32] 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.[33] Thorough cooking to 100°C is expected to destroy Ebola virus rapidly.[33]

DISINFECTION

Filoviruses are sensitive to many ordinary disinfectants, including sodium hypochlorite.[18,19] According to the World Health Organization, 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.[34]

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.[35] Calcium hypochlorite (bleach powder), at concentrations of 0.02% to 2%, is another acceptable disinfectant.[36] Experimentally, filoviruses are also inactivated by UV light[31,37] and Gamma irradiation.[38,39]

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.[40] 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.[34] Human remains should be cremated or buried in a sealed casket with minimal handling.[41]

PREVENTION AND CONTROL

DISEASE REPORTING

Ebola virus infection is not a World Organization for Animal Health (WOAH)-listed disease. However, any animal infections should be reported to WOAH as an emerging disease (see 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,[42-54] although other susceptible hosts, including people, can transmit these viruses once infected. Indoor housing is probably the most effective measure for protecting pigs. However, other methods to prevent bat contact may also have some benefits, like using wire screens to prevent entry into open-sided pig sheds and removing fruit trees that may attract bats. Biosecurity plans should consider transmission via infected humans and fomites. Limited evidence suggests that rodents do not play a role in filovirus transmission.[1,55-57]

At present, there is little or no evidence suggesting that pigs would shed filoviruses 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.[58-62] Reston virus in pigs seems to disappear from tissues in one month.[22] Nevertheless, information about pig infections is still limited, and the potential for prolonged persistence should be considered.

DISEASE CONTROL

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

TRANSMISSION

EBOLA VIRUS

In humans, transmission of Ebola virus probably occurs through direct or indirect contact with infected animals and bushmeat consumption.[63,64] Secondary human-to-human transmission is due to contact with infectious blood, secretions, or other body fluids.[63] How bats transmit filoviruses to each other or other animals is uncertain.[65] Virus titers appear to be very low in wild bats, and nucleic acids have only been detected by nested RT- PCR.[42,66]

A series of experiments in pigs 3 to 6-weeks-old described viral shedding after combined intranasal, oral, and conjunctival inoculation with Ebola virus.[14-16] Infectious virus and viral RNA were found in oral and nasal secretions, but viral shedding from the gut was sporadic and inconsistent. Viremia was documented occasionally, but not in all animals. Moderate levels of infectious virus were found in the bladder of one pig 5 to 6-weeks-old with viremia, but urine was not tested.[14]

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 unknown whether it can be sustained in swine.[67] Transmission from pigs to nonhuman 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]

Little is known about Sudan virus and Bundibugyo virus in pigs. Following oronasal inoculation with Bundibugyo virus, viral RNA was detected in nasal swab samples from 8/11 pigs from 1 to 6 dpi.[24] Infectious virus was also recovered from nasal wash samples and group oral fluids.[24]

RESTON VIRUS

Reston virus-infected pigs were detected during PRRS outbreaks in the Philippines and China, but 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 there was more than one spillover event from another reservoir host or that pigs have maintained these viruses for many years.[20,22]

Experimentally, a Philippine pig Reston virus isolate replicated in 5-week-old pigs inoculated subcutaneously or oronasally but did not cause clinical signs.[22] More specifically,

- Most oronasally inoculated pigs shed the virus in nasopharyngeal secretions, with peak titers occurring at 6 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.

In another study, when pigs were inoculated with a non-human-primate adapted Reston virus strain, viral RNA was detectable in nasal swabs and washes (1 to 14 dpi) and oral swabs (3 to 10 dpi).[27] In group oral fluids, Reston virus RNA was documented in two cohorts from 4 to 10 dpi and 3 to 7 dpi. Live virus was recovered from two blood samples from a single animal. A sham inoculated control animal housed with the infected pigs became seropositive, and live virus was recovered from a nasal swab (3 dpi), oral swab (6 dpi), and nasal wash (10 dpi).[27] Infectious virus was isolated mainly from respiratory and lymphatic tissues.

PATHOGENESIS

According to the CDC, humans acquire Ebola virus 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, the Ebola virus migrates to regional lymph nodes and the liver, spleen, and adrenal glands. 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 impaired clotting, leading to multiorgan failure and shock.[6]

Little is known about the pathogenesis of orthoebolavirus infection in pigs.

DIAGNOSIS

Although filovirus isolation requires high biocontainment (BSL-4), most diagnostic assays can be conducted with inactivated virus.[68] 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 Ebola virus in human clinical specimens.[6]

Standard, contact, and droplet precautions have been recommended for contact with human filovirus-infected patients.[69] Precautions needed for collecting livestock samples are unclear. Necropsies are considered high-risk procedures, and specific advice should be obtained from health departments and the CDC.[41]

Tests used to detect Reston and Ebola virus in pigs have been developed mainly for research.[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).[67]

TESTS TO DETECT NUCLEIC ACIDS, VIRUS, OR ANTIGENS

EBOLA VIRUS

Filoviruses isolation must occur in laboratories with biocontainment facilities capable of handling dangerous human pathogens.[45] In humans, virus isolation in Vero or MA-104 cells[19,70] is used as a confirmatory test for reverse transcriptase polymerase chain reaction (RT-PCR) assays and antigen detection enzyme-linked immunosorbent assays (ELISA).[45]

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.[45]

Antigen detection ELISAs are one of the primary tests used to diagnose human clinical cases.[70] 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 nucleoprotein (NP).[45]

Electron microscopy can detect filovirus particles, which are distinctive in tissues or blood.[18,45,70] 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 has been 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.[71]

RESTON VIRUS

Reston virus replicates less efficiently in Vero cells than Ebola virus.[72] However, virus isolation 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 re-isolate 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.[67]

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.[14-16,67]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[70,73] being used in pigs.

TESTS TO DETECT ANTIBODY

Antibodies to orthoebolaviruses are cross-reactive.[74] The most likely causative agent can be distinguished with comparative serological assays against a panel of different orthoebolaviruses.[75,76]

EBOLA VIRUS

In humans, IgM and IgG ELISAs may be used for some clinical specimens and to monitor the immune response

in confirmed in Ebola virus patients.[6]

In pigs experimentally infected with Ebola virus, tests to detect antibodies have been described, including IgM capture ELISA using cell lysate antigen,[15,67] IgG ELISA using gamma-irradiated sucrose gradient purified whole Ebola virus,[14] and CPE-reduction virus neutralization assays.[67] Furthermore, Pickering and colleagues validated three assays for detecting 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).[77] An immunoblot assay was used to confirm indirect ELISA results.[77]

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.[78] A high seroprevalence rate was detected among pigs in infected regions but not in an uninfected area or outside the Philippines.

Experimentally, antibodies were detected with an indirect ELISA targeting viral NP in Reston virus-infected pigs.[22] All 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

EBOLA VIRUS

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 contained 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 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. Group oral fluids have also been used successfully for RNA detection.[27]

EPIDEMIOLOGY

SPECIES AFFECTED

EBOLA VIRUS

Current evidence suggests that bats are the primary reservoir hosts for ebolaviruses.[42-54] African orthoebolaviruses cause illness in humans and nonhuman primates. Pigs can be infected experimentally with Ebola virus, shed the virus, and develop clinical signs.[14-16] Bundibugyo virus inoculation does not cause clinical illness in pigs, but they can shed the virus and develop gross and histopathological lesions.[24] No experiments have been conducted on swine infection with Sudan virus or Taï Forest, so whether pigs might also be susceptible to these viruses is unknown.

The susceptibility to orthoebolaviruses has been investigated in invertebrates, birds, monkeys, rodents, and other small mammals, but has revealed surprisingly little, as described by Caron and colleagues.[65] Ebola virus antibodies have been detected in dogs.[79-81] Although the role of pigs in Ebola virus epidemiology remains unclear, factors that could facilitate zoonotic transmission have been suggested in Uganda,[82] 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., the possibility of undetected infections)
- Temporal correlation between Ebola virus 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.[83] A recent study failed to detect antibodies to Reston virus in *Rousettus amplexicaudatus*,[83] which had previously been described.[44]

In nature, Reston virus has been detected only in cynomolgus macaques and domesticated pigs co-infected with PRRS virus.[3,20-22,78] Antibodies have been detected in humans, but people do not become clinically ill (see *Public Health*). Experimentally, ferrets have been used as a small animal model to assess Reston virus pathogenicity.[84]

GEOGRAPHIC DISTRIBUTION

EBOLA VIRUS

Human outbreaks of Ebola virus disease have originated in the Democratic Republic of the Congo, the Republic of the Congo, Gabon, and Guinea. Movements of infected people can disperse the virus to additional regions.[46,85] 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.[46] All known outbreaks have occurred within 400 miles ofeach other, indicating the range of the virus may be limited.[85] 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[46] or two[68] 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.[46,85]

Bat species infected with Ebola virus in the wild[42,47] have broad geographic ranges, including the entire tropical forest regions of equatorial central Africa.[86] A swine serosurvey from Uganda found that pigs sampled in June were likelier to have antibodies to Ebola virus than pigs sampled in October.[87] This corresponded to the dry period in Uganda, and other studies have suggested that spillover to humans[88,89] and great apes[30] 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

EBOLA VIRUS

Illness or death due to natural Ebola virus infection has not been reported in pigs. Studies on Ebola prevalence in pigs are summarized below.

- In Uganda, seven percent 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.[87] Sera from four ELISA-positive pigs reacted in Western blot, and one sample had fully neutralized antibody to Sudan virus via virus

neutralization.[87]

- A study of domestic pigs in Sierra Leone found that only 0.75% (3/400) were clearly positive using an indirect ELISA to detect antibodies to Ebola virus NP.[90] These samples were confirmed as positive via immunoblot, reacting with Ebola virus NP. No samples reacted with Ebola virus GP or contained neutralizing antibodies. The authors speculated that pigs may have had contact with an antigenically related orthoebolavirus and that serological cross-reactivity occured.[90] A similar phenomenon is suspected in humans, where unknown filoviruses may account for serological cross-reactivity against known Ebola virus (as described by Fischer et al.[91]).

- In Guinea, 4.5% (14/308) of pigs were seropositive for Ebola virus using an NP-based ELISA, and confirmed with Western blot.[91] Again, cross-reactivity between filoviruses was suspected in serum.[91] Most reactive samples were from a rural site surrounded by mango trees, although no bats were observed during sampling.[91]

- IgG antibodies to Ebola virus, Taï Forest virus, Reston virus, and Lloviu virus were detected in 3.6% (5/139) of swine samples from Ghana using a GP-based ELISA.[92] Two positive serum samples reacted to all four filovirus species using Western blott.[92] Human Ebola virus cases have never been reported in Ghana.

- A survey of 888 swine serum samples in Ghana showed that 25.1% (223) were ELISA-positive using an NP-based test. Prevalence was highest in rural areas. Cross-reactivity between the GPs of Ebola, Bundibugyo, and Reston was confirmed.[93]

RESTON VIRUS

- During the Philippines outbreak, antibodies to Reston virus were found in 71% to 79% of pigs on infected farms using an immunofluorescence assay. 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 PRRS, despite the detection of antibodies to PRRS virus.[20]

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 2024, the family *Filoviridae* contains eight genera (*Cuevavirus, Dianlovirus, Oblavirus, Orthoebolavirus, Orthomarburgvirus, Striavirus, Tapjovirus,* and *Thamnovirus*).[5]

The filovirus ribonucleoprotein (RNP) complex consists of NP (nucleoprotein), VP24 (complex-associated protein), VP35 (polymerase cofactor), VP30 (transcriptional activator), and L (large protein). The RNP complex is surrounded by VP40 (matrix protein) and GP (spike glycoprotein). Three soluble glycoprotein peptides are expressed from the *GP* gene: sGP, ssGP, and Δ-peptide.[5,94] Key functions of orthoebolavirus proteins are further described by Cantoni and colleagues.[95]

A guinea pig and mouse-adapted Ebola virus study found that very few mutations were required to become pathogenic in a novel host.[97] In particular, mutations of VP24 (which plays a role in nucleocapsid assembly, virus release, and immune response suppression),[8] seem vital for adaptation.[97]

CHARACTERISTICS OF ORTHOEBOLAVIRUSES

The genus *Orthoebolavirus* contains six species: *Orthoebolavirus zairense* (Ebola virus), *Orthoebolavirus bundibugyoense* (Bundibugyo virus), *Orthoebolavirus sudanese* (Sudan virus), *Orthoebolavirus taiense* (Taï Forest virus, formerly *Cote d'Ivoire ebolavirus*), *Orthoebolavirus restonense* (Reston virus), and *Orthoebolavirus*

bombaliense (Bombali virus).

The genomes of different orthoebolavirus species can differ from each other by more than 23%.[5,96] Reston virus most likely originated in Africa and diverged from Sudan virus about 1500 years ago.[95] Full-length Reston virus genomes from primates, pigs, and humans are generally quite similar.[94,95] The highest degree of diversity occurs in the *GP* gene (< 10%). Reston virus isolates from different swine farms in the Philippines demonstrated up to 4.5% divergence in one year.[23]

Phylogenetic analysis shows that Reston viruses can be divided into five distinct lineages. Swine isolates from three different farms in the 2008–2009 outbreak in the Philippines were assigned to three different lineages.[94]

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 PRRS virus.[20] Pigs on outbreak farms had antibodies to Reston virus, but 98 pigs from an unaffected region were seronegative.[78] Whether pigs in other parts of the Philippines are free of Reston virus remains to be determined.[78] Reston virus nucleic acids were later detected in pigs on three farms in Shanghai, China, that had also 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 to 6-week-old pigs experimentally infected with Ebola virus, neither IgG antibody titers nor neutralizing antibodies were detected by day seven when the pigs were euthanized.[14] IgM titers to Ebola virus could be found using ELISA on day 5/6 in a second study that used pigs of the same age.[15]

In 3 to 4-week-old pigs experimentally infected with Ebola virus, IgG antibody titers and neutralizing antibodies were measured during a contact 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.

In pigs oronasally infected with Bundibugyo virus, IgM and IgG titers were detected in 64% (7/11) animals from 6 to 11 dpi. An IgA response was detected in nasal wash fluid samples from six pigs from four to 16 dpi. IgA development was also documented in group oral fluid samples from 10 to 21 dpi. For all sample types, antibody response was measured by a commercially available Bundibugyo GP ELISA.[24]

Six-week-old pigs inoculated with a non-human-primate adapted Reston virus were positive for IgA (nasal wash fluid and group oral fluids) between 10 and 29 dpi.[27] IgM and IgG were also detected by 10 dpi.

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.[98,99]

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.[68]A number of Ebola virus vaccines, based on a wide variety of platforms, have been tested in laboratory rodents and nonhuman primates,[99] including standard inactivated vaccines[18] and classical subunit vaccines,[100] 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.[99]

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.[99] Vaccine types in development for humans may not be the optimal approach in livestock. 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.[68]

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.[101]

GAPS IN PREPAREDNESS

Overall, little is known about the possible role of pigs in Ebola virus and Reston virus transmission. Potential routes of transboundary spread should be explored. Reston virus should be considered a livestock pathogen with zoonotic potential. Genomic mutations could impact morbidity and mortality in pigs and the likelihood of human-to-human transmission.

REFERENCES

1. Miranda ME, Miranda NL. Reston ebolavirus in humans and animals in the Philippines: a review. *J Infect Dis*. 2011;204 Suppl 3:S757-60. doi:10.1093/infdis/jir296
2. Demetria C, Smith I, Tan T, et al. Reemergence of Reston ebolavirus in cynomolgus monkeys, the Philippines, 2015. *Emerg Infect Dis*. 2018;24(7):1285-1291. doi:10.3201/eid2407.171234
3. Pan Y, Zhang W, Cui L, Hua X, Wang M, Zeng Q. Reston virus in domestic pigs in China. *Arch Virol*. 2014;159(5):1129-32. doi:10.1007/s00705-012-1477-6
4. Peñas JA, Miranda ME, de Los Reyes VC, Sucaldito MNL, Magpantay RL. Risk assessment of Ebola Reston virus in humans in the Philippines. *Western Pac Surveill Response J*. 2019;10(3):1-8. doi:10.5365/wpsar.2017.3.004
5. Kuhn JH, Amarasinghe GK, Basler CF, et al. ICTV Virus Taxonomy Profile: Filoviridae. *J Gen Virol*. 06 2019;100(6):911-912. doi:10.1099/jgv.0.001252
6. Centers for Disease Control and Prevention (CDC). Ebola (Ebola Virus Diseae). Accessed July 8, 2021. https://www.cdc.gov/vhf/ebola/index.html
7. Yamaoka S, Ebihara H. Pathogenicity and virulence of ebolaviruses with species- and variant-specificity. *Virulence*. 2021;12(1):885-901. doi:10.1080/21505594.2021.1898169
8. Rojas M, Monsalve DM, Pacheco Y, et al. Ebola virus disease: an emerging and re-emerging viral threat. *J Autoimmun*. 2020;106:102375. doi:10.1016/j.jaut.2019.102375
9. Breman JG, Heymann DL, Lloyd G, et al. Discovery and description of Ebola Zaire virus in 1976 and relevance to the West African epidemic during 2013-2016. *J Infect Dis*. 2016;214(suppl 3):S93-S101. doi:10.1093/infdis/jiw207
10. Bell BP, Damon IK, Jernigan DB, et al. Overview, control strategies, and lessons learned in the CDC response to the 2014-2016 Ebola epidemic. *MMWR Suppl*. 2016;65(3):4-11. doi:10.15585/mmwr.su6503a2
11. Formenty P, Hatz C, Le Guenno B, Stoll A, Rogenmoser P, Widmer A. Human infection due to Ebola virus, subtype Côte d'Ivoire: clinical and biologic presentation. *J Infect Dis*.1999;179 Suppl 1:S48-53. doi:10.1086/514285
12. Goldstein T, Anthony SJ, Gbakima A, et al. The discovery of Bombali virus adds further support for bats as hosts of ebolaviruses. *Nat Microbiol*. 2018;3(10):1084-1089. doi:10.1038/s41564-018-0227-2
13. Martell HJ, Masterson SG, McGreig JE, Michaelis M, Wass MN. Is the Bombali virus pathogenic in humans? *Bioinformatics*. 2019;35(19):3553-3558. doi:10.1093/bioinformatics/btz267
14. Kobinger GP, Leung A, Neufeld J, et al. Replication, pathogenicity, shedding, and transmission of Zaire ebolavirus in pigs. *J Infect Dis*. 2011;204(2):200-8. doi:10.1093/infdis/jir077
15. Nfon CK, Leung A, Smith G, Embury-Hyatt C, Kobinger G, Weingartl HM. Immunopathogenesis of severe acute respiratory disease in Zaire ebolavirus-infected pigs. *PLoS One*. 2013;8(4):e61904. doi:10.1371/journal.pone.0061904
16. Weingartl HM, Embury-Hyatt C, Nfon C, Leung A, Smith G, Kobinger G. Transmission of Ebola virus from pigs to nonhuman primates. *Sci Rep*. 2012;2:811. doi:10.1038/srep00811
17. Bausch DG. Ebola virus as a foodborne pathogen? Cause for consideration, but not panic. *J Infect Dis*. 2011;204(2):179-81. doi:10.1093/infdis/jir201
18. Schou S, Hansen AK. Marburg and Ebola virus infections in laboratory nonhuman primates: a literature review. *Comp Med*. Apr 2000;50(2):108-23.
19. Peters CJ, Jahrling PB, Ksiazek TG, Johnson ED, Lupton HW. Filovirus contamination of cell cultures. *Dev Biol Stand*. 1992;76:267-74.
20. Barrette RW, Metwally SA, Rowland JM, et al. Discovery of swine as a host for the Reston ebolavirus. *Science*. 2009;325(5937):204-6. doi:10.1126/science.1172705
21. Miranda ME, Ksiazek TG, Retuya TJ, et al. Epidemiology of Ebola (subtype Reston) virus in the Philippines, 1996. *J Infect Dis*. 1999;179:S115-S119. doi:10.2307/30117612

22. Marsh GA, Haining J, Robinson R, et al. Ebola Reston virus infection of pigs: clinical significance and transmission potential. *J Infect Dis*. 2011;204 Suppl 3:S804-9. doi:10.1093/infdis/jir300

23. Carroll SA, Towner JS, Sealy TK, et al. Molecular evolution of viruses of the family Filoviridae based on 97 whole-genome sequences. *J Virol*. 2013;87(5):2608-16. doi:10.1128/JVI.03118-12
24. Lewis C, Pinette M, Lakin S, et al. Experimental infection of Bundibugyo virus in domestic swine leads to viral shedding with evidence of interspecies transmission. *Transbound Emerg Dis*. 2024. doi:doi/10.1155/2024/5350769
25. Tian K, Yu X, Zhao T, et al. Emergence of fatal PRRSV variants: unparalleled outbreaks of atypical PRRS in China and molecular dissection of the unique hallmark. *PLoS One*. 2007;2(6):e526. doi:10.1371/journal.pone.0000526
26. Haddock E, Saturday G, Feldmann F, et al. Reston virus causes severe respiratory disease in young domestic pigs. *Proc Natl Acad Sci U S A*. Jan 2021;118(2)doi:10.1073/pnas.2015657118
27. Lewis CE, Pinette MM, Lakin SM, et al. Domestic pigs are susceptible to experimental infection with nonhuman primate-derived Reston virus without the need for adaptation. *Sci Rep*. 2024;14(1):715. doi:10.1038/s41598-024-51280-8
28. Senthilkumaran C, Kroeker AL, Smith G, et al. Treatment with Ad5-porcine interferon-α attenuates ebolavirus disease in pigs. *Pathogens*. 2022;11(4)doi:10.3390/pathogens11040449
29. Piercy TJ, Smither SJ, Steward JA, Eastaugh L, Lever MS. The survival of filoviruses in liquids, on solid substrates and in a dynamic aerosol. *J Appl Microbiol*. 2010;109(5):1531-9. doi:10.1111/j.1365-2672.2010.04778.x
30. Leroy EM, Rouquet P, Formenty P, et al. Multiple Ebola virus transmission events and rapid decline of central African wildlife. *Science*. 2004;303(5656):387-90. doi:10.1126/science.1092528
31. Bowen ET, Simpson DI, Bright WF, Zlotnik I, Howard DM. Vervet monkey disease: studies on some physical and chemical properties of the causative agent. *Br J Exp Pathol*. 1969;50(4):400-7.
32. Chepurnov AA, Chuev Iu P, P'Iankov O V, Efimova IV. [The effect of some physical and chemical factors on inactivation of the Ebola virus]. *Vopr Virusol*. 1995;40(2):74-6.
33. European Food Safety Authority (EFSA). An update on the risk of transmission of Ebola virus (EBOV) via the food chain - part 2. *EFSA J*. 2015;13(3):4042.
34. World Health Organization (WHO) Division of Emerging and Other Communicable Diseases - Surveillance and Control, Centers for Disease Control and Prevention (CDC): National Center for Infectious Diseases Division of Viral and Rickettsial Diseases Special Pathogens Branch. Infection Control for Viral Haemorrhagic Fevers in the African Health Care Setting. World Health Organization. Updated 1998. Accessed July 8, 2021. https://apps.who.int/iris/handle/10665/65012
35. Rouquet P, Froment JM, Bermejo M, et al. Wild animal mortality monitoring and human Ebola outbreaks, Gabon and Republic of Congo, 2001-2003. *Emerg Infect Dis*. 2005;11(2):283-90. doi:10.3201/eid1102.040533
36. Kerstiens B, Matthys F. Interventions to control virus transmission during an outbreak of Ebola hemorrhagic fever: experience from Kikwit, Democratic Republic of the Congo, 1995. *J Infect Dis*. 1999;179 Suppl 1:S263-7. doi:10.1086/514320
37. Sagripanti JL, Lytle CD. Sensitivity to ultraviolet radiation of Lassa, vaccinia, and Ebola viruses dried on surfaces. *Arch Virol*. 2011;156(3):489-94. doi:10.1007/s00705-010-0847-1
38. Elliott LH, McCormick JB, Johnson KM. Inactivation of Lassa, Marburg, and Ebola viruses by gamma irradiation. *J Clin Microbiol*. 1982;16(4):704-708.
39. Lupton HW. Inactivation of Ebola virus with 60Co irradiation. *J Infect Dis*. 1981;143(2):291.
40. U.S. Environmental Protection Agency (EPA). List L: EPA's Registered Antimicrobial Products that Meet the CDC Criteria for Use Against the Ebola Virus. Accessed July 8, 2021. https://www.epa.gov/sites/production/files/2018-01/documents/2018.10.01.listl_.pdf
41. Centers for Disease Control and Prevention (CDC). Update: management of patients with suspected viral hemorrhagic fever--United States. *MMWR Morb Mortal Wkly Rep*. 1995;44(25):475-9.
42. Leroy EM, Kumulungui B, Pourrut X, et al. Fruit bats as reservoirs of Ebola virus. *Nature*. 2005;438(7068):575-6. doi:10.1038/438575a
43. Nakayama E, Saijo M. Animal models for Ebola and Marburg virus infections. *Front Microbiol*.

2013;4:267. doi:10.3389/fmicb.2013.00267
44. Taniguchi S, Watanabe S, Masangkay JS, et al. Reston Ebolavirus antibodies in bats, the Philippines. *Emerg Infect Dis*. 2011;17(8):1559-60. doi:10.3201/eid1708.101693
45. Mehedi M, Groseth A, Feldmann H, Ebihara H. Clinical aspects of Marburg hemorrhagic fever. *Future Virol*. 2011;6(9):1091-1106. doi:10.2217/fvl.11.79
46. Muyembe-Tamfum JJ, Mulangu S, Masumu J, Kayembe JM, Kemp A, Paweska JT. Ebola virus outbreaks in Africa: past and present. *Onderstepoort J Vet Res*. 2012;79(2):451. doi:10.4102/ojvr.v79i2.451
47. Pourrut X, Souris M, Towner JS, et al. Large serological survey showing cocirculation of Ebola and Marburg viruses in Gabonese bat populations, and a high seroprevalence of both viruses in *Rousettus aegyptiacus*. *BMC Infect Dis*. 2009;9:159. doi:10.1186/1471-2334-9-159
48. Swanepoel R, Leman PA, Burt FJ, et al. Experimental inoculation of plants and animals with Ebola virus. *Emerg Infect Dis*. 1996;2(4):321-5. doi:10.3201/eid0204.960407
49. Hayman D, Emmerich P, Yu M, et al. Long-term survival of an urban fruit bat seropositive for Ebola and Lagos bat viruses. *PLoS ONE*. 2010;5(8):e11978. doi:10.1371/journal.pone.0011978
50. Towner JS, Amman BR, Sealy TK, et al. Isolation of genetically diverse Marburg viruses from Egyptian fruit bats. *PLoS Pathog*. 2009;5(7):e1000536. doi:10.1371/journal.ppat.1000536
51. Kuzmin IV, Niezgoda M, Franka R, et al. Marburg virus in fruit bat, Kenya. *Emerg Infect Dis*. 2010;16(2):352-4. doi:10.3201/eid1602.091269
52. Maganga GD, Bourgarel M, Ella GE, et al. Is Marburg virus enzootic in Gabon? *J Infect Dis*. 2011;204 Suppl 3:S800-3. doi:10.1093/infdis/jir358
53. Swanepoel R, Smit S, Rollin P, et al. Studies of reservoir hosts for Marburg virus. *Emerg Infect Dis*. 2007;13(12):1847-1851. doi:10.3201/eid1312.071115
54. Paweska JT, Jansen van Vuren P, Masumu J, et al. Virological and serological findings in *Rousettus aegyptiacus* experimentally inoculated with vero cells-adapted hogan strain of Marburg virus. *PLoS One*. 2012;7(9):e45479. doi:10.1371/journal.pone.0045479
55. Leirs H, Mills JN, Krebs JW, et al. Search for the Ebola virus reservoir in Kikwit, Democratic Republic of the Congo: reflections on a vertebrate collection. *J Infect Dis*. 1999;179 Suppl 1:S155-63. doi:10.1086/514299
56. Bradfute SB, Warfield KL, Bray M. Mouse models for filovirus infections. *Viruses*. 2012;4(9):1477-508. doi:10.3390/v4091477
57. Bray M, Davis K, Geisbert T, Schmaljohn C, Huggins J. A mouse model for evaluation of prophylaxis and therapy of Ebola hemorrhagic fever. *J Infect Dis*. 1998;178(3):651-61.
58. Fisher-Hoch SP, Perez-Oronoz GI, Jackson EL, Hermann LM, Brown BG. Filovirus clearance in nonhuman primates. *Lancet*. 1992;340(8817):451-3.
59. Bausch DG, Towner JS, Dowell SF, et al. Assessment of the risk of Ebola virus transmission from bodily fluids and fomites. *J Infect Dis*. 2007;196 Suppl 2:S142-7. doi:10.1086/520545
60. Rowe AK, Bertolli J, Khan AS, et al. Clinical, virologic, and immunologic follow-up of convalescent Ebola hemorrhagic fever patients and their household contacts, Kikwit, Democratic Republic of the Congo. *J Infect Dis*. 1999;179 Suppl 1:S28-35. doi:10.1086/514318
61. Rodriguez LL, De Roo A, Guimard Y, et al. Persistence and genetic stability of Ebola virus during the outbreak in Kikwit, Democratic Republic of the Congo, 1995. *J Infect Dis*. 1999;179 Suppl 1:S170-6. doi:10.1086/514291
62. Martini GA, Schmidt HA. [Spermatogenic transmission of the "Marburg virus"]. *Klin Wochenschr*. 1968;46(7):398-400.
63. Malvy D, McElroy AK, de Clerck H, Günther S, van Griensven J. Ebola virus disease. *Lancet*. 2019;393(10174):936-948. doi:10.1016/s0140-6736(18)33132-5
64. Alexander KA, Sanderson CE, Marathe M, et al. What factors might have led to the emergence of Ebola in West Africa? *PLoS Negl Trop Dis*. 2015;9(6):e0003652. doi:10.1371/journal.pntd.0003652
65. Caron A, Bourgarel M, Cappelle J, Liégeois F, De Nys HM, Roger F. Ebola virus maintenance: if not (only) bats, what else? *Viruses*. 2018;10(10)doi:10.3390/v10100549
66. Groseth A, Feldmann H, Strong JE. The ecology of Ebola virus. *Trends Microbiol*. 2007;15(9):408-16. doi:10.1016/j.tim.2007.08.001

67. Weingartl HM, Nfon C, Kobinger G. Review of Ebola virus infections in domestic animals. *Dev Biol (Basel)*. 2013;135:211-8. doi:10.1159/000178495

68. Feldmann F, Feldmann H. Ebola: facing a new transboundary animal disease? *Dev Biol (Basel)*. 2013;135:201-9. doi:10.1159/000190049
69. Centers for Disease Control and Prevention (CDC). Guidance for Collection, Transport and Submission of Specimens for Ebola Virus Testing. Accessed July 7, 2021. https://www.cdc.gov/vhf/ebola/laboratory-personnel/specimens.html
70. Grolla A, Lucht A, Dick D, Strong JE, Feldmann H. Laboratory diagnosis of Ebola and Marburg hemorrhagic fever. *Bull Soc Pathol Exot*. 2005;98(3):205-9.
71. Martines RB, Ng DL, Greer PW, Rollin PE, Zaki SR. Tissue and cellular tropism, pathology and pathogenesis of Ebola and Marburg viruses. *J Pathol*. 2015;235(2):153-74. doi:10.1002/path.4456
72. Morikawa S, Saijo M, Kurane I. Current knowledge on lower virulence of Reston Ebola virus. *Comp Immunol Microbiol Infect Dis*. 2007;30(5-6):391-8. doi:10.1016/j.cimid.2007.05.005
73. Vogel G. Infectious diseases: testing new Ebola tests. *Science*. 2014;345(6204):1549-50. doi:10.1126/science.345.6204.1549
74. Kuhn JH, Becker S, Ebihara H, et al. Proposal for a revised taxonomy of the family Filoviridae: classification, names of taxa and viruses, and virus abbreviations. *Arch Virol*. 2010;155(12):2083-103. doi:10.1007/s00705-010-0814-x
75. Hayman DT, Suu-Ire R, Breed AC, et al. Evidence of henipavirus infection in West African fruit bats. *PLoS One*. 2008;3(7):e2739. doi:10.1371/journal.pone.0002739
76. McCormick J. Ebola virus ecology. *J Infect Dis*. 2004;190(11):1893-1894. doi:10.1086/425429
77. Pickering BS, Collignon B, Smith G, Marszal P, Kobinger G, Weingartl HM. Detection of Zaire ebolavirus in swine: assay development and optimization. *Transbound Emerg Dis*. 2018;65(1):77-84. doi:10.1111/tbed.12606
78. Sayama Y, Demetria C, Saito M, et al. A seroepidemiologic study of Reston ebolavirus in swine in the Philippines. *BMC Vet Res*. 2012;8:82. doi:10.1186/1746-6148-8-82
79. Fischer K, Suluku R, Fehling SK, et al. Ebola virus neutralizing antibodies in dogs from Sierra Leone, 2017. *Emerg Infect Dis*. 2020;26(4):760-763. doi:10.3201/eid2604.190802
80. Allela L, Boury O, Pouillot R, et al. Ebola virus antibody prevalence in dogs and human risk. *Emerg Infect Dis*. 2005;11(3):385-90. doi:10.3201/eid1103.040981
81. Haun BK, Kamara V, Dweh AS, et al. Serological evidence of Ebola virus exposure in dogs from affected communities in Liberia: A preliminary report. *PLoS Negl Trop Dis*. 2019;13(7):e0007614. doi:10.1371/journal.pntd.0007614
82. Atherstone C, Smith E, Ochungo P, Roesel K, Grace D. Assessing the potential role of pigs in the epidemiology of Ebola virus in Uganda. *Transbound Emerg Dis*. 2017;64(2):333-343. doi:10.1111/tbed.12394
83. Jayme SI, Field HE, de Jong C, et al. Molecular evidence of Ebola Reston virus infection in Philippine bats. *Virol J*. 2015;12:107. doi:10.1186/s12985-015-0331-3
84. Yan F, He S, Banadyga L, et al. Characterization of Reston virus infection in ferrets. *Antiviral Res*. 2019;165:1-10. doi:10.1016/j.antiviral.2019.03.001
85. Changula K, Kajihara M, Mweene AS, Takada A. Ebola and Marburg virus diseases in Africa: increased risk of outbreaks in previously unaffected areas? *Microbiol Immunol*. 2014;58(9):483-91. doi:10.1111/1348-0421.12181
86. Becquart P, Wauquier N, Mahlakoiv T, et al. High prevalence of both humoral and cellular immunity to Zaire ebolavirus among rural populations in Gabon. *PLoS One*. 2010;5(2):e9126. doi:10.1371/journal.pone.0009126
87. Atherstone C, Diederich S, Pickering B, et al. Investigation of Ebolavirus exposure in pigs presented for slaughter in Uganda. *Transbound Emerg Dis*. 2021;68(3):1521-1530. doi:10.1111/tbed.13822
88. Pinzon JE, Wilson JM, Tucker CJ, Arthur R, Jahrling PB, Formenty P. Trigger events: enviroclimatic coupling of Ebola hemorrhagic fever outbreaks. *Am J Trop Med Hyg*. 2004;71(5):664-74.
89. Pinzon E, Wilson JM, Tucker CJ. Climate-based health monitoring systems for eco-climatic conditions associated with infectious diseases. *Bull Soc Pathol Exot*. 2005;98(3):239-43.

90. Fischer K, Jabaty J, Suluku R, et al. Serological evidence for the circulation of Ebolaviruses in pigs from Sierra Leone. *J Infect Dis*. 2018;218(suppl5):S305-s311. doi:10.1093/infdis/jiy330

91. Fischer K, Camara A, Troupin C, et al. Serological evidence of exposure to ebolaviruses in domestic pigs from Guinea. *Transbound Emerg Dis*. 2020;67(2):724-732. doi:10.1111/tbed.13391
92. Ogawa H, Ohya K, Ayizanga R, et al. Detection of anti-ebolavirus antibodies in Ghanian pigs. *J Vet Med Sci*. 2022;84(11):1491-1494. doi:doi: 10.1292/jvms.22-0186
93. Grayo S, Camara A, Doukouré B, et al. Geographic disparities in domestic pig population exposure to Ebola viruses, Guinea, 2017-2019. *Emerg Infect Dis*. 2024;30(4):681-690. doi:10.3201/eid3004.231034
94. Albarino CG, Wiggleton Guerrero L, Jenks HM, et al. Insights into Reston virus spillovers and adaption from virus whole genome sequences. *PLoS One*. 2017;12(5):e0178224. doi:10.1371/journal.pone.0178224
95. Cantoni D, Hamlet A, Michaelis M, Wass MN, Rossman JS. Risks posed by Reston, the forgotten ebolavirus. *mSphere*. 2016;1(6). doi:10.1128/mSphere.00322-16
96. Bào Y, Amarasinghe GK, Basler CF, et al. Implementation of objective PASC-derived taxon demarcation criteria for official classification of filoviruses. *Viruses*. 2017;9(5). doi:10.3390/v9050106
97. Pappalardo M, Reddin IG, Cantoni D, Rossman JS, Michaelis M, Wass MN. Changes associated with Ebola virus adaptation to novel species. *Bioinformatics*. 2017;33(13):1911-1915. doi:10.1093/bioinformatics/btx065
98. Sobarzo A, Groseth A, Dolnik O, et al. Profile and persistence of the virus-specific neutralizing humoral immune response in human survivors of Sudan ebolavirus (Gulu). *J Infect Dis*. 2013;208(2):299-309. doi:10.1093/infdis/jit162
99. Marzi A, Feldmann H. Ebola virus vaccines: an overview of current approaches. *Exp Rev Vaccines*. 2014;13(4):521-531. doi:10.1586/14760584.2014.885841
100. DeBuysscher BL, Scott D, Marzi A, Prescott J, Feldmann H. Single-dose live-attenuated Nipah virus vaccines confer complete protection by eliciting antibodies directed against surface glycoproteins. *Vaccine*. 2014;32(22):2637-2644. doi.org/10.1016/j.vaccine.2014.02.087
101. Hensley LE, Mulangu S, Asiedu C, et al. Demonstration of cross-protective vaccine immunity against an emerging pathogenic Ebolavirus Species. *PLoS Pathog*. 2010;6(5):e1000904. doi:10.1371/journal.ppat.1000904
102. Birungi D, Tumwine G, Kato CD, Ssekamatte T, Ocaido M, Majalija S. Knowledge, perceptions and effects of Ebola virus disease outbreak on the pig value chain in the agro-pastoralist district of Luwero, Central Uganda. *BMC Infect Dis*. 2021;21(1):669. doi:10.1186/s12879-021-06337-8
103. Glud HA, George S, Skovgaard K, Larsen LE. Zoonotic and reverse zoonotic transmission of viruses between humans and pigs. *APMIS*. 2021;129(12):675-693. doi:10.1111/apm.13178