LASSA VIRUS

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SUMMARY

Etiology

- Lassa virus (LASV) is a bi-segmented, negative-sense, single-stranded RNA virus belonging to the Old World complex, genus *Arenavirus*, family *Arenaviridae*. LASV, which primarily causes infection in humans, was originally isolated from a missionary nurse in Lassa, Nigeria in 1969. The virus is closely related to other Old World arenaviruses including lymphocytic choriomeningitis and Lujo viruses.

Cleaning and Disinfection

- LASV is stable as an aerosol when relative humidity does not exceed 30%. At room temperature, LASV retains infectivity for 10 to 55 minutes.
- LASV can be inactivated by 1% sodium hypochlorite, 2% glutaraldehyde, and most other detergents and disinfectants. Ultraviolet light and gamma irradiation render the virus inactive as do temperatures above 56°C (133°F), pH less than 5.5, and pH greater than 8.5.

Epidemiology

- LASV is not a known pathogen of pigs.
- LASV is endemic in parts of West Africa, where the rodent reservoir host, *Mastomys natalensis* (the multimammate rat), is also found. LASV is zoonotic and causes 100,000–300,000 infections annually.
- LASV causes a mild, flu-like illness in 80% of infected humans. Others may go on to develop Lassa fever characterized by hemorrhage; respiratory distress; vomiting; facial swelling; pain in the chest, back, or abdomen; shock; and sometimes death. Neurological signs, such as tremors and encephalitis have also been described. Hearing loss, which may be permanent, is a common complication occurring in up to one-third of human patients.
- Death in humans is relatively uncommon, with an overall mortality rate of about 1%. However, during epidemics, the case-fatality rate in hospitalized patients may reach 50%. Death is more likely following human-to-human transmission compared to zoonotic transmission.

Transmission

- LASV transmission in pigs is apparently not described.
- Rodent-to-human transmission of LASV occurs via contact with body fluids, excreta, urine, tissues, or blood, as well as inhalation of infectious aerosols. Consumption of infected rodents may also lead to infection.

- Human-to-human transmission of LASV occurs via direct contact with infected body fluids.
- Vertical and horizontal transmission of LASV are both important in the reservoir host, *Mastomys natalensis*. Other host species have recently been documented but routes of transmission in those species have not been explored.

Infection in Swine/Pathogenesis

- Information on LASV infection in swine is not available.

Diagnosis

- No specific tests have been described for LASV detection in swine.
- LASV isolation must be performed in a BSL4 laboratory. Vero cells are most frequently utilized for isolation. Indirect fluorescent antibody testing has traditionally been used to diagnose LASV in the laboratory.
- Polymerase chain reaction/ligase detection reaction (PCR/LDR) and reverse-transcriptase PCR (RT-PCR) have been described for LASV. Differentiation among LASV isolates can be done using such techniques and RT-PCR is considered the "gold standard" test for LASV.
- A commercially available rapid antigen enzyme-linked immunosorbent assay (ELISA), antigen ReLASV®, is available for use in humans. ELISAs for detecting IgM and IgG in patient serum include the IgM/IgG ReLASV®.

Immunity

- Information on LASV immunity in swine is not available.
- Cell-mediated immunity is important in viral clearance and patient survival. Neutralizing antibodies develop late in LASV infection and do not appear important for viral clearance or protection against infection.
- Multiple vaccines have been described including vesicular stomatitis virus-based vaccines, live non-pathogenic strains, and reverse genetics-produced reassortant viruses. No vaccine is currently commercially available for humans or animals.

Prevention and Control

- Prevention and control measures for LASV in swine are not established.
- In endemic areas, humans should take measures to avoid contact with rodents and their excretions, store food in rodent-proof containers, and keep rodents out of homes. Rodents should not be consumed. Human-to-human transmission is greatly reduced by the use of standard barrier precautions.
- Human infections can be treated early with ribavirin to prevent severe disease. Experimental studies with favipiravir (an RNA polymerase inhibitor) in guinea pigs show the drug to be promising, even when given later in infection.

Gaps in Preparedness

- LASV infection in swine has not been reported in the literature; the clinical presentation, if any, has not been described.
- Although LASV is not currently found in the U.S., the distribution of potential reservoirs is not well known.
- Only reference laboratories such as the Centers for Disease Control and Prevention (CDC) are capable of detecting LASV, and the symptoms in humans are non-specific.
- There are no licensed vaccines for LASV in humans or animals.

OVERVIEW

Lassa virus (LASV) is a member of the family *Arenaviridae*, genus *Arenavirus*. It is found within the Old World complex of arenaviruses, and currently circulating LASV strains cluster into at least four major lineages or clades. Three of the lineages/clades are found in Nigeria and the fourth is found in Sierra

Leone, Liberia, and Guinea. LASV is endemic to regions of West Africa; human infection has been documented in Nigeria, Sierra Leone, Liberia, Guinea, Ghana, Benin, Mali, Senegal, Gambia, Côte d'Ivoire, and Burkina Faso. LASV infection has also been reported in the Central African Republic.

LASV is maintained in its major rodent reservoir species, *Mastomys natalensis*, by both vertical and horizontal transmission. LASV has been found in a few other rodent species; however, the significance of their contribution to zoonotic transmission remains to be determined. The symptoms of Lassa fever in humans are varied and nonspecific. LASV causes a mild, flu-like illness in 80% of infected humans.[8] Others may go on to develop Lassa fever characterized by hemorrhage; respiratory distress; vomiting; facial swelling; pain in the chest, back, or abdomen; shock; and sometimes death.[8] Neurological signs, such as tremors and encephalitis have also been described. Hearing loss, which may be permanent, is a common complication occurring in up to one-third of human patients. Death is relatively uncommon, with an overall mortality rate of about 1%. However, during epidemics, the case-fatality rate in hospitalized patients may reach 50%.[8] Zoonotic transmission results in low rates of mortality (1–2%). Early treatment with intravenous ribavirin can improve survival in LASV-infected patients and is used routinely in some hospitals in Sierra Leone and Nigeria. Favipiravir, an RNA-dependent RNA-polymerase inhibitor that has recently been used to treat Ebola patients, has been shown to significantly reduce mortality in LASV-infected guinea pigs and administration was less time sensitive than ribavirin.

LASV was originally isolated from a missionary nurse in 1969 in Lassa, Nigeria. In subsequent decades, human LASV infections have been estimated at 100,000–300,000 annually, with 5,000–10,000 deaths. Human infection occurs in regions where the reservoir host species is native, and households with cases of LASV infection are more likely to house LASV-infected *M. natalensis*. Human infection appears to occur more frequently during the dry season, while the rainy season appears important in maintaining LASV within *Mastomys* rats.

Natural LASV infection has only been documented in humans, *Mastomys natalensis*, and a few other rodent species in LASV-endemic areas of Africa. Experimentally, multiple non-human primate species, guinea pigs, and humanized mice have been infected with LASV. Due to the danger LASV poses, it has been categorized as a biosafety level four (BSL4) pathogen and therefore, all experiments using live virus must occur at the maximum level of biocontainment. This has likely hindered progress on the development of vaccines and diagnostics for LASV.

LASV infection has not been documented in swine. There is no description of clinical signs, immunity, diagnostics, prevention, or control of LASV infection in swine. Human clinical signs may include general malaise, lethargy, low-grade fever, and headache. Approximately 20% of infected individuals have disease that progresses and may include hemorrhage of the gingiva, conjunctiva, or nasal mucosa, pharyngitis, respiratory distress, vomiting, facial edema, arthralgia, pain in the chest, back and abdomen, and shock. Neurological symptoms may include hearing loss, which may become permanent, tremors, and encephalitis. Death may occur within two weeks of onset of symptoms and is due to multiple organ failure. Disease in pregnant women is generally severe and results in fetal death. Barrier nursing precautions are of utmost importance to prevent nosocomial transmission.

Enzyme linked immunosorbent assays (for detecting IgM and IgG, as well as Lassa antigen) are commonly used to diagnose LASV infection in humans and are commercially available (ReLASV®). Reverse-transcriptase polymerase chain reaction (RT-PCR) is considered the gold standard. Virus isolation can only be done in a BSL4 laboratory. None of these assays are currently available for reliable use in the field in LASV-endemic regions. Diagnostics are currently performed only by reference laboratories such as the Centers for Disease Control and Prevention (CDC) in the U.S.

A variety of vaccines have been developed and tested experimentally. They include recombinant vesicular stomatitis virus (rVSV) vectors expressing the glycoproteins or nucleoprotein of LASV, a yellow fever vaccine strain expressing LASV antigen, a reassortant live attenuated virus, and LASV virus-like particles. It is clear that humans who do not develop an appropriate cell-mediated immune response are much more likely to succumb to Lassa fever, and that neutralizing antibodies do not play an important role early in infection, if at all. High levels of viremia negatively correlate with clearance of LASV infection, and those individuals are much more likely to die.

Because clinical signs are generally mild and non-specific, diagnosis of LASV in humans is difficult and the only treatment for LASV, ribavirin, must be administered very early during infection for treatment to be successful. Similarly, the emergence of LASV in swine would likely be difficult to detect and treat. Diagnostic tests developed for humans could possibly be adapted to swine. Control measures for an LASV outbreak in swine have not been established. To prevent infections in humans, rodents and their excretions should be avoided. The rodent reservoir, *Mastomys natalensis*, is not native to the U.S.; however, the virus could enter the U.S. through deliberate or accidental importation of LASV-infected reservoir hosts. Efforts in development of reliable diagnostics and effective vaccines for LASV should continue.

LITERATURE REVIEW

1. Etiology

1.1 Key Characteristics

Lassa virus (LASV) is a member of the Old World complex of viruses, genus *Arenavirus*, family *Arenaviridae*, which also includes lymphocytic choriomeningitis virus (LCMV) and Lujo virus.[1] It is a bi-segmented, single-stranded, negative-sense RNA virus with a pleomorphic structure and a classic granular appearance common to all arenaviruses. The granularity is due to the incorporation of host cell ribosomes during virion assembly.[1-3] Virions range in size from 50–300 nm, are enveloped, and have surface club-shaped spikes, 8–10 nm in length, composed of glycoprotein (GP) one (GP1) and GP2.[4] Variability in virion size is due to variation in copy number of the small (S) and large (L) genome segments incorporated into each virion.[4] The helically symmetrical nucleocapsids of the genome have a string of beads appearance, appearing circular due to the formation of short, double-stranded panhandles by the 3' and 5' ends of the S and L genome segment.[4,5]

1.2 Strain Variability

LASV strains isolated from humans cluster into at least four lineages or clades based on geographic location.[6,7] Three of the four major clades occur within Nigeria and the fourth is found in Sierra Leone, Guinea, and Liberia.[6] Isolates from other countries, including Benin[3], Burkina Faso[8], Côte d'Ivoire[3,8,9], Ghana[10], Central African Republic[2,11], Senegal[12], Gambia[2], and Mali[13,14] may fall into separate lineages or clades. The virulence of individual virus strains has been investigated and non-Nigerian strains have been found to have higher viral copy numbers as well as higher case fatality rates in humans, suggesting greater virulence.[6] However, there is currently insufficient data to determine the differences in virulence among strains.

2. Cleaning and Disinfection

2.1 Survival

LASV is stable outside of the host, especially when relative humidity is below 30%. At 24°C and 32°C (75°F and 90°F), LASV has a half-life of 10–55 minutes.[15]

2.2 Disinfection

LASV is susceptible to inactivation by most detergents and disinfectants.[16] Sodium hypochlorite (0.5–1%[16]), phenolic compounds, 3% acetic acid, lipid solvents and detergents (e.g. SDS), formaldehyde/paraformaldehyde, glutaraldehyde (2%), and beta-propriolactone[16] disrupt virion integrity. Heating to 56–60°C (122–140°F), exposure to gamma or ultraviolet irradiation, exposure to pH less than 5.5 or greater than 8.5[16], autoclaving, incineration, and boiling result in virus inactivation.[15,16]

3. Epidemiology

3.1 Species Affected

The multimammate rat, *Mastomys natalensis*, is the primary reservoir host for LASV. In endemic areas, seroprevalence in *Mastomys natalensis* can reach 50%.[17] Other rodent species found to be reverse-transcriptase polymerase chain reaction (RT-PCR) positive for LASV include *Hylomyscus pamfi* in Nigeria[9], *Mastomys erytholeucus* in Nigeria and Guinea[9], and *Lemniscomys striatus*, *Praomys daltoni*, *Mus minutoides*, and *Praomys rostratus* in Guinea.[17] The incidence of LASV infection in swine is unknown.

3.2 Zoonotic Potential

LASV is zoonotic and poses a significant threat to public health. Between 100,000 and 300,000 people are thought to be infected annually in endemic areas, resulting in approximately 5000–10,000 deaths.[2,3] Humans are accidental hosts. Transmission occurs through direct contact with infected rodents as well as contact with food or fomites and inhalation of aerosolized excretions.[2,18] Risk factors for human LASV infection include hunting, cooking, and eating infected rats, and infestation of the home with infected rats.[2,18] *M. natalensis* is often found inside houses during the dry season in Guinea[3,17]; in houses where human LASV infection was found in Sierra Leone, *M. natalensis* were ten times more infected than in households that did not have LASV infection.[3] *M. natalensis* often lives in human settlements, in close proximity to feed stores and field crops. Housing quality may also impact the risk of exposure to LASV-infected rats and subsequent zoonotic transmission.[18]

Transmission of LASV among humans occurs through direct contact with infected patients' body fluids. The symptoms of Lassa fever in humans are varied and nonspecific. LASV causes a mild, flu-like illness in 80% of infected humans.[8] Others may go on to develop Lassa fever characterized by hemorrhage; respiratory distress; vomiting; facial swelling; pain in the chest, back, or abdomen; shock; and sometimes death.[8] Neurological signs, such as tremors and encephalitis have also been described. Hearing loss, which may be permanent, is a common complication occurring in up to one-third of human patients. Death is relatively uncommon, with an overall mortality rate of about 1%. However, during epidemics, the case-fatality rate in hospitalized patients may reach 50%.[8] Human-to-human transmission of LASV results in higher mortality than zoonotic infection (1–2%[15]). Use of standard barrier nursing precautions significantly reduces the risk of LASV transmission.[2]

3.3 Geographic Distribution

There is no information available on LASV infection in pigs.

Human LASV infection is confined to areas in which the LASV rodent reservoir species, *Mastomys natalensis*, is endemic.[19] The majority of documented cases of human LASV infections are from Nigeria, Sierra Leone, Liberia, and Guinea. Other countries with documented human LASV infections include Mali, Côte d'Ivoire, Ghana, Burkina Faso, Senegal, Central African Republic, Gambia, and Benin.[2] The first reported LASV infection in humans was in 1969 in Lassa, Nigeria, in a missionary nurse.[2,3]

3.4 Morbidity and mortality

There is no information available on morbidity and mortality due to LASV infection in swine.

4. Transmission

There is no information available on transmission of LASV in swine. Maintenance of LASV in the reservoir host, *Mastomys natalensis*, may occur through horizontal transmission during the rainy season, when there is more active mating and breeding.[17]

5. Infection in Swine/Pathogenesis

There is no information available on the pathogenesis of LASV infection in swine.

5.1 Clinical Signs

LASV infection has not been described in swine. Infections in the reservoir host, *Mastomys natalensis*, are subclinical.

5.2 Postmortem Lesions

There is no information available on the pathogenesis of LASV infection in swine

6. Diagnosis

6.1 Clinical History

There is no information on LASV infection in swine.

Clinical history in humans includes travel to or living in an endemic area. Symptoms are generally mild (80% of cases) and flu-like and include general malaise, lethargy, low-grade fever, and headache. In approximately 20% of infected individuals, disease progresses and may include hemorrhage of the gingiva, conjunctiva, or nasal mucosa, pharyngitis, respiratory distress, vomiting, facial edema, arthralgia, pain in the chest, back and abdomen, and shock.[2,8,15,16] Neurological symptoms can include hearing loss, which may become permanent[20], tremors, and encephalitis. Death may occur within two weeks of onset of symptoms and is due to multiple organ failure.[2,8,15,16] Disease in pregnant women is generally severe and results in fetal death.[15]

6.2 Tests to Detect Nucleic Acids, Virus, or Antigens

Virus isolation may be done using Vero cells and must be performed in a BSL4 laboratory, though it is not always successful.[21] Indirect fluorescent antibody testing can be used in conjunction with virus isolation to identify LASV in tissue culture cells.[21]

RT-PCR is considered to be the gold standard for diagnosis of LASV infection in humans.[21] However, should viremia be absent or below the limit of detection, the usefulness of RT-PCR is limited. A conventional RT-PCR assay to detect the nucleoprotein gene (NP) has been used with human clinical samples.[7,21] In conventional RT-PCR field testing for virus prevalence in rodent species, the LASV glycoproteins (GPs) and RNA-dependent RNA polymerase (L) gene have been targeted.[9] Some experimental work has targeted the L gene with a RT-PCR/ligase detection reaction (LDR) for differentiation among isolates.[22] Others have designed multiplex real-time qRT-PCR detecting the GP of LASV in conjunction with other viral hemorrhagic fever-causing viruses in a four-plex assay.[23] Given the geographic overlap of viral hemorrhagic fever-causing viruses and similarity of clinical signs in humans, this may prove to be very useful in the future. Whether such an approach will prove to be useful in swine remains to be determined.

LASV antigen (NP protein) may be detected using a commercially available, though not yet FDA approved, enzyme linked immunosorbent assay (ELISA), ReLASV®.[24] Immunohistochemistry can be used to diagnose LASV in tissue samples.[25]

6.3 Tests to Detect Antibody

There are commercially available IgM and IgG ELISAs (ReLASV®) available for detection of anti-LASV antibody in humans.[24] Whether these ELISAs can be adapted to use in swine is unknown.

6.4 Samples

There is no available information on sampling swine for LASV. Based on data from humans and experimentally-infected animals, blood is an appropriate sample to collect for virus isolation, RT-PCR, indirect fluorescent antibody testing, LASV antigen ELISA, and IgM/ IgG ELISA.[8,16,21] Additionally, samples appropriate for immunohistochemistry include lymph nodes, spleen, and liver, and assessing for LASV antigen by immunohistochemistry.[25]

7. Immunity

7.1 Post-exposure

There is no information available on immunity to LASV in swine.

7.2 Vaccines
There is no information on LASV vaccine development for use in swine.

Multiple vaccines have been developed and used experimentally in animal models. No vaccines are commercially available although there is an urgent need for a human vaccine. Current vaccine platforms include recombinant vesicular stomatitis virus (rVSV) expressing the nucleoprotein (NP) or glycoproteins (GPs) from one or another strain of LASV.[26,27] A live reassortant vaccine has been found to be efficacious in animal models of LASV, including non-human primates.[28,29] Additionally, a yellow fever 17D-based LASV vaccine and an alphavirus virus replicon particle platform have been described.[28] There are concerns regarding the use of any live virus or replication competent virus vector in immunocompromised individuals. A virus-like particle vaccine has also been described[30], which may prove safer in immunocompromised individuals.

7.3 Cross-protection
Experimental data show that CD4+ T-cell clones, derived from LASV antibody positive humans, are cross reactive against variable epitopes of the LASV nucleoprotein.[31] Additionally, in experimental vaccine studies using a rVSV-based LASV glycoprotein vaccine, complete protection was afforded against both homologous and heterologous lethal virus challenge in guinea pigs and cynomolgus macaques.[26,27] The mechanism of cross-protection against heterologous virus infection is not fully understood. Cell-mediated immunity appears more important in clearance of LASV than humoral immunity.[2]

8. Prevention and Control
The potential impact of an LASV outbreak in swine is unclear. Control measures for an LASV outbreak in swine are not established.

In endemic areas, humans can take measures to avoid contact with rodents and their excretions. For example, food should be stored in rodent-proof containers, and rodents should be excluded from homes. Trapping may reduce rodent populations. Rodents should not be consumed. If a rodent infestation is established, specific cleaning protocols can reduce the risk of disease transmission (see http://www.cdc.gov/rodents/cleaning/ for more information).

Measures to prevent human-to-human transmission in healthcare settings include isolation and use of barrier nursing precautions.[8,16] Treatment of LASV infection includes supportive care and ribavirin, if diagnosis is made early enough in infection.[3,8] Favipiravir, an RNA-dependent, RNA-polymerase inhibitor, is currently being tested in animal models of LASV infection and may prove to be a useful treatment option in the future.[32]

9. World Organization for Animal Health (OIE) Terrestrial Animal Health Code
LASV is not covered in the 2015 OIE Terrestrial Animal Health Code.[33]

10. Gaps in Preparedness
LASV infection in swine has not been reported in the literature; the clinical presentation, if any, has not been described.

LASV is not currently found in the U.S.[2,19] However, LASV could become endemic in some areas if *Mastomys natalensis* were to be deliberately or accidentally imported to the U.S. It is not known whether other rodent species in the Americas could act as LASV reservoirs.

The symptoms of LASV in humans are vague, making the infection difficult to detect. Travel history can aid in diagnosis. Furthermore, LASV can only be definitely diagnosed by reference laboratories such as the Centers for Disease Control and Prevention (CDC). Timely diagnosis is a challenge, particularly in developing countries.[3,8,16,32]

There are no licensed LASV vaccines available for use in humans or animals. Multiple experimental vaccines have been shown to protect against lethal LASV infection in animal models and there is a need for a licensed vaccine for people.

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