United States Department of Agriculture

Animal and Plant Health Inspection Service

Veterinary Services

July 2022

Swine Hemorrhagic Fevers: African and Classical Swine Fevers

Integrated Surveillance Plan

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Swine Hemorrhagic Fevers Surveillance Plan, Version 2
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1. Disease Description

a. African Swine Fever

African swine fever (ASF) is a highly contagious viral hemorrhagic disease caused by a large, enveloped, double-stranded DNA virus of the family *Asfarviridae* and genus *Asfivirus.* The ASF virus (ASFV) affects animals in the family Suidae, including domestic pigs, feral pigs, and Eurasian wild boar. African wild swine, such as warthogs and bushpigs, act as reservoir hosts but do not show signs of disease. Infection caused by ASF virus can be peracute, acute, subacute, or chronic. Rarely, pigs that recover from infection can become persistently infected carriers of the virus. Soft ticks of the genus *Ornithodoros* are natural arthropod hosts for the virus. The zoonotic potential is negligible; no evidence suggests that ASF virus affects people. The disease has been successfully excluded from many developed nations with extensive swine production but is endemic in Africa. Outbreaks in countries free of ASF can severely impact producers due to high swine mortality, the curtailment on exports of swine and pork products, and costs to control and eradicate the disease. Currently, no approved vaccine or treatment is available.

b. Classical Swine Fever

Classical swine fever (CSF) is a highly contagious viral septicemia, caused by a small, enveloped RNA virus of the family *Flaviviridae* and genus *Pestivirus,* that only affects swine. CSF has several clinical presentations (acute, chronic, and congenital infection) that are dependent on the host's previous exposure to the virus, viral virulence (high, moderate, and low), and host factors such as age and nutritional status. Young animals are usually affected more severely than older animals and mortality rates may reach up to 90 percent; in older breeding pigs, the course of the infection is often mild or even subclinical. Naïve populations tend to be more severely affected and are more likely to present with the classical acute presentation; however, the classical acute presentation is now rarely seen. Instead, more moderate forms and presentations of the disease predominate. Prevailing strains of CSF virus (CSFV) exhibit moderate to low virulence, making clinical diagnosis difficult especially in older animals. Low virulence strains usually give rise to a mild disease or subclinical infection that can remain undetected for long periods of time. Leukopenia, a drop in white blood cell numbers, is a consistent clinical laboratory finding, except with low virulence strains. Also known as hog cholera, CSF has been eradicated from many developed nations with extensive swine production but is still endemic in much of the world. Although vaccines for CSF are available, outbreaks in countries free of CSF can severely impact producers due to high swine mortality, the curtailment on exports of swine and pork products, and costs to control and eradicate the disease.

2. Purpose and Rationale

The increased spread of ASF in Asia, Europe, and the Caribbean and CSF in the Caribbean and South America raises concern for potential disease introduction into the United States. Detection of these diseases upon introduction may be complicated because the current clinical presentations of ASF and CSF throughout the world resemble those of many other production diseases present in the United States. Therefore, the U.S. Department of

Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) proposes an integrated active surveillance plan for ASF and CSF targeting higher-risk populations, sick pigs, and mortality events with the purpose of enhancing the vigilance for both diseases and the country's preparedness for emergency response. This document outlines an active integrated surveillance plan that builds upon current diagnostic and surveillance methodologies for both diseases.

3. Surveillance Objectives

a. Objective 1. Strengthen detection capabilities and enhance outbreak preparedness for ASF and CSF

Previously, ASF detection relied on passive surveillance and the reporting of suspect disease cases. Now, due to the approval of additional diagnostic specimens (e.g., tonsil, spleen tissue, and whole blood) as valid sample types for real-time Polymerase Chain Reaction (qPCR) testing in the National Animal Health Laboratory Network (NAHLN); APHIS has integrated ASF surveillance with CSF active surveillance into a single active surveillance plan. This integration of swine fever surveillance plans strengthens our ability to detect an ASF or CSF incursion at the national level and assists in preparedness.

A foreign animal disease (FAD) outbreak (including ASF and CSF) could severely impact our high-volume sample collection, laboratory capacity, and data management capabilities. By executing a targeted active surveillance program, these systems are constructed and tested at lower sample sizes. This will reduce the impact of a FAD while still providing confidence in the processes.

Timely and consistent surveillance will also provide a baseline of disease absence data to assist in situational assessment at the outset of an outbreak. Documentation of recent negative test results could provide at least a short-term level of confidence in disease freedom in unaffected areas. These data could also decrease the amount of initial outbreak testing required to assess the scope of the outbreak if performed on a timely and consistent basis.

b. Objective 2. Support claims of disease freedom for ASF and CSF

In addition to improving detection capabilities, this surveillance plan aims to provide continued support for U.S. claims of disease freedom from ASF and CSF. This support is obtained from the surveillance components as outlined below, without additional samples needed from other non-targeted components.

4. Expected Outcomes: Products, Decisions, and Actions

Expected outcomes include:

a. Products. VS will generate surveillance reports quarterly and annually.

b. Evaluation. VS will evaluate the surveillance system once every 3 years.

c. Decisions and actions. APHIS will use the information and results provided in evaluations and reports to support trade claims of disease freedom and to improve our preparedness for a potential introduction of these diseases. Any major change in the introduction threat of ASF or CSF to the United States, in diagnostic capabilities, or in the swine industry will lead VS to a review and potential modification of the plan.

5. Stakeholders and Responsible Parties

Table 1. Stakeholders and their responsibilities and interests

Stakeholder	Interest/Responsibility
USDA-APHIS-VS	Cooperative Data Sharing
Field Operations (FiOPS)	• Field implementation of ASF/CSF surveillance activities, including sample collection and data collection • Situational assessment and implementation of disease response
Strategy and Policy (S&P)	• Development, evaluation, reporting, and revision of the ASF/CSF surveillance plan; data analysis • Risk-based analysis • Policy and budget • Import, export, and international health status management • Surveillance data management • Coordination of disease response
Diagnostics and Biologics (D&B)	• Diagnostic laboratory support, reference laboratory services, sample testing and data reporting, diagnostic test development and validations • Data to support possible vaccine development
National Animal Health Laboratory Network (NAHLN)	• Sample testing and electronic submission of test information
APHIS-Marketing and Regulatory Programs Information Technology	• Development and maintenance of a data management framework infrastructure
Wildlife Services (WS)	• Feral swine surveillance activities
State animal health officials and field staff	• Jointly responsible with VS Area Veterinarian-in-Charge (AVIC) for field implementation, sample collection, data collection, identification of epidemiological changes related to disease, and coordination of disease response
Veterinarians, industry field representatives, and individual producers	• Animal health and production monitoring, rapid disease detection and reporting, sample collection and submission, biosecurity plans, and support for business continuity
Academia	• Support with diagnostic validations, introduction pathways and risk assessments
Agricultural Research Service (ARS)	• Support with diagnostic validations, molecular epidemiology studies and development of new diagnostic and vaccine capabilities
Food Safety and Inspection Service (FSIS)	• Share condemnation information by code
Industry producer groups	• Industry outreach and programming, scientific issues, surveillance data
USDA APHIS International Services and Foreign Agricultural Service, trading partners	• Trade issues and international disease status report updates

Stakeholder	Interest/Responsibility
Commercial diagnostic and reagent companies	• Manufacture and sales of commercial reagents and assays

6. Population Descriptions and Characteristics

This plan focuses on three U.S. swine populations for surveillance, including larger commercial swine herds, higher-risk (less biosecure) swine herds, and feral swine. APHIS, State and industry partners will monitor all three populations. If clinical signs consistent with the ASF or CSF case definitions are observed, an FAD investigation must be initiated immediately.

This monitoring is accomplished through the testing of five surveillance components:

1. Foreign Animal Disease Investigations
2. Case-compatible sick pig Veterinary Diagnostic Laboratory (VDL) submissions
3. Case-compatible slaughter condemnation samples and aggregation point samples from sick or dead pigs
4. Higher-Risk swine including less biosecure farms, waste feeders, herds with potential or known feral swine exposure, and samples from markets known to server higher-risk operations
5. Feral swine

WS will monitor feral swine for CSF and/or ASF by testing serum and whole blood samples collected as part of its activities to reduce crop damage. When feral pigs are found sick or dead due to non-traumatic events (disease, starvation, old age, etc.), VS will test for ASF and CSF by viral antigen testing through FAD investigations. WS will collaborate with the AVIC or State animal health official to initiate FAD investigations.

Since all samples, regardless of surveillance component, tested for ASF will be initially tested with qPCR, which detects a nucleic acid sequence specific to ASFV, testing healthy swine without clinical signs would provide little diagnostic value as healthy animals would not be expected to have detectable ASFV. Therefore, sampling sick swine in all components provides a higher likelihood of detecting the disease. Because mild strains of CSF may fail to generate significant or observable clinical signs, it is important to monitor serum from apparently healthy higher-risk swine and feral swine for antibodies to CSFV.

7. Case Definitions

See Appendixes 1 and 2 for full versions of these case definitions.

Note: VS may review the case definitions throughout an outbreak and may modify it as additional information becomes available or the needs change. Currently, National Veterinary Services Laboratory (NVSL) Foreign Animal Disease Diagnostic Laboratory (FADDL) must confirm both ASF and CSF diagnoses.

African Swine Fever Case Definition and Reporting Criteria

1.1. **Suspect Case:**
  1.1.1. an animal having clinical signs consistent with ASF; **OR**
  1.1.2. an epidemiologic link to ASFV
1.2. **Presumptive Positive Case:** a suspect case with non-negative screening test result for ASFV (PCR, enzyme-linked immunosorbent assay [Ab ELISA])
1.3. **Confirmed Positive Case:**
  1.3.1. an animal from which ASF virus has been isolated; **OR**
  1.3.2. otherwise identified, by at least two different tests[1]
    1.3.2.1. one antigen **AND** one antibody (Ab) assay, especially in the case of subacute or chronic cases; **OR**
    1.3.2.2. two antigen assays.

Classical Swine Fever Case Definition and Reporting Criteria

1.1. **Suspect Case**: an animal having
  1.1.1. clinical signs consistent with CSF; **OR**
  1.1.2. an epidemiologic link to CSFV.
1.2. **Presumptive Positive Case:** a suspect case with a non-negative screening laboratory test result for CSFV (reverse transcriptase-qPCR [RT-qPCR], Ab ELISA)
1.3. **Confirmed Positive Case:**
  1.3.1. an animal from which an approved laboratory has isolated CSF virus; **OR**
  1.3.2. otherwise identified, by at least two different tests[2]
    1.3.2.1. one antigen **AND** one antibody assay, especially in the case of subacute or chronic cases; **OR**
    1.3.2.2. two antigen assays

8. Data Sources and Sampling Methods

a. Passive Foreign Animal Disease Investigations

Anyone interacting with swine (including APHIS or State personnel, producers, and private veterinarians) who observe animals or herds meeting the case definition, or those in which ASF or CSF could otherwise reasonably be suspected, must report suspicious cases to local State and Federal animal health officials. This reporting may initiate an FAD investigation by a foreign animal disease diagnostician (FADD). See VS Guidance 12001[3] for additional information.

The FADD will collect a minimum of serum, tonsil scraping, and whole blood (ethylenediaminetetraacetic acid [EDTA] and heparin) from live affected swine. When possible, the FADD should necropsy at least one pig, and ideally up to 10 pigs, and collect a full set of tissues including tonsil, lymph node, lung, kidney, spleen, liver, heart,

[1] Under unique conditions, animal health officials may consider multiple non-negative ELISAs supported by positive IPT as confirmed positive cases.
[2] Under unique conditions, after confirming a negative vaccine status, animal health officials may consider multiple non-negative ELISAs supported by positive IPTs as confirmed positive cases.
[3] https://www.aphis.usda.gov/animal_health/lab_info_services/downloads/VSG_12001.pdf

serum, and whole blood consistent with the *Foreign Animal Disease Investigation Manual*. An additional oral fluids (OF) sample can be collected and paired with an approved sample type. For further guidance on OF collection refer to Appendix 3. To maximize detection of a potential ASF incursion into the United States, an FADD or WS staff will initiate collection of samples from feral swine suffering non-traumatic morbidity and mortality events via an FAD investigation.

b. Active Surveillance

1. Sick Pigs Submitted to Veterinary Diagnostic Laboratories (VDL)

Most of swine tissue submissions to VDLs originate from clinically ill commercial U.S. swine operations and include cases where ASF and CSF should be considered as part of the differential diagnosis list. Since the specimens come from clinically ill swine, these samples represent the highest surveillance value for improving ASF/CSF detection capability. Laboratory personnel reviewing the case make the decision to test VDL submissions for ASF and CSF. If the submitter suspects ASF or CSF, the appropriate FAD investigation protocols should be followed.

ASF and CSF-approved NAHLN laboratories and the NVSL-approved Dorado Laboratory (in Puerto Rico) will use the following selection criteria to identify eligible cases for ASF and CSF surveillance testing. Any swine accession submitted is eligible for testing if:

- One of the following tissue specimens can be obtained from all tissue submissions for other disease testing (in order of priority):
  - Tonsil or spleen
  - Lymph node(s)
  - Whole blood
- AND one or more of the following lesions or herd history is observed and/or reported:
  - Hyperacute septicemias
  - Skin discoloration
  - Hemorrhagic or swollen lymph nodes
  - Enlarged spleen
  - Kidney petechia
  - Epistaxis
  - Abortions, particularly with congenital deformities
  - Button ulcers in the colon
  - Intestinal hemorrhage
  - Tonsil pathology (tonsillitis, hemorrhagic, necrotic foci, etc.)
  - Undiagnosed central nervous system (CNS) cases (especially congenital tremors and nonsuppurative encephalitis)
  - Herd mortality greater than established baseline mortality and at least one other ASF- or CSF-compatible clinical sign in the barn
  - Other clinically, grossly, or histologically compatible cases that the pathologist submits due to suspicion of ASF or CSF

2. Slaughter and Aggregation Point Surveillance

The primary targets for ASF and CSF targeted slaughter surveillance are slaughter plant condemnations (including butcher, sow, and roaster markets), and samples from ill or dead swine collected at swine aggregation points. Aggregation points can include buying stations for market swine, non-conforming (not fitting the target specifications for the industry) commercial swine and cull sows/boars. Roaster markets may include non-conforming swine or animals specifically raised to a smaller body size. Roaster markets across the country should be sampled, with special attention to roaster markets in Florida and Texas, as well as slaughter facilities in Puerto Rico and the U.S. Virgin Islands. These States have a higher probability of disease introduction due to their proximity to areas that are currently affected with ASF and CSF (the Caribbean and Central America).

VS Field Operations staff or State animal health officials will collect samples including tonsil, spleen, lymph node, or whole blood from sick, dying, or dead animals at aggregation points and terminal livestock markets or slaughter facilities. Selection criteria are listed below for condemnations at slaughter. All samples should be submitted to an ASF and CSF-approved NAHLN laboratory or the NVSL-approved Dorado Laboratory.

Selection criteria:

Federal and State animal health officials will submit slaughter swine specimens to an assigned ASF and CSF-approved NAHLN laboratory or the NVSL-approved Dorado Laboratory, as directed by national swine staff if condemned for the following reasons:

- Skin and ear discoloration (erysipelas-like)
- Septicemia
- Hemorrhagic lymph nodes
- Enlarged spleen
- Kidney petechia
- Nasal bleeding
- Intestinal hemorrhage
- Knuckled over
- Dying
- Febrile (may present as huddling)
- Tonsil pathology (tonsillitis, hemorrhagic, necrotic foci, etc.)
- Central nervous system signs (incoordination, paddling, circling, head tilt, abnormal mentation)

3. Higher-Risk of Introduction than the General Swine Population Surveillance

This component includes domestic pigs in swine populations where risk of ASF or CSF introduction is higher due to garbage feeding, outdoor housing, potential or documented exposure to feral swine, or potential exposure to illegal animal meat products from infected countries. State and Federal animal health officials will collect samples both on farm and at concentration points, such as custom

slaughter floors or auctions where pigs from higher-risk premises are butchered or sold. Florida, Texas, Georgia, Louisiana, New York, New Jersey, Puerto Rico, and the U.S. Virgin Islands are expected to contribute substantially with samples from higher-risk populations as they were determined to be at an elevated risk of ASF/CSF incursion given the current global distribution of disease. FADDL and the NVSL-approved Dorado Laboratory will test tissue and whole blood samples collected from these populations for ASF and CSF by qPCR and serology.

Tissues collected from waste feeding operations present a high-value sample as operators feed swine treated waste, including meat. Feeding meat and other products from infected animals is a known risk factor for ASF and CSF transmission. In the U.S., registered waste feeders are periodically inspected and tested by State and Federal animal health authorities. If, during visits to farms or concentration points, inspectors find sick or dead animals with clinical presentations suggestive of hemorrhagic diseases of swine, they should initiate an FAD investigation consistent with the *Foreign Animal Disease Investigation Manual*. Alternatively, in cases of sick or dead swine that do not exhibit clinical signs consistent with ASF or CSF, investigators should collect and submit tonsil, spleen, lymph nodes, or whole blood to a NAHLN laboratory approved for active surveillance wherein laboratory staff will determine the need for ASF/CSF testing as described in section 8b1. When Federal or State officials identify, new producers of higher-risk swine, they will be given contact information and educational materials that emphasize reporting any disease outbreaks to Federal or State animal health officials.

In the absence of sick or dead swine, inspectors will routinely collect whole blood and serum samples from apparently healthy animals in higher-risk operations and submitted to FADDL or the NVSL-approved Dorado Laboratory for ASF qPCR and CSF serology. For information on the specific case of higher-risk sampling in Puerto Rico and the U.S. Virgin Islands, see Appendix 4.

In the event of an FAD outbreak resulting in an overwhelming increase in sample submission to FADDL, please refer to Appendix 5 for guidance on higher-risk sample submission.

Selection criteria:

The following clinical signs should be used as a guide for sampling sick pigs:

- Fever
- Increased pulse and respiratory rate
- Lethargy/listlessness
- Anorexia
- Recumbency
- Vomiting
- Diarrhea
- Eye discharges
- Hemorrhage
- Abortions

- White pigs commonly exhibiting reddening of the skin
- Incoordination
- Undiagnosed central nervous system (CNS) cases (especially congenital tremors and nonsuppurative encephalitis)

4. <u>Feral Swine Surveillance</u>

In designated very high-risk counties within high-risk States, including Texas, Louisiana, Georgia, Florida, Puerto Rico, and the U.S. Virgin Islands, APHIS Wildlife Services will collect both whole blood for ASF and CSF qPCR testing as well as serum for ASF and CSF serologic testing. In all other States, feral swine testing is limited to CSF serology.[4] All samples should be submitted to NVSL-Ames for testing.

Surveillance for ASF and CSF in feral swine will also be conducted through FAD investigations when feral pigs are found sick or dead due to non-traumatic events. Wildlife Services will initiate an FAD investigation and the AVIC and/or State animal health official will approve. Tonsil and spleen samples (or lymph nodes if tonsil and spleen are not available) will be submitted to FADDL and duplicate samples of those same tissues *may* be screened for ASF and CSF at an approved NAHLN laboratory upon the direction of the local animal health officials. For additional information, refer to section 8a.

9. Sample Numbers

Table 2 displays proposed annual sample numbers by component below. VS has based these sample numbers on the sample collection numbers from the previous ASF/CSF surveillance system with modifications based on feedback from the VDLs. Note that sick pig VDL submissions will be tested for both ASF and CSF from the same submitted tissue. State specific targets can be found in the Field Collection Manual for State and Federal Veterinarians and Animal Health Technicians Participating in Classical Swine Fever and African Swine Fever Surveillance.

[4] Due to the presence of low pathogenic CSF in the Caribbean basin, continued sampling of both feral swine and high-risk domestic swine populations for CSF serological titers allows animal health officials to monitor for possible silent incursions into the U.S. swine population.

Table 2. Proposed sample numbers by component.

Sample component	Tissue and Whole Blood samples	Serum samples	ASF PCR tests	CSF PCR tests	CSF tests on serum	ASF tests on serum	Total samples
Sick pig VDL submissions	6,500		6,500	6,500			6,500
Slaughter surveillance	1,500		1,500	1,500			1,500
Higher-risk surveillance	4,200	4,000	4,200	200	4,000		8,200
Feral swine surveillance	5,000	10,000	5,000	5,000	10,000	5,000	15,000
Total	**17,200**	**14,000**	**17,200**	**13,200**	**14,000**	**5,000**	**31,200**

10. Data

a. Data Platforms

Platforms for data collection include the following, depending upon the data source and submitter:

- Manual spreadsheets maintained by WS
- Emergency Management Response System 2 (EMRS)
- NAHLN Laboratory Messaging System (LMS)
- Searchable Test Results Application for NVSL Diagnostics (STRAND) database
- Veterinary Services Integrated Surveillance Module (VSISM)/Comprehensive Laboratory Submission Module (CLSM) for laboratory submission data
- Mi-Corporation (MiCo) mobile application for laboratory submission data

b. Initial Data Collection and Management Processes for ASF/CSF surveillance

Table 3 summarizes the current surveillance components and business processes envisioned for a combined ASF-CSF active surveillance program.

Table 3. Current surveillance components and business processes for ASF-CSF surveillance

Sampling Component	Collector	Sample type	Testing Lab	Data Transfer Method
FAD Investigation (domestic and feral swine)	FADD – State or Federal or NAHLN laboratory-initiated investigation	Tonsil, spleen, lymph node, serum, whole blood	FADDL/ Approved NAHLN Lab	10-4 Form, results via STRAND and recorded in EMRS
Sick pig VDL	NAHLN diagnosticians select samples from case-compatible laboratory submissions	Tonsil, spleen, lymph node-compatible signs required, whole blood	Receiving NAHLN Lab	VDL accession -> LIMS -> Messaging
Slaughter swine	FSIS or Slaughter plant personnel	Tonsil, spleen, lymph node, whole blood	Assigned NAHLN Lab	CLSM OR MiCo – results messaged electronically
Higher-risk – swine fed waste	VS-State field staff; visits and mortality calls	Tonsil, spleen, lymph node, serum, whole blood	NAHLN Lab; FADDL-serum (CSF)	CLSM OR MiCo OR 10-4 Form – results messaged electronically
Higher-risk – market ante-mortem sick/dead swine	VS-State field staff; visits and mortality calls	Tonsil, spleen, lymph node, whole blood	Assigned NAHLN Lab	CLSM OR MiCo– results messaged electronically
Feral swine	WS personnel	Serum, whole blood	FADDL	Spreadsheet to VS from WS – results reported via STRAND

This integrated ASF and CSF active surveillance plan uses multiple existing ASF and CSF surveillance sampling components, which are each associated with a data collection and transfer method.

Foreign animal disease investigation information for feral and domestic swine is entered into EMRS.

For surveillance samples collected by Federal or State animal health officials, field data entry begins with VSISM a Microsoft Dynamics Customer Relationship Management (CRM) application with a web interface, CLSM, for laboratory submission entry. CLSM supports recording sample collections and test requests based on species and commodity group, not disease. This allows field users to record the sample collection once, then specify which diseases the individual specimens are to be tested for, such as ASF and CSF. The MiCo mobile application is a new mobile and desktop platform that allows the user to enter submission data regardless of network status. When a network connection is established, all information input into MiCo is automatically uploaded to VSISM. Regulatory personnel will enter sample data via a web based CLSM data portal or MiCo mobile application that will capture complete and standardized field data through dropdowns and restricted entry fields.

Currently, use of the CLSM field-based data collection module and the MiCo mobile application is limited to regulatory personnel (USDA and State field staff) collecting targeted samples. Similar data structure and minimum data requirements are applied to epidemiological data associated with case-compatible sick pig submissions to NAHLN laboratories by accredited veterinarians. VS and NAHLN laboratory Information Technology

(IT) personnel have developed standardized message structures, so that minimum case data and ASF-CSF test results are messaged to Enterprise Messaging Services (EMS), for routing to LMS, and then pulled into the Data Integration Services (DIS) for matching to VSISM ASF-CSF field data. VS requires all participating laboratories to collect and record entries for any mandatory data fields, either from laboratory accession forms or through communications with veterinarians submitting samples selected for FAD testing. VS will require that all mandatory data be messaged for laboratories to qualify for reimbursement of ASF-CSF testing costs.

VS utilizes DIS to integrate the data received from laboratories via messaging into LMS and VSISM to generate complete surveillance records.

For feral swine, WS will share mainland feral swine field submission data with VS via spreadsheets for inclusion in surveillance data reporting. Feral swine submissions from Puerto Rico and the U.S. Virgin Islands are entered into EMRS. NVSL-FADDL uses STRAND to report negative ASF-CSF test results.

Any presumptive positive and confirmed positive test for ASF and CSF will be handled as an FAD with personal reporting (phone call and direct emails) to those involved and need to know.

c. Minimum Data Elements Required for ASF-CSF Surveillance Samples

In order to leverage surveillance data in the best possible way to meet the goals of the surveillance program, APHIS is actively working to improve consistency in reporting of sample related ASF and CSF surveillance data (i.e., the data associated with sample results that is critical in assessing targeted surveillance effectiveness). Samples with documented (recorded) risk profiles are many times more valuable than random surveillance samples lacking documentation of known risk factors. Further, the sample pool should represent a wide range of operations found within that defined subpopulation (adequate coverage).

In order to document desired risk profiles, this program must capture the following minimum data elements for collected samples (minimum data elements may vary by component):

- Age of animal(s) sampled
- Animal ID (or group/pen description) tied to bar-coded tube or container #
- Date(s) samples collected, tested, and reported (+/- ship date)
- Herd clinical signs and history (if any), especially those compatible with selection criteria
- Laboratory performing testing
- State of production site, slaughter plant or market (if a Premises identification number (PIN) or latitude-longitude is available all available data must be included)
- Production type
- Submitter (if available)
- Sample selection criteria/clinical signs (if available)
- Sample type (e.g., serum, tonsil, spleen, lymph node, whole blood, etc.)
- Slaughter plant code (for example, FSIS number)
- Submission component
- Test(s) requested
- Test result and interpretation

d. Future Unified Electronic Data Collection Alternatives

APHIS will continue to work closely with NAHLN laboratory IT specialists and other subject matter experts to explore options geared toward avoiding duplication of data entry and improving electronic data collection and transmission.

11. Data Analysis, Interpretation, and Metrics

VS will analyze data for the following:

a. Representativeness

- Geographic
    1. Diagnostic laboratory surveillance – the geographic distribution of diagnostic laboratory surveillance samples should generally reflect the population distribution of the U.S. swine herd. Further, the number of operations sampled should reflect the number of operations within each State.
    2. Slaughter surveillance – the geographic distribution of slaughter surveillance samples should generally reflect the distribution of swine slaughter plants targeted in this plan (roaster markets, aggregation points). Large commercial plants are primarily located in the upper Midwest region, while smaller plants are distributed across the United States. Due to the emphasis on sampling in Florida, Texas, and Puerto Rico, sample numbers from these States are expected to be higher than other States.
    3. Higher-risk surveillance – the geographic distribution of higher-risk surveillance samples should be distributed across the country with an emphasis on Florida, Texas, Georgia, Louisiana, New York, New Jersey, Puerto Rico, and the U.S. Virgin Islands. Additionally, States that allow waste feeding should have a higher number of samples in this component than would be expected based on their swine population. If detailed information is available, VS will evaluate geographic representativeness at the county level, with a greater number of samples expected to come from counties near landfills or international ports.
    4. Feral swine surveillance – the geographic distribution of feral swine samples should reflect the distribution of feral swine populations. Most samples should come from States with established feral swine populations.
- Production type/age
    1. Diagnostic laboratory surveillance – samples within this component should reflect the age and production type distribution of commercial swine operations; without this data, analysis based on production type and age analysis has proven to be impossible
    2. Slaughter surveillance – production type should be represented by a distribution of samples between commercial, non-conforming, and higher-risk production animals. Age distribution is expected to be 4 months and older to capture animals specifically marketed to roaster markets, as well as non-conforming commercial swine that grow slower than their cohort.
    3. Higher-risk surveillance – samples within this component should primarily be non-commercial (operations that do not raise swine in biosecure facilities)

production types for both ASF and CSF. This should include swine raised out of doors, waste feeders, and transitional herds (domestic swine are produced under less secure conditions that may allow a greater degree of exposure to wildlife), with waste feeders making up most of the CSF samples.

4. Feral swine CSF active surveillance – age distribution is expected to be primarily adult animals, with smaller proportions of juvenile and sub-adults. Production type does not apply to this component.

- Temporal
    1. Diagnostic laboratory surveillance – sick pig submissions should be spaced evenly - weekly and monthly throughout the year.
    2. Slaughter surveillance – submissions should be spaced evenly monthly throughout the year. Roaster submissions may be more seasonal, depending on regional temperature differences and timing of celebrations/holidays.
    3. Higher-risk surveillance – waste feeder samples are more likely to be seasonal to reduce stress on animals during temperature extremes.
    4. Feral swine active surveillance – WS collects these samples throughout the year, providing a temporally representative sample.

b. Probability of Detection

Probability of detection is monitored for each component and for each disease. Tables 4 and 5 list the estimated initial prevalence detection levels and number of potentially infected animals.

Table 4. Estimated initial prevalence detection levels and potential number of infected animals – ASF surveillance

Component	No. of samples	Detectable prevalence*	Sub-population size	Number of infected pigs needed to detect at least one case*	Population sampled
Sick pig VDL	6,500	0.0004	15,000[5]	6	Commercial swine experiencing morbidity and mortality per year which are submitted to the diagnostic laboratory
Slaughter	1,500	0.0021	11,150,000[6]	23,415	Swine condemned at slaughter across market hog and roaster hog slaughter in the U.S.
Higher-risk	4200	0.0008	5,054,302[7]	4,043	Swine raised in non-commercial settings, such as waste feeders, outdoor raised swine, swine with known or suspected feral swine exposure, and show swine
Feral	5,000	0.00064	~6,000,000[8]	3,840	Estimated number of feral swine in the U.S.

*Detectable prevalence of disease with 0.95 probability assuming a test sensitivity of 0.95

[5] Consultation with NAHLN ASF/CSF-approved laboratories
[6] Based on NASS Quick Stats estimate of swine slaughter in the U.S. in 2018 (https://quickstats.nass.usda.gov/) and FSIS condemnation data
[7] NASS Quick Stats (https://quickstats.nass.usda.gov/)
[8] History of Feral Swine in the Americas, Last Modified: Mar 23, 2018. (www.aphis.usda.gov/aphis/ourfocus/wildlifedamage/operational-activities/feral-swine/sa-fs-history)

Table 5. Estimated initial prevalence detection levels and potential number of infected animals – CSF surveillance

Component	No. of samples	Detectable prevalence *	Sub-population size	Number of infected pigs needed to detect at least one case*	Population sampled
Sick pig VDL	6,500	0.0004	15,000[3]	6	Commercial swine experiencing morbidity and mortality per year which are submitted to the diagnostic laboratory
Slaughter	1,500	0.0021	11,150,000[4]	23,415	Swine condemned at slaughter across market hog and roaster hog slaughter in the U.S.
Higher-risk	4,200	0.0008	5,054,302[5]	4,043	Swine raised in non-commercial settings, primarily waste feeders and swine with known or suspected feral swine exposure
Feral	10,000	0.00032	~6,000,000[9]	1,922	Estimated number of feral swine in the U.S.

*Detectable prevalence of disease with 0.95 probability assuming a test sensitivity of 0.95

c. Consistency of Clinical Signs Reported with the Case Definition

1. Diagnostic laboratory surveillance – reason for submission should be distributed among the consistent clinical signs, with minimal to no samples tested with no reason for submission or 'general swine submission.'
2. Slaughter surveillance – submissions should be from condemned animals for clinical signs or post-mortem lesions consistent with the reasons for submission listed above.
3. Higher-risk surveillance – reason for submission should be distributed among the consistent clinical signs, with minimal to no samples tested with no reason for submission or 'general swine submission.'

d. Timeliness

- Collectors should submit samples as quickly as possible, but no later than 48 hours after collection for all components, with proper sample storage and packaging.
- Test result turnaround time
    - Laboratories should test tissue samples as quickly as possible and should not hold or delay testing for any longer than 1 week.
    - Laboratories should test serology samples within 7-10 days of receipt.

[9] History of Feral Swine in the Americas, Last Modified: Mar 23, 2018. (www.aphis.usda.gov/aphis/ourfocus/wildlifedamage/operational-activities/feral-swine/sa-fs-history)

Appendix 1. African Swine Fever Case Definition

1. Disease Information [10]

1.1. General Disease and Pathogen Information: African swine fever (ASF) is an infectious disease of both domestic and wild pigs caused by the African swine fever virus (ASFV), the only member of the *Asfaviridae* family. It can be transmitted through direct or indirect contact or by ticks of the genus *Ornithodoros*. Infection can be peracute, acute, subacute, or chronic. Pigs that recover from infection can become persistently infected carriers of the virus.

1.2. Clinical Signs

1.2.1. Peracute: Caused by highly virulent strains. Pigs are typically found dead, sometimes without clinical signs of disease or any post-mortem lesions.

1.2.2. Acute: Caused by highly virulent strains. Clinical signs include fever, increased pulse and respiratory rate, lethargy, anorexia, and recumbency. Jaundice, vomiting, bloody diarrhea, eye discharge, bloody nasal discharge, and abortions may be observed. Pigs commonly exhibit reddening, hemorrhage, and/ or petechiation of the skin. One to two days before death the pig may develop anorexia, depression or listlessness, cyanosis, and incoordination. Death occurs 2-13 days after infection. Mortality rates approach 100 percent. Commonly seen post-mortem lesions include enlarged, and often friable spleen, enlarged liver, renal petechiae/ hemorrhages, hemorrhagic and enlarged lymph nodes (most commonly gastrohepatic and renal), and hemorrhages/ petechiae in other organs including urinary bladder, lungs, heart, stomach, and intestines.

1.2.3. Subacute: Caused by moderately virulent strains. Clinical signs are similar to the acute form but are less severe. The duration of illness is 5-30 days and mortality rates are lower (30-70 percent). Death occurs 15-45 days after infection. Like clinical signs, post-mortem lesions are similar to those seen with the acute form, but typically less severe.

1.2.4. Chronic: Caused by low virulence strains. Clinical signs develop over 2-15 months, are variable, and may include weight loss, fever, respiratory signs, skin necrosis, pericarditis, lung adhesions, and joint swelling. Mortality rates are low. Post-mortem lesions can include emaciation and focal caseous necrosis and mineralization of the lungs.

2. Laboratory Criteria

2.1. Agent Isolation and Identification: Collect whole blood (EDTA and heparin), spleen, lymph nodes, and tonsils. Keep samples as cold as possible without freezing. Tests include qPCR, immunohistochemistry (IHC), and virus isolation (VI).

2.2. Agent Characterization: Genome sequencing is critical to differentiate viral strains.

[10] In the 2018 ASF outbreaks in both China and Russia, the disease presented in the acute form.

2.3. Serology: Antibody detection in serum is evaluated by ELISA, indirect fluorescent antibody (IFA), and immunoperoxidase test (IPT). Antibodies develop 7-10 days post-infection and can persist for life. Pigs infected with highly virulent ASFV strains can die before antibody production occurs.

3. Case definition and Reporting Criteria

3.1. Suspect Case:

3.1.1. an animal having clinical signs consistent with ASF; **OR**

3.1.2. an epidemiologic link to ASFV

3.2. Presumptive Positive Case: A suspect case with a non-negative screening laboratory test result for ASFV (PCR, Ab ELISA)

3.3. Confirmed Positive Case:

3.3.1. an animal from which ASF virus has been isolated; **OR**

3.3.2. otherwise identified, by at least two different tests

3.3.2.1. one antigen **AND** one antibody assay, especially in the case of subacute or chronic cases; **OR**

3.3.2.2. two antigen assays.[11]

[11] Under unique conditions, animal health officials may consider multiple non-negative ELISAs supported by positive IPTs as confirmed positive cases.

Appendix 2. Classical Swine Fever Case Definition

1. Disease Information

1.1. General Disease and Pathogen Information: Classical swine fever (CSF) is an infectious disease of both domestic and wild pigs caused by the CSF virus (CSFV), a small, enveloped RNA virus of the family Flaviviridae and genus *Pestivirus.* The incubation period is typically 7 to 10 days, though it can range from 2 to 15 days. CSF has several clinical presentations (acute, chronic, and congenital infection) that are dependent on previous exposure to the virus, viral virulence (high, moderate, and low), and host factors such as age and nutritional status. Young animals are usually affected more severely than older animals and mortality rates may reach up to 90 percent. In older breeding pigs, the course of the infection is often mild or even subclinical. Naïve populations tend to be more severely affected and are more likely to present with the classical acute presentation; however, the classical acute presentation is now rarely seen. Instead, more moderate forms and presentations of the disease predominate. Prevailing strains of CSF virus exhibit moderate to low virulence, making clinical diagnosis difficult especially in older animals. Low virulence strains usually give rise to a mild disease or subclinical infection that can remain undetected for long periods of time. Leukopenia is a fairly consistent clinical laboratory finding, except with low virulence strains.

1.2. Clinical Signs

1.1.1. Acute: Illness usually seen in weaned suckling pigs less than 12 weeks of age, unresponsive to antibiotics, and characterized by fever, severe depression, skin hyperemia, conjunctivitis, and staggering gaits followed by posterior paresis, abortion (rare), and/or diarrhea.

1.1.2. Chronic: Pigs recovered from acute infection may progress into a chronic infection during which they experience anorexia, fever, diarrhea, and/or dermatitis, and which may result in the occurrence of runts in the herd. Chronic disease is characterized by subdued acute infection followed by a brief recovery before relapse of fever, anorexia leading to wasting, and death 1-3 months after onset.

1.1.3. Congenital infection: Congenital infection can result in reduced reproductive performance and/or abortions/stillbirths. Weak piglets may be the only indication of disease in a herd. Pigs born to sows infected after day 50-70 of gestation may be born with congenital tremors or be persistently infected and appear normal for several months before dying. Survival periods of 11 months after birth have been observed in the literature. (Sows infected prior to day 50-70 of gestation may abort or give birth to stillbirths, mummies, or pigs with congenital defects).

2. Laboratory Criteria

2.1. Agent Isolation and Identification: Collect whole blood (EDTA and heparin), tonsils, spleen, and lymph nodes (retropharyngeal, submandibular, mesenteric). Keep samples as cold as possible without freezing. Tests include RT-qPCR, genomic sequencing, and virus isolation.

2.2. Agent Characterization: Genome sequencing is critical to differentiate vaccinated strains, which occasionally persist in vaccinated animals, from wild type CSF virus.

2.3. Serology: Antibody detection in serum is evaluated by ELISA, typically followed by an immunoperoxidase virus neutralization test (IP-VN) and/or immunoperoxidase test (IP) for confirmation of inconclusive or non-negative results. Due to immunosuppression with virulent strains, antibodies are not detectable before 18 days post infection and last at least several years. With chronic infections, antibodies are potentially briefly detectable at the end of the first month but, if present, quickly disappear. Congenitally infected pigs are persistently viremic and seldom produce specific antibodies. The more sensitive ELISA assays recommended for screening cross react with bovine viral diarrhea virus (BVD).

3. Case Definition and Reporting Criteria:

3.1. Suspect Case: an animal having

3.1.1. clinical signs consistent with CSF; **OR**

3.1.2. an epidemiologic link to CSFV.

3.2. Presumptive Positive Case: a suspect case with a non-negative screening laboratory test result for CSFV (RT-qPCR, Ab ELISA)

3.3. Confirmed Positive Case:

3.3.1. an animal from which an approved laboratory has isolated CSF virus; **OR**

3.3.2. otherwise identified, by at least two different tests[12]

3.3.2.1. one antigen AND one antibody assay, especially in the case of subacute or chronic cases; **OR**

3.3.2.2. two antigen assays

[12] Under unique conditions, after confirming a negative vaccine status, animal health officials may consider multiple non-negative ELISAs supported by positive IPTs as confirmed positive cases.

Appendix 3: Procedures for Conducting Oral Fluids Sampling for On-Farm Foreign Animal Disease Investigations for African Swine Fever (ASF)

1. Purpose and Background

ASF is a highly contagious and deadly viral disease affecting both domestic and feral (wild) pigs in all age groups. ASF can be clinically indistinguishable from endemic diseases, allowing the virus to circulate for some time within the United States swine herd before detection, which could increase demands on regulatory and industry resources to conduct surveillance and control activities for the virus.

The swine industry routinely uses oral fluids as an aggregate sample type to detect antigen and/or antibodies for endemic diseases in the United States. Oral fluids are relatively easy to collect by farm personnel. Following a request from swine industry stakeholders, VS is evaluating the utility of oral fluids for inclusion in its ASF surveillance and response policies. The initial phase of this evaluation includes the submission of oral fluids samples in select States during on-farm FAD investigations for which ASF is one of the differential diagnoses.

2. Objectives

The objectives of this phase of the evaluation are to:

- Introduce FADDs to field experience with oral fluids collection;
- Develop experience within participating NAHLN laboratories on protocols for oral fluids processing, testing, and reporting for ASF;
- Provide additional evidence of negative results concordance between sample types as a path towards evaluating oral fluids samples for use in targeted ASF surveillance and response activities.

3. Sampling Conditions

Oral fluids samples that are collected should meet the following conditions:

- The pigs on which the investigation is being conducted have demonstrated clinical signs consistent with ASF.
- An official FAD investigation has been initiated.
- If oral fluids have been collected along with a matched approved sample, the additional ropes from the kit may be placed randomly throughout the barn and the fact that these samples do not have a matching approved sample should be noted on the VS Form 10-4, box 22 (“Additional Data”). The labeled numbers on each sample tube that do not have a matching approved sample should be listed within this additional information.
- If testing occurs at a NAHLN laboratory, duplicate samples including both oral fluids and associated approved sample types must be sent to the NVSL - FADDL.
- The oral fluids samples will be tested for ASF by qPCR only.
- To the extent possible, oral fluids samples should be collected from each of the pens from which an approved sample (e.g., blood or approved tissues from dead or euthanized pigs) is collected, up to six pens per barn.

- One rope should be placed per 25 animals in a pen. If there are less than 25 animals in a pen from which samples will be collected, one rope per pen should be placed.
- The number of pigs in the pen at the time of oral fluids collection must be recorded on the VS Form 10-4, box 22 ("Additional Data"). If available, the number of clinically ill and asymptomatic pigs within each pen should be noted.
- If dead animals that are being sampled for ASF have been removed from the pen or barn and cannot be associated with any one pen, oral fluids should not be collected for evaluation as "matched" approved samples to these mortalities as comparison and confirmation cannot be performed.

4. Oral Fluids Collection Supplies

The supplies used to collect oral fluids from pigs can either be provided to the FADD in a commercially available kit or can include the individual components listed below.

Supplies include:

- 3-strand twisted undyed cotton rope from 1/2 inch (if collecting nursery pigs) to 5/8 inch (if collecting grow/finish pigs) in diameter;
- Clean plastic bag, single-use plastic boot, or semen collection bags;
- Side cutters, knife or scissors;
- Snap-cap, screw-top tube, or red-top tubes (note tubes CANNOT contain additives such as EDTA);
- Permanent marker;
- Disposable gloves.

5. Oral Fluids Collection Procedures

After State and Federal animal health officials have determined that a possible FAD report should be investigated for ASF, the FADD coordinating the visit to the farm should notify farm personnel of his/her intent to collect oral fluids samples as part of the investigation, even though these samples will not be approved samples that can confirm the absence or presence of disease. Upon arrival at the farm, the FADD should observe biosecurity practices to determine whether he or she should enter the barns in which the affected animals reside or died to collect samples or observe animals. If the FADD will be entering the barns to sample animals as part of the investigation, he or she can perform the oral fluids collection process. If the FADD will not be entering the barns or if there are other factors that warrant having others collect the rope sample, the FADD may direct farm personnel to properly collect oral fluids samples.

Oral fluids samples should only be collected from pens from which approved samples (such as blood samples or approved tissues) are collected, or from pens located randomly throughout the barn if oral fluids have already been collected from pens from which approved samples have been collected. If approved samples cannot be matched to a specific pen, oral fluids should not be collected.

Before collecting an oral fluids sample, the number of pigs in the pen and their current clinical status (dead/ill/healthy) should be observed and recorded. One rope should be hung in the pen for every 25 pigs in the pen.

The rope(s) to be hung in the pen should be cut so that it will be at shoulder height of the pigs in the pen when tied in a knot and placed in a clean area of the pen, away from feed or water. A

knot should be tied to secure the rope(s) to the pen divider or wall and the rope strands should be unraveled. If more than one rope will be hung in a pen, ropes should be placed spaced apart so that different pigs can easily chew on each rope.

Pigs should be allowed to chew on the rope for at least 20 or 30 minutes (a longer time may be needed if pigs are uninterested in the rope or untrained to the rope and the duration should be noted). FADDs may find it most efficient to hang ropes first and then collect approved samples while oral fluids are being collected.

After the sampling time has passed, the FADD or his or her designee (wearing disposable gloves) may insert the wet end into a disposable bag (or alternate disposable vessel). While the rope is still tied, the collector can "strip" the rope by exerting downward pressure to force liquid out of the rope and into one corner of the disposable bag (if the bag within a kit has the tube already attached, the fluid should pool in the bottom of the tube and can be disconnected once the target sample volume has been reached). The sample should then be visually inspected for any obvious contamination prior to acceptance. If substantial contamination is noted, a subsequent sample may be needed. A minimum sample volume of 4-5 mL is recommended to allow for samples to be split into quantities of approximately 2-2.5 mL for shipping to the NAHLN laboratory and FADDL.

Once the FADD has obtained sufficient fluid in the bag, he or she can snip a corner of the bag off and transfer the fluid into the tube. After fluids are extracted, ropes and disposable bags should be immediately discarded.

Oral fluids samples should be packaged and submitted along with the other approved samples, consistent with the instructions provided in the current version of the *Foreign Animal Disease Investigation Manual*. Samples for shipment to the testing laboratory must be prepared in compliance with the same Federal guidelines required for serum samples, including a properly labeled, insulated and leak-proof container, an absorbent material, and ice packs to keep the samples chilled, as well as the VS 10-4 submission form. If testing occurs at a NAHLN laboratory, duplicate samples including both oral fluids and associated approved samples must be sent to NVSL-FADDL.

Additional information about sampling procedures, including a video demonstration, is available from Iowa State University's Center for Food Security and Public Health at the following links:

Job Aid: http://www.cfsph.iastate.edu/pdf/oral-fluid-collection-in-pigs

Video Demonstration: http://www.cfsph.iastate.edu/video.php?link=oral-fluid-collection-in-pigs

The VS 10-4 submission form should be completed and included in the shipment in accordance with the instructions provided in the current version of the *Foreign Animal Disease Investigation Manual*. Additional information that should be included in the VS Form 10-4, box 22 ("Additional Data") includes:

- A list of oral fluids tube numbers and the corresponding approved samples to which the sampled fluids match;
- The number of pigs in the pen and their current clinical status (dead/ill/healthy) at the time of oral fluids collection for each tube;

- Whether any of the oral fluids samples were collected from random pens as "additional samples" that do not have a match (provided that matched samples were also collected and are included in the submission), and which samples those are.

6. Support

For additional information or guidance, FADDs may contact the VS Swine Health Commodity Staff at: VS.SP.ASEP.Swine@usda.gov

7. References

- Foreign Animal Disease Investigation Manual
- Iowa State University Center for Food Safety and Public Health, "Oral Fluid Collection in Pigs"
- Iowa State University Center for Food Safety and Public Health, "Oral Fluid Collection in Pigs (video)"

Appendix 4: Higher-Risk Sampling in Puerto Rico and the U.S. Virgin Islands

Puerto Rico

All registered garbage feeding facilities and other high-risk facilities will be subject to surveillance testing on a 4-month basis. When found, unregistered waste feeders should also be included in testing efforts. Investigators will collect whole blood and serum samples from apparently healthy animals from this component and submit samples to the NVSL-approved Dorado Laboratory (in Puerto Rico) for ASF and CSF testing. If, during these visits, sick or dead animals are found with clinical presentations suggestive of hemorrhagic diseases of swine, investigators will initiate an FAD investigation as described above in section 8a. Until further notice, the APHIS will treat all sick pig cases identified through this component as an FAD. Refer to section 8a for further details.

In the specific case of illegal boat landings (IBL) in Puerto Rico, swine production sites within 3 km of such illegal landings should be placed under a quarantine and undergo routine ASF and CSF surveillance. Upon IBL detection, Federal or State animal health professionals will place the premises under a 20–24-day quarantine. Veterinary health professionals will perform site visits on day 7 and 14 to document observational surveillance and discuss any concerns with the premises owner. If any sick or dead pigs are identified during observational surveillance, samples will be collected for immediate diagnostic testing. 20 to 24 days following the IBL detection comprehensive diagnostic testing will occur as outlined in table A4. All samples collected should be sent to the NVSL-approved Dorado Laboratory for ASF and CSF testing.

If sick or dead pigs are found, at any time, in the 3 km radius, the AVIC will initiate an FAD investigation and an FADD will collect a full set of tissues including tonsil, lymph node, spleen, serum, and whole blood for submission to FADDL for ASF and CSF testing in accordance with the *Foreign Animal Disease Investigation Manual*.

Table A4: Sample Size Required to Achieve a 95% Confidence of Disease Detection*

Population Size	No. of samples
10	10
20	16
30	19
40	21
50	23
60	24
70	25
80	25
90	26
100	26

*Detectable prevalence of disease with 0.95 probability assuming a test sensitivity of 0.95

US Virgin Islands

Due to the pervasive nature of untreated garbage feeding in the U.S. Virgin Islands, for the purpose of surveillance, APHIS has categorized all swine production units in the U.S. Virgin Islands as higher-risk facilities. As such, APHIS and/or State employees will visit all known facilities every 6 months to sample a minimum of 5 pigs, targeting those that are sick or dead.

Tonsil, spleen, lymph node, and/or whole blood samples will be collected and sent to the NVSL-approved Dorado Laboratory in Puerto Rico for ASF and CSF PCR testing. When found, previously unidentified production facilities should be included in testing efforts. If an APHIS or State employee suspects ASF or CSF during a farm inspection, an FAD investigation should be initiated as described in section 8a.

Appendix 5: NVSL FADDL Outbreak Response Sample Reallocation

In the event of an FAD outbreak, an influx of samples may be sent to NVSL FADDL resulting in the reprioritization of routine ASF and CSF surveillance samples and delays in result reporting for the Higher-Risk component. FADDL will immediately notify APHIS staff of the impending testing requirements associated with the outbreak and the status of routine ASF/CSF surveillance samples. Should FADDL deprioritize ASF/CSF surveillance samples, FADDL will notify APHIS staff and indicate the effect on the timelines of test results.

If deemed necessary, all samples collected from apparently healthy animals through the Higher-Risk surveillance component will be immediately rerouted to a NAHLN laboratory approved for ASF/CSF active surveillance. States should submit Higher-Risk samples to the NAHLN laboratory that is currently accepting samples collected in their State through other active surveillance components (Slaughter and Aggregation point and the Sick Pig VDL components). State quotas for Higher-Risk swine will remain unchanged during this time. NAHLN laboratories are currently not approved to perform CSF or ASF serologic testing; therefore, these laboratories will test whole blood samples for ASF and CSF via qPCR. Serum sample collection will be temporarily suspended from the Higher-Risk component sampling guidelines.

All sample information collected and submitted to NAHLN laboratories must be submitted through CLSM or MiCo, not via 10-4 forms. A 10-4 form is only acceptable for samples submitted to FADDL For guidance on MiCo use please review this training video: https://www.youtube.com/watch?v=S75zoTnGnvk&list=PL2_jEtoY8jiibq__NjekK2IN9dtFMjsPs&index=113