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Swine Hemorrhagic Fevers: African and Classical Swine Fever

Integrated Surveillance Plan

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1. Disease Description

a. African Swine Fever

African swine fever (ASF) is a highly contagious viral hemorrhagic disease affecting animals in the family Suidae, including domestic pigs, feral pigs, and Eurasian wild boar. African wild swine, such as warthogs and bush pigs, act as reservoir hosts but do not show signs of disease. Infection caused by ASF virus can be peracute, acute, subacute, or chronic. 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 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 rarely seen anymore. Instead, more moderate forms and presentations of the disease predominate. Prevailing strains of CSF virus are moderate to low virulence, making clinical diagnosis difficult especially in older animals. Low virulent 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 fairly 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 and Europe and CSF in the Caribbean and South America increases concern for potential disease introduction into the United States (U.S.). Detection of these diseases in the U.S., if they are introduced, may be complicated because the current clinical presentations of both diseases throughout the world resemble those of many other production diseases present in the U.S. Therefore, the U.S. Department of

Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) Veterinary Services (VS) 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 initial active integrated surveillance plan that builds upon current diagnostic and surveillance methodologies for both diseases.

3. Surveillance Objectives

a. Objective 1: To strengthen detection capabilities and enhance outbreak preparedness for ASF and CSF

   Current detection of ASF relies on reporting of suspect disease cases based on a passive disease reporting system. With the approval of additional diagnostic specimens (i.e., tonsil and spleen tissue) as valid sample types for real-time PCR (rPCR) testing in the National Animal Health Laboratory Network (NAHLN), ASF surveillance can now be integrated with current CSF surveillance efforts to implement an active surveillance plan. This integration of swine fever surveillance plans strengthens the ability to detect an ASF and CSF incursion at the national level and assists in preparedness.

   A foreign animal disease (FAD) outbreak (including ASF and CSF), could severely test our high-volume sample collection, laboratory capacity, and data management capabilities. By executing a targeted active surveillance program, these systems can be constructed and tested at lower sample sizes to provide confidence in the processes.

   Timely and consistent surveillance may 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. This 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 can be obtained from the streams as outlined below, without additional samples needed from other non-targeted streams.

4. Expected Outcomes: Products, Decisions, and Actions

Expected outcomes include:

a. Products: Surveillance reports will be summarized quarterly and annually.

b. Evaluation: The surveillance system will be evaluated after the first year and then once every 3 years thereafter.

c. Decisions and actions: The information and results provided in evaluations and reports will be used to support trade claims of disease freedom and to adjust our preparedness for a potential introduction of these diseases. Any major change in the introduction threat of ASF or CSF to the U.S., in diagnostic capabilities, or in the swine industry should lead 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

Stakeholder	Interest/Responsibility
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
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. All populations will be monitored by observation, and if clinical signs consistent with the case definitions for either ASF or CSF are observed, an FAD investigation should be initiated immediately.

Commercial swine operations will be monitored through the testing of

- Case-compatible sick pig veterinary diagnostic laboratory (VDL) submissions,
- Case-compatible slaughter condemnation samples
- Tissue samples from sick or dead pigs found at aggregation points
- Swine in higher-risk (less biosecure) farms
- Waste feeder submissions
- Aggregation point samples from markets known to serve higher-risk operations
- Herds with potential or known feral swine exposure

Feral swine

- Samples collected by Wildlife Services (WS)

Since all samples tested for ASF will be initially tested with real-time PCR (rPCR - which detects antigen), testing healthy swine without clinical signs would provide little diagnostic value as healthy animals would not be expected to have detectable ASF virus. Therefore, sampling sick swine in all streams provides a higher likelihood of detecting the disease.

While rPCR sampling for both ASF and CSF will be limited to animals with clinical illness, serum samples will be drawn from selected herds for use in CSF serological surveillance. Because mild strains of CSF may fail to generate significant clinical signs, it is important to continue to monitor serum from this segment for CSF antibody titers, continuing past CSF surveillance protocols.

7. Case Definition

Please see Appendix 1 and 2 for full ASF and CSF case definitions, which are still undergoing further review.

Note: In any ASF or CSF outbreak, case definitions may be edited after the first presumptive positive or confirmed positive case (index case). The case definition will be reviewed throughout the outbreak and may be modified with additional information or the changing needs of the eradication effort.

African Swine Fever Case Definition and Reporting Criteria:

1.1. *Suspect case*: An animal having clinical signs and epidemiologic information consistent with ASF.

1.2. *Presumptive positive case*: A suspect case with a non-negative screening laboratory test result for ASF virus (PCR) at the National Veterinary Services Laboratories (NVSL) Foreign Animal Disease Diagnostic Laboratory (FADDL) or a National Animal Health Laboratory Network (NAHLN) laboratory approved for ASF Preparedness and Surge Testing, or

1.2.1. A suspect case that is positive for ASFV antibodies by two different antibody tests at NVSL FADDL.

1.3. *Confirmed positive case*: An animal from which ASF virus has been isolated and identified at NVSL FADDL or a laboratory designated by the Secretary of Agriculture or,

1.3.1. A presumptive positive case with a positive confirmatory ASFV antigen test at NVSL FADDL

Classical Swine Fever Case Definition and Reporting Criteria:

1.1. *Suspect case*: A pig or herd that has:

1.1.1. Clinical signs, history, or epidemiology consistent with CSF; **OR**

1.1.2. An inconclusive or positive real-time PCR (rPCR)/reverse transcriptase PCR (RT-PCR) performed on a sample taken during routine surveillance, without the presence of clinical criteria, for which either additional laboratory diagnostics (sequencing information or confirmatory testing) or epidemiological investigation results are pending; **OR**

1.1.3. A positive antibody enzyme-linked immunosorbent assay (ELISA) performed on a sample taken during routine surveillance with subsequent positive results to immunoperoxidase (IP) and IP-virus neutralization (VN) tests with neither epidemiological information nor known clinical signs consistent with CSF.

1.2. *Presumptive positive case*:

1.2.1. A suspect case with a positive repeated rPCR/RT-PCR or genomic sequencing consistent with CSF virus conducted at FADDL after an initial positive rPCR/ RT-PCR on a sample from a pig with or without clinical signs and/or epidemiological evidence of CSF conducted; **OR**

1.2.2. A pig or herd with epidemiological information and/or clinical criteria consistent with CSF; **AND**

Positive rPCR or RT-PCR; **OR**

Positive ABC test on tissue samples; **OR**

Positive IP-VN test.

1.3. *Confirmed positive case*: A pig from which CSF virus has been isolated with sequence confirmation at NVSL-FADDL, or a laboratory designated by the Secretary of Agriculture.

8. Data Sources and Sampling Methods

When sampling dead swine for any stream, a full set of tissues should be collected including tonsil, lymph node, lung, kidney, spleen, liver, heart, serum, and whole blood. Currently, only tonsil, spleen, lymph node, and whole blood samples are approved for ASF testing in NAHLN laboratories; likewise for CSF, only tonsil, spleen, and lymph node samples are approved for testing. In the event of a non-negative result at a NAHLN laboratory, the other tissue types submitted by collectors can be forwarded to FADDL for testing.

a. Passive Foreign Animal Disease Investigations

Any animal or herd meeting the case definition should be reported as suspicious cases to local State and Federal animal health officials by those interacting with swine [including USDA Food Safety and Inspection Service in-plant personnel, producers, and private veterinarians]. This reporting will initiate an FAD investigation by a foreign animal disease diagnostician (FADD). See VS Guidance 12001[1] for additional information. At a minimum, specimens to be collected from live affected swine are serum, tonsil scraping, and whole blood (ethylenediaminetetraacetic acid [EDTA] and heparin). When possible, at least one pig, and ideally up to 10 pigs, should be necropsied and the following minimum tissues collected: tonsil, spleen, lymph nodes, and kidney. To maximize detection of a potential ASF incursion into the U.S., samples may also be collected from feral swine suffering non-traumatic morbidity and mortality events via a foreign animal disease investigation.

b. Active Surveillance

1. Sick pigs submitted to VDLs

The majority 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 must come from clinically ill swine, these samples represent the highest surveillance value for improving ASF-CSF detection capability.

The following selection criteria will be used to identify eligible cases for ASF and CSF surveillance testing at ASF and CSF VS Swine Surveillance-Contracted NAHLN laboratories. 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
  - Spleen

[1] https://www.aphis.usda.gov/animal_health/lab_info_services/downloads/VSG_12001.pdf

    - Lymph node(s)
- 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
    - 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. <u>Slaughter and Aggregation Point Surveillance</u>

Slaughter plant (including butcher, sow, and roaster plant) condemnations and samples from ill or dead swine collected at swine aggregation points are the primary targets for ASF and CSF targeted slaughter surveillance. Aggregation points can include buying stations for market swine, sub-standard swine from commercial operations, and non-conforming cull sows/boars. Sick, dying, or dead animals should be sampled by VS Field Operations or State animal health officials at aggregation points and terminal livestock markets or slaughter facilities; samples to collect include tonsil, spleen, and lymph node. Selection criteria are listed below for condemnations at slaughter and all samples should be submitted to an ASF and CSF-approved NAHLN laboratory.

Specifically for CSF, slaughter surveillance also targets roaster markets, livestock auctions, and slaughter plants in States with larger swine populations and a higher probability of CSF introduction. 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. These two States have a higher probability of disease introduction due to their proximity to areas that are currently affected with CSF (the Caribbean and Central America). Serum, tonsil, spleen, and lymph node samples should be collected; serum samples should be submitted to FADDL for CSF serological testing.

Selection criteria:

Slaughter swine condemned for the following reasons should be submitted to an assigned VS Swine Surveillance-Contracted NAHLN laboratory, as directed by national swine staff:

- Skin and ear discoloration (erysipelas-like)

- Septicemia
- Hemorrhagic lymph nodes
- Enlarged spleen
- Kidney petechia
- Nasal bleeding
- 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 stream includes higher-risk farms because of suspected or known risk factors, such as known feral swine exposure, custom slaughter plants, and garbage feeding operations.

Tissues collected from waste feeding operations present a high-value sample as swine on these operations are fed 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. During these inspections, sick or dead pigs should be sampled by VS Field Operations or State animal health officials for ASF and CSF testing (at minimum, whole blood, tonsil, tonsil scraping, spleen, and lymph node should be collected). If found, unregistered waste feeders should also be included in testing efforts. These waste feed origin samples should be sent to FADDL for testing via the FADI process.

Aggregation points described above may also contain swine from higher-risk herds. Sick, dying, or dead animals should be sampled by VS Field Operations or State animal health officials at aggregation points; samples to collect include whole blood and tonsil scraping (if alive), spleen, tonsil, and lymph node. These samples should be sent to an ASF- and CSF-approved NAHLN laboratory for testing.

Swine herds with known or suspected feral swine exposure may serve as a sentinel for diseases circulating in feral swine. Therefore, if sick or dead animals are observed on these premises, at minimum, whole blood, spleen, tonsil, tonsil scraping, and lymph node samples should be collected. These samples should be sent to FADDL for testing via the FADI process.

Swine in Florida, Texas, and Puerto Rico have a higher probability of disease introduction due to their proximity to areas that are currently affected with CSF (the Caribbean and Central America). These animals may be raised on a small scale, as transitional swine, located near landfills, or have greater exposure to humans or animals that arrive via illegal boat landings (yolas). For Puerto Rico, swine sites within 3 km of illegal boat landings are eligible for routine CSF

surveillance testing on the fourth visit 28 days after notification of illegal boat landing. Serum samples should be collected from live animals and tonsil samples should be collected from dead or euthanized animals. All samples should be sent to FADDL for testing.

In addition to the sampling recommendations for ASF and CSF, producers of higher-risk animals should be given contact information and educational materials that emphasize reporting any disease outbreaks to inspectors for rapid follow-up.

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
- Abortions
- White pigs commonly exhibiting reddening of the skin
- In-coordination
- Undiagnosed CNS cases (especially congenital tremors and nonsuppurative encephalitis)

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

Active surveillance in feral swine is limited to continued testing for CSF in serum samples to rule out previous exposure.[2] All samples should be submitted to FADDL for testing.

9. Sample numbers

Initial annual proposed sample numbers by stream are displayed in the table below. The number of samples is based on current sample collection numbers in the CSF surveillance system with modifications based on feedback from the VDLs. Note that the sick pig VDL submissions will be tested for both ASF and CSF from the same submitted tissue.

[2] 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 stream.

Sample stream	Tissue samples	Serum samples	ASF PCR	CSF PCR	CSF Serum	Total samples
Sick pig VDL submissions	6,500		6,500	6,500		6,500
Slaughter surveillance	1,500		500	1,000		1,500
Higher-risk surveillance	200	4,000	200		4,000	4,200
Feral swine surveillance		3,000			3,000	3,000
Total	**8,200**	**7,000**	**7,200**	**7,500**	**7,000**	**15,200**

10. Data

a. Data platforms

Data platforms for data collection should include the following:

- Manual spreadsheets maintained by WS
- NAHLN Lab 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
- Emergency Management Response System (EMRS)

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

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

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

Sampling Stream	Collector	Sample type	Testing Lab	Data Transfer Method
FAD Investigation (domestic and feral swine)	FADD – State or Federal	Whole blood, tonsil, spleen, lymph node, serum	FADDL duplicates to NAHLN	10-4, results via STRAND and recorded in EMRS
Sick pig VDL	Accredited veterinarian -> NAHLN lab	Tonsil, spleen, lymph node-compatible signs required	Receiving NAHLN Lab	D-lab accession -> LIMS -> Messaging
Slaughter swine	FSIS or plant personnel	Tonsil, spleen, lymph node	Assigned NAHLN	Modified CLSM web portal entry
Higher risk – swine fed waste	VS-State field staff; visits and mortality calls	Serum, tonsil, spleen, lymph node	NAHLN labs; FADDL-serum (CSF)	CLSM – results messaged
Higher-risk – market ante-mortem sick/dead swine	VS-State field staff; visits and mortality calls	Tonsil, spleen, lymph node	Assigned NAHLN	CLSM – results messaged
Feral swine (CSF only)	WS personnel	Serum	FADDL	Spreadsheet to VS from WS – results reported via STRAND

This integrated ASF and CSF active surveillance plan uses several existing CSF surveillance sampling streams. While CSF surveillance data management is currently partially managed via the Veterinary Services Laboratory Submission IT system, newly captured swine surveillance data collection is being migrated to the newly developed CLSM-VSISM data system. VSISM is built on the CRM framework within a common modular species and disease-agnostic software structure to allow multi-disease sampling during a single visit. Because of this structure, adding ASF surveillance capabilities in alignment with CSF data collections is relatively straightforward. Users will have the opportunity to migrate to the new IT management system for both diseases in one effort as the new joint surveillance effort is launched. For regulatory veterinarian sampling, sampling data will be entered via a web-based CLSM data portal which will capture complete and standardized field data through dropdowns and restricted entry protocols.

Currently, the CLSM field-based data collection module is limited to use by regulatory personnel collecting targeted samples. However, similar data structure and minimal requirements will be applied to epidemiological data associated with case-compatible sick pig submissions to NAHLN labs by accredited veterinarians. VS and NAHLN lab IT personnel will develop standardized message structures, so that minimum case data and ASF-CSF test orders and results will be messaged via LMS into the VSISM CSF-ASF dataset. Labs will be required to collect and record entries for any mandatory data fields, either from lab accession forms, or through communications with veterinarians submitting samples selected for FAD testing. VS will require that all mandatory data be uploaded via LMS for labs to qualify for reimbursed ASF-CSF testing costs.

NVSL-FADDL LIMS systems are incapable of electronic messaging at this time. Initially, required WS feral swine field submission data will be shared with VS via spreadsheets for inclusion in surveillance data reporting. VS program managers will receive negative ASF-CSF test results for WS feral swine samples from NVSL-FADDL via STRAND.

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

One significant weakness in reviewing CSF surveillance data for the past 12 years has been inconsistent reporting of sample-related 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 data elements for collected samples (minimum data elements may vary by stream):

- 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 (if available)
- Lab performing testing
- Premises identification number (PIN) of production site, slaughter plant, or market (if no PIN, then latitude-longitude so that PIN can be looked up or assigned)

- 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 stream
- Test(s) requested
- Test result and interpretation

d. Future Unified Electronic Data Collection Alternatives:

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

11. Data Analysis, Interpretation, and Metrics

Data will be analyzed 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 population. 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). Commercial plants are primarily located in the upper Midwest region, while smaller plants are distributed across the U.S. 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, and Puerto Rico. Additionally, States that allow waste feeding are expected to have a higher number of samples in this stream than would be expected based on their swine population. If detailed information is available, geographic representativeness can be evaluated 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 feral swine population. Most samples should come from States with established feral swine populations.
- Production type/age
    1. Diagnostic laboratory surveillance – samples within this stream should reflect the age and production type distribution of commercial swine operations.

2. Slaughter surveillance – production type should reflect the different parts of this stream, with approximate 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 stream should primarily be non-commercial production types for both ASF and CSF. This should include backyard herds, waste feeders, and transitional herds, with waste feeders making up a majority of the CSF samples.
4. Feral swine 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 stream.

- Temporal
  1. Diagnostic laboratory surveillance – sick pig submissions should be spaced approximately evenly throughout weeks and months of the year.
  2. Slaughter surveillance – submissions should occur approximately evenly by month. 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 surveillance – these samples are collected throughout the year, providing a temporally representative sample.

b. Probability of detection

Probability of detection should be monitored for each stream 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

Stream	No. of samples	Detectable prevalence*	Sub-population size	Detect at least one case in X infected	Population sampled
Sick pig VDL	6,500	0.0005	15,000[3]	8	Commercial swine experiencing morbidity and mortality per year which are submitted to the diagnostic laboratory
Slaughter	500	0.0065	11,150,000[4]	72,475	Swine condemned at slaughter across market hog and roaster hog slaughter in the U.S.
Higher risk	200	0.0153	5,054,302[5]	76,500	Swine raised in non-commercial settings, such as waste feeders, outdoor raised swine, swine with known or suspected feral swine exposure, and show swine

*Detectable prevalence of disease with 0.95 probability

[3] Consultation with NAHLN ASF/CSF approved laboratories
[4] Based on NASS Quick Stats estimate of swine slaughter in the U.S. in 2018 (https://quickstats.nass.usda.gov/) and FSIS condemnation data
[5] NASS Quick Stats (https://quickstats.nass.usda.gov/)

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

Stream	No. of samples	Detectable prevalence*	Sub-population size	Detect at least one case in X infected	Population sampled
Sick pig VDL	6,500	0.00065	15,000[2]	98	Commercial swine experiencing morbidity and mortality per year which are submitted to the diagnostic laboratory
Slaughter	1000	0.0040	11,150,000[3]	44,600	Swine condemned at slaughter across market hog and roaster hog slaughter in the U.S.
Higher risk	4000	0.0153	5,054,302[4]	773,308	Swine raised in non-commercial settings, primarily waste feeders and swine with known or suspected feral swine exposure
Feral	3000	0.0018	~6,000,000[6]	10,800	Estimated number of feral swine in the U.S.

*Detectable prevalence of disease with 0.95 probability

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 submitted 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

- Samples should be submitted as quickly as possible, but no later than 48 hours after collection for all streams.
- Test result turnaround time
  - Tissue samples should be tested as quickly as possible, but should not be held and tested for any longer than 1 week.
  - Serology samples (CSF) should be tested within 7-10 days of receipt.

Appendix 1. African Swine Fever Case Definition

DRAFT – UNDER REVIEW

1. Clinical Signs[7]

1.1. *Clinical signs:* 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

[6] 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)

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

the genus *Ornithodoros*. Infection can be peracute, acute, subacute, or chronic. Pigs that recover from infection can become persistently infected carriers of the virus.

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

1.1.2. *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 in-coordination. 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.

1.1.3. *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.

1.1.4. *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, tonsils, and kidneys. Keep samples as cold as possible without freezing. Tests include: virus isolation (VI), direct fluorescent antibody (DFA), sequencing, and real-time PCR (rPCR).

2.2. *Serology:* Antibody detection in serum by ELISA, indirect fluorescent antibody (IFA), and immunoperoxidase test (IPT). Antibodies develop 7-10 days post-infection and can persist for life. Pigs with virulent ASF virus can die before antibody production occurs.

3. Case definition and Reporting Criteria:

3.1. *Suspect case:* An animal having clinical signs and epidemiologic information consistent with ASF.

3.2. *Presumptive positive case:* A suspect case with a non-negative screening laboratory test result for ASFV (PCR) at National Veterinary Services Laboratories (NVSL) Foreign

Animal Disease Diagnostic Laboratory (FADDL) or a National Animal Health Laboratory Network (NAHLN) laboratory approved for ASF Preparedness and Surge Testing, or

3.2.1. A suspect case that is positive for ASFV antibodies by two different antibody tests at NVSL FADDL.

3.3. *Confirmed positive case:* An animal from which ASF virus has been isolated and identified at NVSL FADDL or a laboratory designated by the Secretary of Agriculture or,

3.3.1. A presumptive positive case with a positive confirmatory ASFV antigen test at NVSL FADDL

Note: In any ASF outbreak, case definitions may be edited after the first presumptive positive or confirmed positive case (index case). The case definition will be reviewed throughout the outbreak and modified on the basis of additional information or the changing needs of the eradication effort.

Appendix 2. Classical Swine Fever Case Definition

DRAFT – UNDER REVIEW

1. **Clinical Signs:**

1.1. *Clinical signs:* Classical swine fever (CSF) is 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. 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 rarely seen anymore. Instead, more moderate forms and presentations of the disease predominate. Prevailing strains of CSF virus are moderate to low virulence, making clinical diagnosis difficult especially in older animals. Low virulent 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.

1.1.1. *Acute:* Illness usually seen in weaned suckling pigs less than 12 weeks of age that is 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, dermatitis, and may result in the occurrence of runts in the herd. Characterized by subdued acute infection followed by 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, abortions/stillbirths, or 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. (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 identification:* Tonsils, spleen, kidney, lymph nodes, or distal ileum should be transported without preservatives under cool conditions (not frozen). Whole blood (heparin or EDTA treated) from clinically ill pigs is also a suitable sample. Methods of detection include immunohistochemistry (IHC) using CSF-specific monoclonal antibody

(ABC staining) on tissue samples, real-time and conventional reverse-transcriptase PCR (rPCR and RT-PCR), and virus isolation followed by ABC staining and/or rPCR or RT-PCR.

2.2. *Serological tests:* Serological tests include the neutralizing peroxidase-linked assay (also called immunoperoxidase virus neutralization test - IP-VN), immunoperoxidase test (IP), and E2 antibody ELISA. 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.

3. Case definition and Reporting Criteria:

3.1. *Suspect case:* A pig or herd that has:

3.1.1. Clinical signs, history, or epidemiology consistent with CSF; ***OR***

3.1.2. An inconclusive or positive rPCR/RT-PCR performed on a sample taken during routine surveillance, without the presence of clinical criteria, for which either additional laboratory diagnostics (sequencing information or confirmatory testing) or epidemiological investigation results are pending; ***OR***

3.1.3. A positive antibody ELISA performed on a sample taken during routine surveillance with subsequent positive results to IP and IP-VN tests with neither epidemiological information nor known clinical signs consistent with CSF.

3.2. *Presumptive positive case:*

3.2.1. A suspect case with a positive repeated rPCR/RT-PCR or genomic sequencing consistent with CSF virus conducted at the Foreign Animal Disease Diagnostic Laboratory (FADDL) after an initial positive rPCR/RT-PCR on a sample from a pig with or without clinical signs and/or epidemiological evidence of CSF conducted; ***OR***

3.2.2. A pig or herd with epidemiological information and/or clinical criteria consistent with CSF; ***AND***

Positive rPCR or RT-PCR; ***OR***
Positive ABC test on tissue samples; ***OR***
Positive IP-VN test.

3.3. *Confirmed positive case:* A pig from which CSF virus has been <u>isolated</u> with <u>sequence confirmation</u> at the National Veterinary Services Laboratories, FADDL, or a laboratory designated by the Secretary of Agriculture.