CHIKUNGUNYA VIRUS

SUMMARY

Etiology
- Chikungunya virus (CHIKV) is an Old World alphavirus within the family \textit{Togaviridae} that mainly causes disease in humans.
- There are three genotypes: West African, East Central South African (ECSA), and Asian. The ECSA genotype has caused human epidemics in Africa and the Indian Ocean Region. The Asian genotype circulates in Asia and has recently emerged in the Americas (Caribbean, Latin America, and the U.S.).

Cleaning and Disinfection
- The efficacy of most disinfectants against CHIKV is not known. As a lipid-enveloped virus, CHIKV is expected to be destroyed by detergents, acids, alcohols (70% ethanol), aldehydes (formaldehyde, glutaraldehyde), beta-propiolactone, halogens (sodium hypochlorite and iodophors), phenols, quaternary ammonium compounds, and lipid solvents. Exposure to heat (58°C [137°F]), ultraviolet light, or radiation is also sufficient to render togaviruses inactive.

Epidemiology
- Humans act as hosts during CHIKV epidemics. Animal species including monkeys, rodents, and birds are also capable hosts.
- Natural CHIKV infection has not been documented in pigs. There is some evidence that pigs can mount an antibody response to the virus.
- In humans CHIKV causes fever, myalgia, and polyarthritis that can persist for years. A maculopapular, pruritic rash, lasting about one week, is seen in about half of human patients. Neonates infected with CHIKV can develop serious disease affecting the heart, skin, and brain. Bleeding and disseminated intravascular coagulation have also been observed in humans. Morbidity is high, but CHIKV rarely causes death.
- CHIKV has been detected in more than 60 countries worldwide including Asia, Africa, Europe, and the Americas.

Transmission
- Two transmission cycles exist for CHIKV. In Africa, the virus persists via a sylvatic cycle involving non-human primates and mosquitoes of the \textit{Aedes furcifer-taylori} group. Humans in rural areas are sporadically affected during ‘spill-over’ events. A sylvatic cycle may also exist in some parts of Asia.
• The urban cycle exists where a human-mosquito-human cycle is maintained. This has resulted in large CHIKV outbreaks in Africa, related to the range expansion of *Ae. albopictus*, and is the main cause of CHIKV persistence linked to *Ae. aegypti* in Asia.

**Infection in Swine/Pathogenesis**

• There is no information available on the pathogenesis or signs of CHIKV infection in swine.

**Diagnosis**

• In humans, the Centers for Disease Control and Prevention (CDC) recommends testing via quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) within the first 7 days of illness.
• Serologic testing should be performed if qRT-PCR results are negative 5–7 days after symptom onset, since viremia decreases over time. A positive serologic test can be confirmed by the plaque reduction neutralization test (PRNT).

**Immunity**

• There is no vaccine for CHIKV in humans or animals although multiple candidates utilizing a variety of platforms are in development.
• Long lasting cross-protection between CHIKV lineages occurs in humans.

**Prevention and Control**

• There are no specific prevention and control measures for CHIKV in swine.
• Prevention in humans involves vector control and insect repellent use.

**Gaps in Preparedness**

• Little is known about CHIKV infection in swine. Natural infections have not been reported and clinical signs of disease have not been described. There are no diagnostic tests validated for pigs. In addition, there is no CHIKV vaccine. The efficacy of most disinfectants against CHIKV is not known, and there are no Environmental Protection Agency (EPA)-registered products.
**OVERVIEW**

Chikungunya virus (CHIKV) is an arbovirus in the family **Togaviridae**. It is an Old World alphavirus belonging to the Semliki Forest antigenic complex, which also includes Bebaru virus, Mayaro virus, O’nyong nyong virus, Ross River virus, Getah virus, Semliki Forest virus, and Una virus. CHIKV is a single-stranded, positive-sense RNA virus, enveloped with a spherical shape and icosahedral symmetry. Mutations in the surface envelope glycoproteins E1 and E2 have been identified in recent epidemic strains.

Three CHIKV genotypes have been described. The West Africa and East Central South African (ECSA) lineages evolved in Africa are thought to have diverged about 350 years ago. The ECSA genotype was first isolated from humans in Tanzania in the early 1950s. The sylvatic transmission cycle, which exists largely between mosquitoes of the *Aedes furcifer-taylori* group and non-human primates, leads to sporadic human infections in rural parts of Africa. Periodic epidemics also occur in urban environments due to a human-mosquito-human cycle. Range expansion of the vector *Ae. albopictus* has led to CHIKV epidemics in Africa, while *Ae. aegypti* has traditionally been associated with urban CHIKV in Asia.

A novel ECSA strain infected nearly two million people throughout the Indian Ocean region in the early 2000s. The Indian Ocean Lineage (IOL) strain was subsequently imported to Europe and autochthonous transmission was identified in Italy and France. The Asian genotype, first identified in the late 1950s in Thailand, is responsible for the emergence of CHIKV in the Americas. Since 2013, the virus has spread throughout the Caribbean, Latin America, and the U.S. with nearly 1.4 million suspected cases. In 2014, autochthonous CHIKV transmission was described in Florida; however, transmission in the Americas seems currently to be waning.

CHIKV mainly causes disease in humans. Fever and myalgia are common, as is polyarthritis that may persist for years. A maculopapular, pruritic rash, lasting about one week, is seen in about 40–50% of patients. Neonates infected with CHIKV can develop serious disease affecting the heart, skin, and brain. Bleeding and disseminated intravascular coagulation have also been observed. Although morbidity is high, CHIKV infection rarely results in death. Animal species that act as hosts include monkeys, rodents, and birds. Other vertebrate hosts are likely but have not been identified. Natural CHIKV infection has not been documented in pigs; however, some studies have shown that pigs can mount an antibody response to the virus.

Clinical diagnosis of CHIKV is not possible because of the similar presentation to other tropical febrile diseases. In humans, the Centers for Disease Control and Prevention (CDC) recommends testing via quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) within the first 7 days of illness. Serologic testing should be performed if qRT-PCR results are negative 5–7 days after symptom onset, since viremia decreases over time. A positive serologic test can be confirmed by the plaque reduction neutralization test (PRNT). CHIKV RT-PCR, IgM enzyme linked immunosorbent assay (ELISA), and PRNT are performed at CDC, although some state and territory laboratories may provide CHIKV testing as well as several commercial reference laboratories, although no assays are approved by the Food and Drug Administration (FDA).

There is no treatment for CHIKV and there is no vaccine, although multiple vaccine candidates utilizing a variety of platforms are in development. In humans, CHIKV is prevented through mosquito control. This includes habitat management (eliminating standing water, etc.), use of pesticides (larvicides and adulticides), and biological and genetic methods of control. To reduce the potential for bites, humans can wear Environmental Protection Agency (EPA)-registered insect repellants and treat clothing and gear. Screening windows and doors helps keep mosquitoes out of homes. There are no specific measures to prevent CHIKV infection in swine.
Although neutralizing antibody to CHIKV has been detected in pigs infected experimentally, clinical signs of disease have not been described. An outbreak in pigs may be difficult to detect, and there are no diagnostic tests validated for animals. Vaccine development should continue. Cleaning and disinfection protocols have not been established and there are no EPA-registered disinfectants for CHIKV.
LITERATURE REVIEW

1. Etiology

1.1 Key Characteristics
Chikungunya virus (CHIKV) is an arbovirus belonging to the genus *Alphavirus*, family *Togaviridae.*\(^1\) Alphaviruses can be divided into two groups: New World viruses and Old World viruses, to which CHIKV belongs. Old World alphaviruses are best known for causing rheumatic disease in humans.\(^2\) Eight antigenic complexes have been described. The Semliki Forest complex contains CHIKV, as well as Bebaru virus, Mayaro virus, O’nyong nyong virus, Ross River virus, Getah virus, Semliki Forest virus, and Una virus.\(^1\)

CHIKV is a single-stranded, positive-sense RNA virus. Its genome contains nearly 12,000 nucleotides.\(^1\) The virus is enveloped with a spherical shape and icosahedral symmetry. CHIKV is approximately 70nm in size.\(^1\) CHIKV contains four non-structural proteins, nsP1–4, which are found in infected cells and encode the viral replication machinery of the virus.\(^1\) Structural proteins include the capsid protein (C), two surface envelope glycoproteins (E1 and E2), and two leader peptides for E1 and E2 (denoted E3 and 6K).\(^1\)

1.2 Strain Variability
1.2.1 African Genotypes
It is thought that CHIKV evolved in Africa and diverged into two principal lineages—West African and East Central South African (ECSA)—about 350 years ago.\(^3\) The ECSA genotype was first isolated from humans in Tanzania in the early 1950s.\(^4,5\) Since that time, CHIKV has continued to circulate in Africa, with periodic outbreaks being detected. Notable CHIKV epidemics occurred in the Democratic Republic of the Congo in 1999–2000\(^6\) and in Gabon in 2006–2007.\(^7\)

In 2004, a novel ECSA strain was identified in Kenya. The virus, later classified as the Indian Ocean Lineage (IOL) strain, spread to the Indian Ocean region in 2005.\(^8\) A second, related ECSA was introduced to India in 2006.\(^9\) Altogether, nearly two million people were infected with CHIKV. Around this time, imported CHIKV cases began to surface in Europe. In Italy and France, autochthonous transmission was documented, originating from infected travelers.\(^10,11\) Imported cases also occurred in the Americas, but no local transmission was detected. In part, the success of epidemic IOL strains came from mutations in the E1\(^12,13\) and E2\(^14,15\) envelope glycoprotein genes, which allowed the virus to replicate in both *Ae. aegypti* and *A. albopictus* mosquitoes and expand its geographic range. Epidemic transmission has now slowed in most of Asia. Historic accounts show that African-origin CHIKV has caused global pandemics every 40–50 years.\(^16\)

1.2.2 Asian Genotype
In the late 1950s, a CHIKV variant identified in Thailand was classified as a new genotype, though it originated from the ECSA lineage\(^17\) and was likely introduced to Asia decades earlier.\(^18\) The so-called Asian genotype has since become widespread in Southeast Asia and—as in Africa—causes occasional human outbreaks.

In late 2013, the first outbreak of CHIKV with autochthonous transmission was documented in the Americas on the island of St. Martin.\(^19\) Unexpectedly, the Asian genotype was identified as the cause. The Caribbean virus was most closely related to CHIKV strains identified in China in 2012 and the Philippines in 2013. These isolates, along with strains from Micronesia, have since been termed Cosmopolitan Asian CHIKV (CACV).\(^20\) The Caribbean CHIKV strain is known to have acquired three adaptive amino acid substitutions in nsP1, E1, and E3.\(^20\) By mid-2015, CHIKV had reached much of the Caribbean, Latin America, and the U.S., with nearly 1.4 million suspected cases.\(^21\) In the U.S., nearly
3000 cases were reported to ArboNET in 2014, including 12 autochthonous cases from Florida. In 2015, nearly 700 cases of CHIKV disease were reported to ArboNET, and all were in travelers returning from affected areas. As of 2016, CHIKV transmission in the Americas seems to be waning.

2. Cleaning and Disinfection

2.1 Survival
It is apparently not know whether CHIKV survives outside of the body and in insect vectors.

2.2 Disinfection
In general, lipid-enveloped viruses including togaviruses are destroyed by detergents, acids, alcohols (70% ethanol), aldehydes (formaldehyde, glutaraldehyde), beta-propiolactone, halogens (sodium hypochlorite and iodophors), phenols, quaternary ammonium compounds, and lipid solvents. Exposure to heat (58°C [137°F]), ultraviolet light, or radiation is also sufficient to render togaviruses inactive.

A citrate-based product (marketed as Clinister®) has been shown experimentally to inhibit CHIKV at a concentration of 1.5 mg/ml. However, there are no Environmental Protection Agency (EPA)-registered disinfectants for CHIKV.

3. Epidemiology

3.1 Species Affected
While humans serve as reservoirs during urban epidemics, several animal species are capable CHIKV hosts. Monkeys, rodents, and birds are thought to act as reservoirs for the virus, and it is likely that other vertebrates hosts have not yet been identified.

3.1.1 Pigs and Evidence of CHIKV Exposure
Although natural CHIKV infection has not been documented in pigs, there is some evidence that pigs can mount an antibody response to the virus.

- A 1964 study conducted in Thailand found hemagglutination-inhibiting and neutralizing antibodies to CHIKV in nearly 35% of swine samples (n not reported).
- From 1963–67 serum was obtained from animals, including pigs at slaughter, in Malaysia and 48/328 (12.8%) demonstrated evidence of CHIKV infection via the plaque reduction neutralization test (PRNT). However, antibodies to either Getah virus or Sindbis virus were also detected in most samples; CHIKV neutralizing antibody was detected in only one pig that did not react to other viruses. Twelve wild pigs were also tested and no antibodies to CHIKV were detected.
- In 1966, a study of pigs at slaughter in the Philippines identified antibodies to CHIKV in 5/91 (5.5%) serum samples tested via complement fixation. However, all reactors to CHIKV also reacted to Semliki Forest virus.
- In Eastern Europe, a 1975 study of domestic animals including 86 pigs found no antibody response to CHIKV using the hemagglutination inhibition assay.
- In Bihar, India, serum was collected from pigs in the late 1970s for arboviral testing following an epidemic of encephalitis in humans. Using the hemagglutination inhibition and complement fixation assays, 10 (2.5%) and 2 (0.5%) of pigs were found to react only to CHIKV.
- In a recent study, pigs were among North American bird and mammalian species experimentally infected with two strains of CHIKV. Following subcutaneous injection of the virus, no pigs developed detectable viremia or clinical signs of disease, although neutralizing antibodies were identified 14 days post-infection via PRNT.
3.1.2 Pigs and Evidence of Exposure to Other Old World Alphaviruses

Pigs are susceptible to at least some other Old World alphaviruses. Though infrequent, Getah virus causes clinical disease in swine. Serosurveys have demonstrated that pigs may be subclinically infected with Ross River virus, particularly during times of human epidemics. Antibodies to Sagiyama virus, which is closely related to Ross River virus, have also been demonstrated in pigs. Pigs have been identified as potential hosts for Ndumu virus, another member of the family Togaviridae.

3.2 Zoonotic Potential

Chikungunya is mainly a disease of humans that causes large sporadic epidemics. Both sylvatic and urban transmission cycles have been described (see section 4 for more information). In adults, fever and myalgia are very common, followed by polyarthralgia/polyarthritis which occurs in more than 95% of patients. In some instances, arthritic disease can persist for years. A maculopapular, pruritic rash, lasting about one week, is seen in about 40–50% of patients. Neonates infected with CHIKV can develop serious disease affecting the heart, skin, and brain. Bleeding and disseminated intravascular coagulation have also been observed. Most people infected with CHIKV develop disease; however, death is uncommon.

3.3 Geographic Distribution

In humans, CHIKV infection occurs in more than 60 countries throughout Asia, Africa, Europe, and the Americas. The mosquito vector *Ae. aegypti* is found in the tropics and sub-tropics, while the range of *Ae. albopictus* includes temperate and even cold regions. *Ae. albopictus* is responsible for the establishment of CHIKV in Europe and the Americas.

3.4 Morbidity and Mortality

No information was found on morbidity and mortality due to CHIKV in swine.

4. Transmission

In Africa, CHIKV cycles between non-human primates and *Aedes* spp. found in forest environments, such as *Ae. furcifer* and other members of the *Ae. furcifer-taylori* group. Humans in rural populations are occasionally affected through ‘spill over’ events. There is some evidence that a sylvatic cycle also exists in Asia, particularly in Malaysia and the Philippines.

The urban cycle exists where large populations of humans and urban *Aedes* spp. exist, maintaining a human-mosquito-human cycle. The urban cycle has resulted in large CHIKV outbreaks in Africa, related to the range expansion of *Ae. albopictus*, and is the main cause of CHIKV persistence linked to *Ae. aegypti* in Asia. Vertical transmission has also been documented in humans.

No information was found regarding CHIKV transmission in swine.

5. Infection in Swine/Pathogenesis

5.1 Pathogenesis

No information was found regarding the pathogenies of CHIKV in swine.

5.2 Clinical Signs

Natural CHIKV infection has not been documented in swine. In one experimental study, pigs inoculated with CHIKV subcutaneously did not develop viremia or clinical signs of disease, although an antibody response was detected via PRNT.

5.3 Postmortem Lesions

No lesions caused by CHIKV have been documented in swine.
6. Diagnosis

6.1 Clinical History
In humans, infection with CHIKV may be suspected when fever and joint pain are present. Clinical diagnosis is difficult because of the similar presentation to other tropical febrile diseases including dengue, malaria, typhoid, scrub typhus and others.36

6.2 Tests to Detect Nucleic Acids, Virus, Antigen, and Antibody
Chikungunya virus is a BSL-3 agent and should be handled accordingly. High levels of viremia occur in humans and serum from suspected cases should be treated as potentially infectious.37

According to the Centers for Disease Control and Prevention (CDC), within the first 7 days of illness, the preferred test for CHIKV diagnosis in humans is quantitative reverse transcriptase polymerase chain reaction (qRT-PCR). Serologic testing should be performed if qRT-PCR results are negative 5–7 days after symptom onset, since viremia decreases over time. Virus-specific IgM antibodies may not be detectable via enzyme linked immunosorbent assay (ELISA) until 7 days after the onset of illness. A positive result can be confirmed by PRNT.37

CHIKV RT-PCR, IgM ELISA, and PRNT are performed at CDC.37 Testing may also be available at some state and territory laboratories. CDC provides virus primer/probe sequences, an RNA-positive control, and RT-PCR proficiency panels to laboratories that have demonstrated proficiency using the CDC West Nile virus RT-PCR assay.37

Several commercial reference laboratories offer CHIKV testing in humans, but none of the assays are FDA-cleared. According to CDC37, available tests for CHIKV include:
- qRT-PCR, IgM and IgG IFA assays (Focus Diagnostics, U.S.), and
- IgG and IgM ELISA (ARUP Laboratories, U.S.).

CDC lists37 the following human CHIKV IgM antibody test kits available for purchase in the U.S.:
- Anti-CHIKV IgM human ELISA kit (Abcam, UK),
- Anti-CHIKV ELISA (IgM) (Euroimmun, Germany), and
- Anti-CHIKV IIFT (IgM) (Euroimmun, Germany).

6.3 Samples
6.3.1 Preferred Samples
Suitable samples for RT-PCR, IgM ELISA, and PRNT testing in humans include serum, cerebrospinal fluid, urine, and possibly others.

6.3.2 Oral Fluids
Since CHIKV has not been reported in pigs, there is no information on the use of oral fluids for diagnostic testing.

7. Immunity

7.1 Post-exposure
In humans, an IgM response is detectable 3–8 days after symptom onset and persists for 1–3 months. IgG can be detected via ELISA within 4–10 days of symptom onset and lasts for years, perhaps for life.2
7.2 Vaccines
There is no commercially available vaccine for CHIKV in humans or animals. At least 15 vaccine candidates are currently in development, utilizing a number of platforms (inactivated, live attenuated, live vectored, chimeric, virus-like particle [VLP], subunit protein, and DNA).38,39

7.3 Cross-protection
Since CHIKV lineages are composed of a single serotype, long lasting cross-protection between lineages occurs.38 Some cross-protection also occurs between CHIKV and other alphaviruses. There is evidence that in mice previously infected with Ross River virus, inoculation with CHIKV results in lower mean peak viremia and protection against clinical disease. Similarly, when antiserum from Ross River virus-infected mice was transferred into naïve mice, they were protected against CHIKV disease.40 Serological studies in pigs have suggested antigenic cross-reaction between CHIKV and Semliki Forest virus28, Getah virus, and Sindbis virus.27

8. Prevention and Control
There are no specific measures for preventing CHIKV infection in pigs.

In general, preventing human infection with CHIKV is best accomplished through mosquito control. Mosquitoes lay eggs in standing water (in gutters, old tires, buckets, etc.). Any potential source of standing water should be emptied, and water in bird baths, fountains, wading pools, etc. should be changed at least once per week.41 Pesticides, including larvicides and adulticides, can be part of a mosquito control program but also have toxic effects. For more information, see https://www.epa.gov/mosquitocontrol.

Biological and genetic methods of mosquito control are being explored. These include infection with the bacterium Wolbachia, which provides the mosquito with varying degrees of antiviral protection42, release of mosquitoes genetically modified to resist arboviral infection, and release of mosquitoes carrying a lethal gene in order to reduce the vector population.43

To prevent mosquito bites, CDC recommends using an EPA-registered insect repellant with the active ingredients of DEET, picaridin, IR3535, oil of lemon eucalyptus, or para-methane-diol.44 Clothing and gear can also be treated with the insecticide permethrin.44 When outdoors, people should wear long-sleeved shirts and long pants. Since many mosquito species are active at dusk and dawn, staying inside during those hours may reduce mosquito exposure—however, some mosquitoes, such as the CHIKV vector Aedes, bite during the day as well. To keep mosquitoes out of the home, screen all windows and doors.

There is no antiviral treatment for CHIKV infection. Supportive care for humans includes rest, fluids, anti-pyretics, non-steroidal anti-inflammatory drugs, etc. Experimentally, a number of antivirals have demonstrated in vitro efficacy against CHIKV; however, most of these drugs have not been tested in animal models or have been shown to be less effective in vivo.45

CHIKV is not included in the 2015 OIE Terrestrial Animal Health Code. There are no restrictions for importation of animals from countries or zones infected with this virus.

10. Gaps in Preparedness
If CHIKV were to be introduced to the U.S., the extent to which local transmission could occur is unclear. Aedes spp. expansion into North America may be somewhat seasonal, although the mosquito’s estimated range encompasses at least the southern 1/3 of the U.S. according to the CDC.46 Changes in mosquito
populations and habitat could lead to the expansion of arboviruses such as CHIKV.\textsuperscript{47} The likelihood of CHIKV endemicity is also affected by the potential to establish sylvatic transmission cycles involving non-human primates in the U.S.\textsuperscript{18}

Currently, natural CHIKV infection in swine has not been reported. Although neutralizing antibody has been detected in pigs infected experimentally, clinical signs of disease have not been described. The signs in swine may be vague, as in humans, and an outbreak may be difficult to detect. There are no diagnostic tests validated for animals. In addition, there is no CHIKV vaccine. Cleaning and disinfection protocols for swine facilities have not been established and there are no EPA-registered disinfectants for CHIKV.
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