Key points for detection and diagnosis of African swine fever.

Led by Shanghai Veterinary Research Institute, Chinese Academy of Agricultural Sciences.

Sept 9, 2019

Preface

African swine fever (ASF) is an acute, thermal and highly contagious infectious disease of pigs caused by African swine fever virus (ASFV). The morbidity and mortality can reach 100%. The World Organization for Animal Health (OIE) listed it as a statutory report of animal diseases, while China listed it as animal disease Category I.
ASF was first reported in Kenya in 1921, and became epidemic in Africa, Europe and South America in 1950s and 1960s. Since the first outbreak of African swine fever in China in early August, 2018, the disease has spread to 23 provinces in China, bringing huge losses to China's pig breeding industry and national economy. Due to the complex infection mechanism of ASFV, there is no effective vaccine yet for preventing ASF worldwide, and therefore, early identification and accurate detection are the key to control the spread and occurrence of the disease.

With reference to the relevant documents and technical manuals of OIE, Ministry of Agriculture and Rural Affairs, China Center for Animal Health and Epidemiology and China Center for Animal Disease Prevention and Control, and combining with our research results, this booklet is compiled from three technical points: early identification, on-site investigation and accurate detection of ASF, which provide concise and practical related operation techniques and methods for frontline epidemic prevention personnel engaged in on-site investigation and detection of ASF.

Contribution:
Lead team:Swine Respiratory Infectious Diseases Team, Shanghai Veterinary Research Institute
Leader: zhiyong Ma
Team member: Jianchao Wei etc.

Other participation teams:
Innovation team of severe porcine infectious Diseasess of Harbin Veterinary Research Institute
Leader:： Huaji Qiu
Team member: Yuzi Luo etc.

Lanzhou Veterinary Research Institute Ectoparasites and Insect-norne Diseases Team
Leader:： Zhijie Liu

Team member ： Mi lin etc.

Langmu Pharmaceutical Research Institute Chinese Veterinarian and Clinical Team
Leader:： Jianxi Li
Team member ： Dongan Cui

Contents

1, Introduction

African classical swine fever virus (ASFV) is a DNA virus with envelope, which is the only member of African classical swine fever virus family and African classical swine fever virus genus. The ASFV particle is about 200 nm in diameter, and possesses a regular icosahedron structure, which is composed of multi-layer concentric circle structure, assembled with its genomic DNA, matrix layer, inner

membrane, nucleocapsid and outer capsule from inside out. Its genome is linear double-stranded DNA, which is about 170-193 kb in size and contains 150-167 open reading frames (ORFs). It consists of a central conserved region of about 125 kb and two variant ends containing five polygenic families (MGFs). According to the partial nucleotide sequence of gene B646L encoding major capsid protein p72, ASFV can be divided into 24 genotypes.

The ASFV strain initially introduced into China belongs to genetic Type II. ASFV could not induce neutralizing antibody immunity. The capsid protein p72 and membrane proteins p54, p30 and p12 of ASFV have moderate antigenicity. In infected or recovered pigs, more than 50 virus proteins can induce antibody response, which can be used as antigens for serological diagnosis. Under natural infection conditions, the latent period of ASF is 3-19 days, and infected pigs can produce the ASFV during the latent period, and will produce more ASFV drastically through secretions and excretions following the appearance of the clinical symptoms.

ASFV is quite tolerant to acid and alkali, which is stable in serum-free medium at pH4-10, but can be inactivated within several minutes when pH is lower than 4 or higher than 11.5. ASFV has strong resistance to the environment, which can survive for more than 6 months in carcasses, for several years at low temperature, and can also survive for nearly 4 months in gangrenous blood, for weeks to months in chilled meat, for a long time in cured and smoked pork products, and remains infectious in feces for several weeks.

ASFV has weak resistance to heat and can be inactivated at 60℃ for 20 minutes or 56℃ for 70 minutes. General disinfection measures can effectively kill ASFV. The most effective disinfectants are detergents such as hypochlorite, alkalis and glutaraldehyde.

The natural transmission of ASF is slow, however, ASF is highly contagious. Direct contact, feeding, tick bite and injection can spread epidemic the diseases. Digestive tract (mouth) and respiratory tract (nose) are the main infection routes of ASFV. Flies, mosquitoes, rats, etc. can also transmit ASFV.

Source materials for infection: secretions and excretions of wild boars and diseased domestic pigs carrying ASFV, slops containing dead pig tissues or contaminated by ASFV, pork containing ASFV and its products, and soft ticks.

Transmission route: ASFV can be transmitted by direct contact between infected pigs and healthy susceptible pigs. ASFV can be transmitted indirectly by feeding contaminated slops, contaminated feed materials, bedding, vehicles, equipment and clothing. The soft ticks of the genus Physalis, especially *O. moubata* (Africa) and *O. erraticus* (Europe), are the hosts and vectors of ASFV. The digestive tract and respiratory tract are the main infection routes.

Susceptible animals: domestic pigs and wild boars. Other mammals, including humans, are not infected with ASFV. At present, the reported diseased pigs are mainly pigs fed with slops polluted by ASFV. Pigs of different breeds, ages and genders are also susceptible to ASFV.

Incubation period: domestic pigs range from 3 days to 19 days.

ASF is a disease that can cause a series of syndromes in domestic pigs and wild boars

of different species and ages. Acute swine fever is characterized by high fever, hemorrhage of reticuloendothelial system and high mortality. ASFV has three types of strains: strong virulence, moderate virulence and low virulence, which can clinically be divided into most acute type, acute type, subacute type and chronic type.

The most acute type: the sick pig has a fever with its body temperature of 41℃-42℃, showing anorexia, anorexia, depression, skin congestion and other symptoms, and usually dies within 1-4 days. Some pigs may die asymptomatically. The morbidity and mortality of both can reach 100%.

Acute type: The sick pig has a fever with its body temperature of 41℃-42℃, showing anorexia, unwillingness to move around, redness of skin, vomiting, nasal bleeding, bloody stool, constipation, etc. Female pigs will abort pregnancy. The mortality rate can reach 90%-100%.

Subacute type: similar to acute type. The sick pigs develop moderate fever and decreased appetite, bleeding and swelling of skin, and die in 7-20 days after infection with 30%-70% death rate.

Chronic type: The sick pigs lose weight and grow poorly, showing intermittent fever, and the skin in ears, abdomen and inner thighs is necrotic or ulcerated, and the joints are enlarged. The infected pigs may also have respiratory symptoms.

Laboratory diagnosis of ASFV is carried out by etiological diagnosis (including virus isolation, detection of virus antigen and genomic DNA) or antibody detection. select Appropriate diagnostic methods can be chosen based on the disease situation in the region or country as well as the capacity of the diagnostic laboratory.

Identification of pathogens: The national authorized laboratory diagnosis must be aimed at virus isolation, and also, pig alveolar macrophages or bone marrow culture should be inoculated, antigen in tissue smear or frozen section should be detected by fluorescence antibody test (FAT), and genomic DNA should be detected by PCR. PCR is an excellent, sensitive and rapid detection technique for ASFV, especially when tissues are not suitable for virus isolation and antigen detection. In suspicious cases, the materials were passed down in porcine alveolar macrophage culture, and the above process was repeated.

Serological test: Pigs that have tolerated ASF natural infection usually produce anti-ASFV antibodies 7-10 days after infection, and these antibodies last for a long time. For regional endemic, or the initial outbreak caused by low virulence strains, the research on such an occurrence needs to include the detection of special antibodies and tissue exudates in the sample serum. Many methods, such as indirect fluorescent antibody (IFA), enzyme-linked immunosorbent assay (ELISA) and western blotting, can be used for antibody detection.

2, Early identification

In order to determine ASF epidemic as quickly as possible, all the staff in pig farms should be familiar with the clinical symptoms of ASF, and be very clear of where are the pig farms, slaughterhouses, pig trading places and harmless treatment sites within three kilometers, and also pay close attention to the abnormal death of the pigs around, and identify the potential dangers of the epidemic in a timely manner.

All the staff members should always pay close attention to the abnormal situation of

pigs in all segments of the pig farm, including the developing state of pigs, feeding conditions, changes in body temperature, changes in body appearance and performance of female pigs. Currently, most cases of ASFV infection in China belong to the acute or subacute types. Generally, symptoms of acute or subacute types mainly include fever, lethargy, anorexia, skin bleeding, and problems in circulatory system, respiratory system and digestive system accompanied by neurological disorders.

Mental state: It is vital for us to timely identify the pigs with abnormal eyes (pigs may not produce ASFV at this time) and the pigs with depressed spirits, and collect the sample promptly for inspection and detection. Pig farms should encourage front-line staff to pay close attention to any abnormal pigs, and reward the staff members who identify the abnormal pigs.

Hunt for food: Pay attention to the feed intake of group and individual pigs. If the hunt for food is slightly reduced (excluding feed factors), samples should be collected and sent for inspection as soon as possible. Pigs that the feed intake has dropped significantly are generally accompanied by an increase in body temperature.

Change of body temperature: The body temperature of normal nursing pigs and fattening pigs is generally between 39℃ and 40℃. The body temperature of normal basic pigs ranges from 38℃ to 39℃ (the temperature of pregnant pigs rises to 39℃ to 40℃ during estrus and a few days before and after delivery). If pigs that body temperature exceeds the normal range are identified, they should be sampled for inspection promptly. Different thermometers must be used from pig to pig to avoid cross contamination when measuring body temperature. Far-infrared thermometer can be used for temperature monitoring. Personnel carrying out the tasks should wear proper gloves and protective clothing for the said procedures, and should try to avoid entering the infected area if possible.

Body surface changes: Red skin of the pigs indicates that pigs may suffer a fever. In addition, some pigs have bad blood coagulation during body injection. For these abnormal pigs, samples should be taken promptly for detection.

Performance of multiparous pigs: Besides the above-mentioned clinical manifestations, multiparous pigs will also abort pregnancy. Abortion caused by ASF is different from black fetus or white fetus, which are common in other diseases, and the fetus is homogeneous and red.

Upon observing any suspicious symptoms of the pigs, samples should be sent for inspection promptly (the sampling priority: nasal swab > saliva > vaginal swab > anal swab).

3, On-site screening

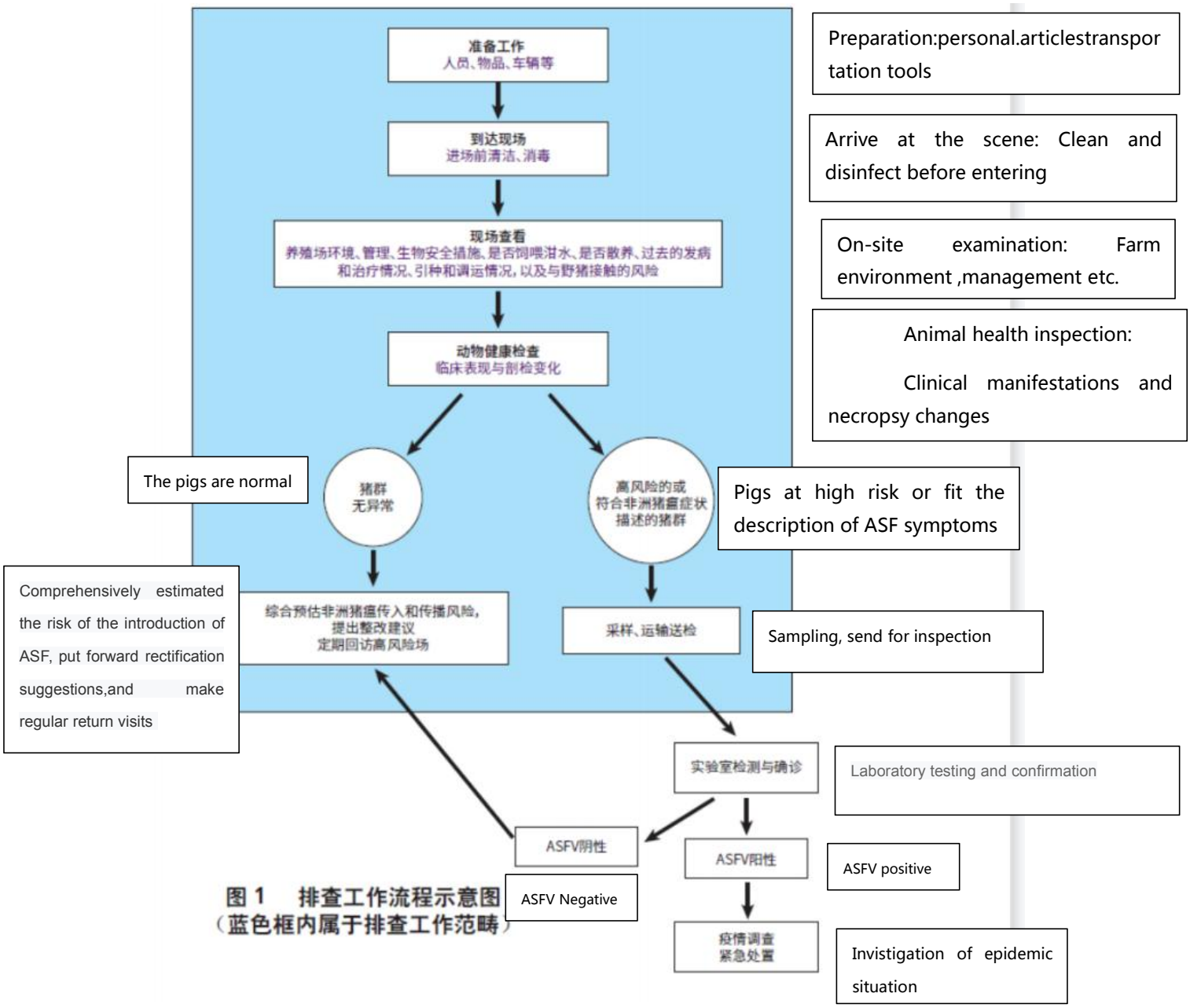


图 1　排查工作流程示意图
（蓝色框内属于排查工作范畴）

Figure 1. Schematic diagram of investigation work flow (China Center for Animal Disease Prevention and Control).

3,1 Preparation

3,1,1 Personnel

Understand the basic knowledge of ASF (basic biological characteristics, epidemiological characteristics, clinical manifestations, pathological changes of autopsy, etc.).

Understand the high risk factors of ASF infection and transmission in Africa (feeding slop, low biosafety level, animal trafficking, circulation of pork and its products).

Understand the biosafety operation requirements, and avoid the spread caused by human factors. Farmers and other personnel should coordinate well as they may face great challenges.

3,1,2 Transportation tools

Vehicles must be thoroughly cleaned and disinfected. The vehicle should not be used to carry other irrelevant articles. Disposable plastic sheets should be used in the car and trunk to avoid cross pollution.

3,1,3 Preparation of articles

3,1,3,1 List of materials required for entering the infected area:

Rubber boots: Disposable and biosafety;

Protective clothing and mask;

Shoe cover or boot cover; Disposable latex gloves;

Disinfectant and water spray (disinfectant suitable for ASFV);

Waste bags (including biohazard waste bags);

Self-sealing bag (for holding mobile phones or other devices);

Facial disinfectant wipes;

Sealing tape;

Goggles, Detergent and brush.

3,1,3,2 List of materials required for sampling

General materials:

marker (pen); printed data table, pen, writing board, a sharp box for holding needles and blades; Autoclave bag, Swabs for environmental sampling and tubes for the swabs;

Materials required for sample packaging and transportation:

Container/centrifugation tubes/vials (clearly marked); Absorbent paper;

Containers or bags with ease to seal can be used as secondary packaging (i.e. leak-proof), containers for storing animal samples and blood collection tubes;

Refrigerator (+4℃);

Portable -80℃ freezer/dry ice/liquid nitrogen tank (only required when sampling away from a well-equipped laboratory);

Materials for tie-up animals (e.g., lassos, wooden boards);

Materials needed for blood collection:

disinfectant absorbent cotton (alcohol cotton);

Sterile blood collection tube without anticoagulant (10 ml) (red cover); Sterile blood collection tube containing EDTA (10 ml) (purple cover); According to the size of the pig and sampling positions (neck, vein and ear vein), select vacuum blood collection

vessels or 10-20 ml syringes.

3,2 Requirements for Entry and exit from the possibly infected farms.

3,2,1 Vehicles arriving at the farm are parked near the entrance of the farm and are not allowed to enter the farm.

Wear personal protective equipment at the designated place (clean area) of the farm (if necessary, follow the requirements of the breeding farm for personal protection), and prepare materials and disinfectants, etc.

Before entering the production area of the farm, take off and remove unnecessary clothes and articles (clips, ties, watches, etc.), empty the clothes pockets, and enter the production area according to the entry procedures of the farm (bathing or other methods).

Electronic devices (mobile phones, etc.) should be placed in sealed plastic bags for subsequent cleaning and disinfection. While in the farms, mobile phones must be placed in plastic bags and used with the bags on.

Other non-disposable goods shall enter the site after disinfection. If necessary, the materials should be disinfected according to the procedures for entering the farm before entering the site.

Disinfection should be carried out on clean and dry ground (preferably concrete ground), and clean and non-clean areas should be separated with a clear boundary.

3,2,2 Wear personal protective equipment in clean area before entering.

Some farms may ask all personnel to take showers and wear the provided clothes from the farm before entering the farm.

Other farms may require all personnel to put on disposable protective clothing and boots, and also wear gloves, which are sealed with tape.

If you need to wear waterproof clothing, waterproof clothing should be covered on the outer layer of boots. Wear another layer of gloves to facilitate necessary replacement in between.

The cover of the boots should cover at least the bottom and the lower part of them. Before entering the site, wear a mask and carefully check the item list.

3,2,3 Preparation before exit

Clean and disinfect all articles that have come into contact with the farm in non-clean areas.

Disinfect the surface of the container containing the sample, and then place it in a clean area. Remove the boots' cover and put it in a waste bag in a non-clean area, then thoroughly scrub the boots (especially the soles).

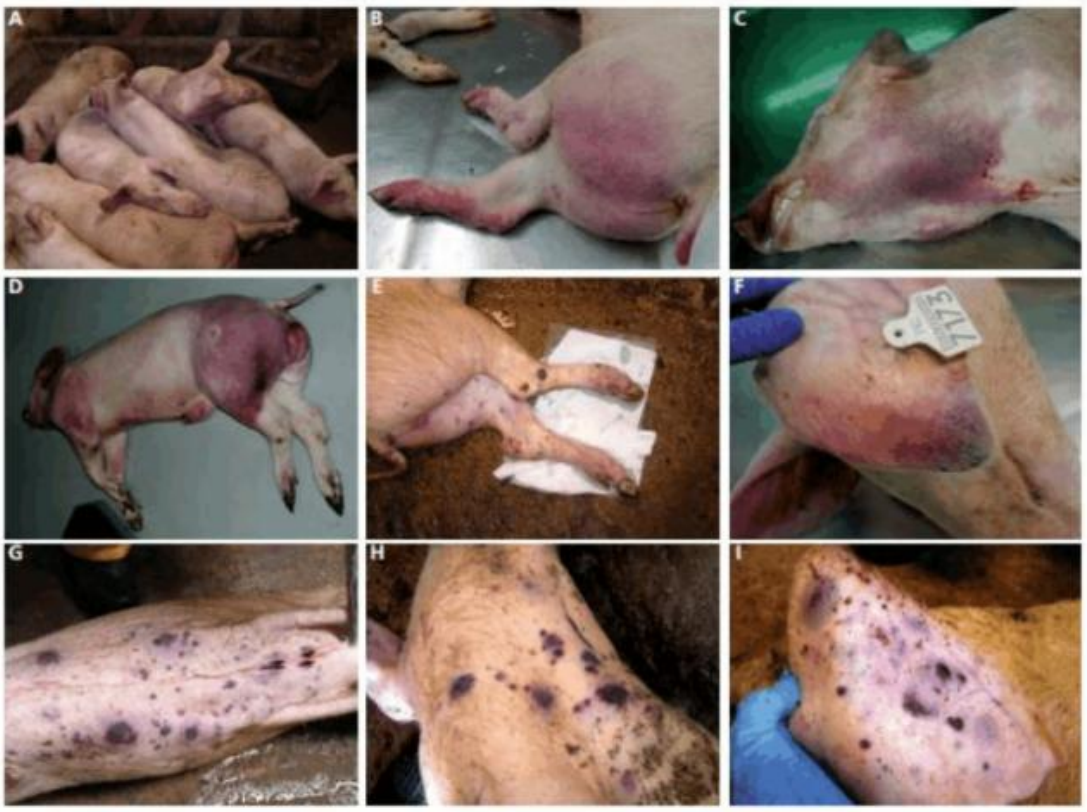
Take off gloves and put them in waste bags in non-clean areas.

Take off the disposable protective clothing and put it in a waste bag in a non-clean area. Take off your boots, disinfect them and put them in a clean bag. Hands and goggles must also be disinfected, and the face should be cleaned with disinfectant wipes. Personnel entering the production area can leave only after bathing in the designated area.

Non-disposable goods (rubber, boots, etc.) and containers containing samples shall be placed in double-layer bags and sealed with adhesive tape. Only then, you may use your regular shoes back. Bags taken out of the farm should be placed on plastic sheets

prepared in advance in vehicles.
Vehicles exposed to possible infected articles should be cleaned and disinfected.
Clean and disinfect the tires and surfaces of the vehicles before leaving potentially contaminated areas. Remove all visible dirt. Don't forget to clean any hidden areas, such as the wheel arches, the tire boards and the bottoms. After removing all dirt, spray the surface with disinfectant.
Dispose all the wastes in the vehicle and clean up all dirt (all the wastes should be properly disposed). Wipe the steering wheel, gear lever, pedal and handbrake with a cloth soaked with disinfectant.
3,2,4 Notes following the exit
If you don't raise pigs at home, you can go home to shower and wash your hair thoroughly. Soak the clothes in disinfectant for 30 minutes; If pigs are raised at home, you should shower in other places before going back home.
If you enter a farm with suspected infection or confirmed cases by ASFV, you should not go to any place with pigs within three days.
Disinfect the inside and outside of the vehicle again. Remove all plastic sheets from the vehicle and dispose of them properly.
Selection of disinfectants: ASFV has weak resistance to heat, and common disinfection measures can kill the virus, however, the virus can survive for more than 6 months or even several years in infected pig tissues at low temperature conditions. The most effective disinfectants are detergents, such as hypochlorite, alkalis and glutaraldehyde. The following treatments can inactivate the virus: 8/1000 sodium hydroxide for 30 minutes, 2%-3% hypochlorite chloride for 30 minutes, 3/1000 formalin for 30 minutes and 3% O-phenylphenol for 30 minutes.
Alkalis (sodium hydroxide, potassium hydroxide, etc.), chlorides and phenolic compounds e.g., are suitable for disinfection of buildings, wooden structures, cement surfaces, vehicles and similar facilities or equipment, while alcohol and iodides are suitable for disinfection of personnel.
Equipment that is not easy to disinfect may be exposed to sunlight for disinfection.
3,3 Clinical diagnosis
The main clinical manifestations of the pigs with ASF are quite different, and hence difficult to be diagnosed, however, usually, some or all of the following typical symptoms may occur, which include high fever, vomiting, diarrhea or constipation, some bloody stools, weakness and difficulty in standing, red, purple or blue skin on different parts of the body surface (especially ears, nose, abdomen and buttocks), some coughing and dyspnea, and pregnancy abortion, stillbirth or weak birth of the female pigs. Following the above clinical symptoms, the pigs usually die within 2-10 days. Dissection of the carcasses revealed that many organs and tissues of internal organs were bleeding, the spleen was significantly enlarged, the color became dark, and the texture became brittle. In some farms where ASF occurred for the first time, the pigs suffered very acute fever, and when those suffered the most acute fever, the pigs might die suddenly without any symptoms, or without special clinical manifestations and pathological changes.
3,3,1 Clinical symptoms

Based on its virulence, ASFV can be divided into three main strains: high virulence, moderate virulence and low virulence. The clinical manifestations of ASF ranged from acute (very urgent) to asymptomatic (not obvious). At present, most cases of ASFV infection in China belong to acute or subacute types. ASF is usually characterized by sudden death and elevated body temperature before death. Clinical manifestations ranged from rapid death within 7 days of infection to chronic infection lasting for several weeks or months (**Figure 2**).



A: The pigs looked visibly weak and feverish and probably huddled together for warm

B-E: There are marked areas of hyperemia on the skin of the extremities in the neck, chesk and extremities

F:The tips of ears are cyan

G-I: Necrotic lesions on the skin of the abdomen ,neck and ears

Figure 2 Clinical symptoms of acute African swine fever.

Note: Reference from the Food and Agriculture Organization of the United Nations (FAO) <<Animal

Production and Animal Health Manual>>.

Major clinical symptoms:

●Sudden death without symptoms;

●High morbidity and mortality;

●High fever with body temperature to 40.5-42℃;

● There are bleeding spots and cyanosis on the ears, limbs and skin of the abdomen back;

●Vomiting, diarrhea or constipation, bloody feces;

●Weak, stiff gait, unwilling to stand, etc.;

●Occasionally, mucous purulent secretion is found in eyes and nose.

Other clinical symptoms:

●Depression and loss of appetite;

●Dyspnea, wet cough;

●Joint pain and swelling;

●Pregnancy abortion, stillbirth and weak piglet of pregnant pigs.

3,3,2 Anatomical and pathological changes

The characteristic pathological changes of ASF are usually visible bleeding from multiple organ.

The most obvious pathological changes include:

● Lymph nodes (especially gastrointestinal and kidney) are enlarged, edema and bleeding of the whole lymph node, and the shape is similar to blood clot;

●Spleen is significantly enlarged, usually 3-6 times as large as normal spleen, and its color becomes dark and its texture becomes brittle.

●Blood stasis on the surface of kidney (spotty hemorrhage) (**Figure 3**).

Figure 3 Common pathological changes of acute African swine fever.

Note: Reference from the Food and Agriculture Organization of the United Nations (FAO) <<Animal Production and Animal Health Manual>>.

The autopsy changes may also include:

●subcutaneous hemorrhage;

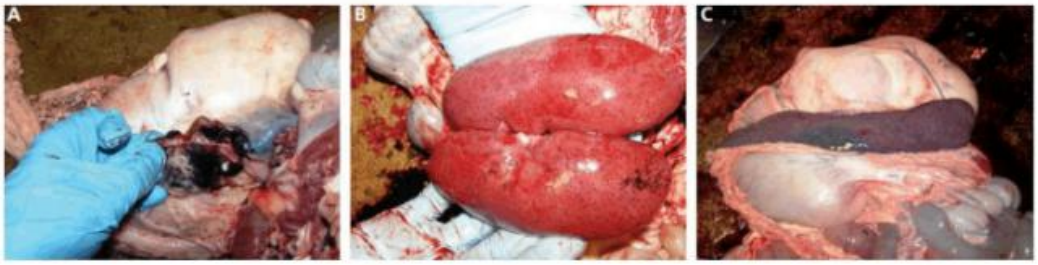
●Pericardial hydrops, coelom hydrops and ascites;

●Bleeding points on the surface of heart (epicardium), bladder and kidney (cortex and renal pelvis);

● There may be congestion and petechia in the lungs, foam in the trachea and bronchus, and severe alveolar and interstitial pulmonary edema.

●Stasis, ecchymosis (large bleeding), excessive blood coagulation in stomach, small intestine and large intestine;

●Liver congestion and gallbladder bleeding (**Figure 4**).



A: When infected with ADFV, gastric lymph nodes and liver and kidney lymph nodes are significantly hemorrhages and swells. Non-diseased tissue should appear white without inflammation

B:Kidneys infected with ADFV have obvious petechiae on the skin, and healthy kidney tissue is uniformly colored light brown with no irregular changes on the surface

C: The kidneys of pigs infected with ADFV are usually swollen, fragile, showing signs of infarction, and healthy spleens are uniform in color and texture

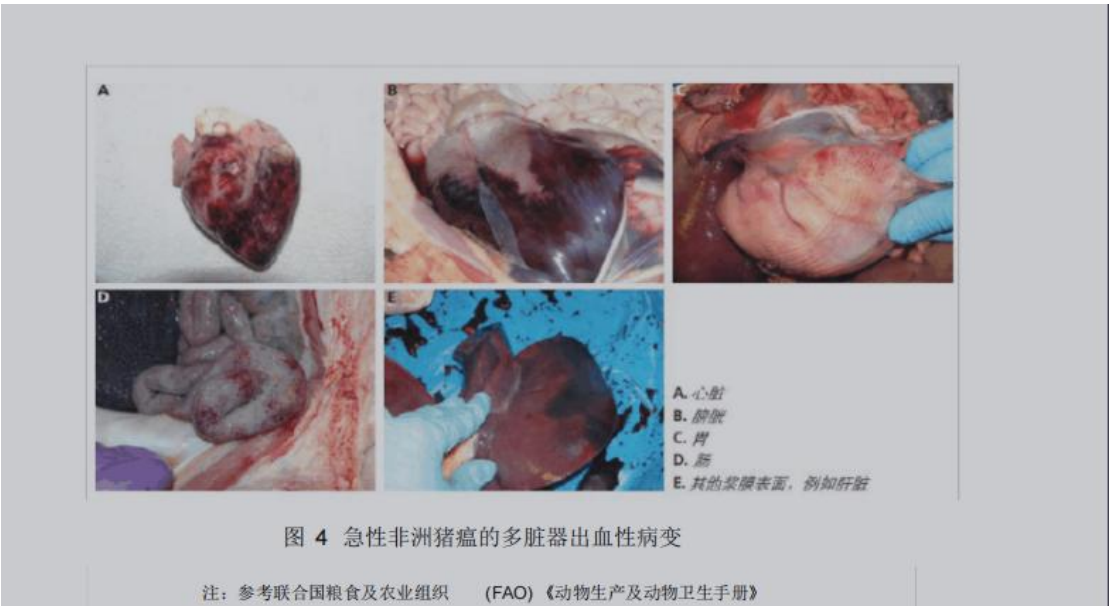


Figure 4 Multiple organ hemorrhagic lesions of acute African swine fever.

Note: Reference from the Food and Agriculture Organization of the United Nations (FAO) <<Animal Production and Animal Health Manual>>.

3,3,3 Differential diagnosis

ASF has various clinical manifestations and different degrees in severity. The clinical symptoms of ASF are very similar to those of classical swine fever (CSF), swine erysipelas, Salmonella, Actinobacillus, other septicemia, and porcine dermatitis nephrotic syndrome (PDNS), and sometimes they are easily mixed up (**Table 1**).

Table 1 Differential diagnosis of African swine fever.

Note: Reference from the Food and Agriculture Organization of the United Nations (FAO) <<Animal Production and Animal Health Manual>> and Manual on Field Investigation of African Swine Fever by China Center for Animal Disease Prevention and Control.

3,3,4 Actions to be taken promptly in case of suspected cases

In case of any suspected cases, the epidemic situation shall be reported immediately according to <<the requirements of Technical Specifications for Prevention and Control of African Classical Swine Fever and Emergency Plan for African Classical Swine Fever>> (see Appendix I).

3,4 Specimen collection, packaging and transportation

3,4,1 Method of sampling

Effective and accurate collection of ASF samples is a key step to realize rapid and accurate detection of ASF. In case of outbreak (passive monitoring), samples should be taken from the sick pigs, dead pigs and the environment around. In routine monitoring, samples should be taken from ASFV susceptible pigs and the environment (such as the pig station and the gate of the field, etc.). If it is aimed at the population in precise clearance, the number of the samples can be appropriately increased. Attention should be paid to avoid cross contamination during sampling, and disposable gloves should be replaced after collecting each pig. After touching pigs, troughs and railings, replace disposable gloves promptly. When collecting blood, a fresh needle must be replaced for every pig collected so as to avoid the spread of ASFV. Relevant samples submitted to the diagnostic laboratory should have clear and long-lasting labels, and the samples are of good quality. All protective articles used in the collection process of the samples should be either incinerated or treated with high-pressure inactivation.

Sample type: nose and mouth swab.

Collect the mouth and nose swabs in the same sampling tube for each pig, so as to improve the accuracy of detection and also reduce the workload. It is recommended to store the sample at 4℃ and send it for detection immediately. If no detection facility nearby is available, it should be sent to a qualified laboratory within 24 hours for detection.

Whole blood sampling: Sterile operation should be maintained during the process.

Before blood collection: use alcohol cotton to disinfect the blood collection site around.

After blood collection: disinfect locally and press with dry cotton balls to stop bleeding.

Whole blood was collected from jugular vein, anterior vena cava or auricular vein by vacuum blood collection vessel containing anticoagulant (EDTA- purple cap).

If the animal is dead, blood can be collected from the heart immediately. Avoid using vacuum blood collection tubes containing heparin (green cover).

Serum sample: Blood samples were collected from jugular vein, anterior vena cava and auricular vein by using vacuum blood collection tube without anticoagulant (red cover), or during autopsy.

Leave it on the bench for separating, and then serum can be collected. If the serum is red, this indicates that the samples undergo hemolysis. Usually the blood samples of dead animals are prone to hemolysis.

Samples can be detected immediately after separation. If storage is needed, it should be stored at -20℃ for antibody detection. For virus detection, better store it at -80℃ freezer.

Organ and tissue samples: Samples of spleen, lymph node, liver, tonsil, heart, lung and kidney can be collected.

Spleen and lymph nodes are rich with the virus. For animals that have died for a long time, bone marrow samples can be collected, and intra-articular tissue fluid can also be collected.

It is recommended to keep the sample at 4℃, which should be sent to the laboratory for detection promptly. If samples cannot be delivered to the laboratory in time, the samples can be stored in cold storage or liquid nitrogen.

The virus can be inactivated by soaking the sample in 10% buffered formalin for more than 30 minutes, which can then be used for PCR detection.

The minimum required quantity of the sample: 1 ml serum; 1 ml whole blood; Tissue sample 10g.

Environmental samples: Collect soft ticks and other media and environmental swabs from farms, such as feces, slop and feed.

Soft ticks and other vector samples: Blunt ticks can be used to detect ASFV.

How to collect the ticks: (a) manual collection, (b) carbon dioxide trapping and (c) vacuum suction capture. After collection, ticks should be kept alive or stored directly in liquid nitrogen to avoid DNA degradation.

Environmental sample: take the block as a unit, and carry out regional sampling on the risk level. For example, all walls, floors, equipment and other surfaces in pig farms shall be fully covered for sampling.

Production area: including walls, floors, fans, trenches, equipment, waterlines, material lines, etc. of all sections in pig farms in each section. Sampling shall cover all positions that are difficult to collect, including inaccessible corners and the bottom of the trough.

Outside the production area: collect samples from any possible infected areas, including all areas that may be polluted, such as piggery, gate of the field, material tower, sewer, staff dormitory, storage room, bathroom, office, kitchen, vehicle, water source, etc.

It is recommended that all samples be stored below 4℃ unless specified.

3,4,2 Recording, preservation, packaging and transportation of the samples

In order to ensure accurate diagnosis, samples must be carefully packed, marked and recorded promptly and immediately sent to the nearest laboratory designated by the Ministry of Agriculture and Rural Affairs.

Delivering of the Sample must comply with the regulations set by the Ministry of Agriculture and Rural Affairs <<Highly pathogenic animal pathogenic microorganism (virulent) species transportation packaging specification>> and other regulations.

Before sampling, you must call the designated laboratories to ensure that the correct sample submission procedures are followed, so as to ensure that the samples can be stored immediately and be readily detected.

Ensure the safety of samples during transportation, avoid infecting other animals during transportation, and avoid contamination of the samples.

The samples to be transported must be kept cold with sufficient cooling materials (such as ice packs) to prevent degradation.

Sampling information record: the sampling sheet should be filled in when collecting samples. The samples must be marked item by item with pen or signature pen (in triplicate).
Samples and seals shall be filled in with a signature pen.
The external seal of heat preservation container shall be filled with pen or signature pen.
Small plastic centrifuge tubes can be marked with markers.
The sampling sheet and medical history data should be packed in plastic packaging bags and sent to the laboratory along with the samples, and each sample should correspond to the source animal.
Sample information should at least include the following contents:
Name of livestock owner and address of farm;
Variety and quantity of animals raised in farms;
The species and quantity of suspected infected animals or susceptible animals;
Date of first case and second case;
Distribution of infected animals in herds;
The number of dead animals, the number and age of animals with clinical symptoms;
Clinical symptoms and their duration, death and time, etc.
Feeding types and standards, including feed sources, etc.
List and description of samples to be sent for inspection, including types of diseased materials and preservation methods;
History of animal immunization and treatment;
The name, address, zip code, telephone number and email address of the sender;
Date of the samples being delivered;
Signature who collected and sent the samples and official stamp of the company on the information sheet.
Sample packaging requirements: The collected samples should be carefully packed, marked and sent to the laboratory.
The “triple packaging system” should be used for samples to ensure the biological safety during transportation and avoid contamination of the samples.
It is recommended to use the “Triple Packaging System” for samples, mark correctly, and prevent leakage, with a sample sheet attached.
Direct Packaging: Samples should be stored in sealed and sterile main containers, and different containers should be selected according to the characteristics of the samples to be tested and the purpose of the test, as shown in **Figure 5**. Each sample container shall be marked with the sample name, sample number, sampling date, etc., so as to clearly identify which animal it comes from.
Secondary packaging: Absorbent materials should also be placed in secondary containers. If multiple fragile primary containers are placed in a single secondary container, they must be individually packaged or separated to prevent cross contact. The secondary packaging container shall be sealed with the signature of the sampler, indicating the sealing date and the placement direction of the package.
Hard outer packaging: The volume of a liquid sample shall not exceed 4 liters, and in the case of solid sample, the weight shall not exceed 4 kilograms (excluding the

weight of ice). Samples must be kept at 4℃ or lower and sent to the nearest animal disease prevention and control institution for preservation. Caution: Never freeze whole blood or serum mixed with blood clots.

Outer packaging label and marking: “Class B infectious substance” label should be have “Class B infectious substance” marked next to its correct shipping name; The full name, address and telephone number of the person in charge of sampling;

The full name, address and telephone number of the laboratory contact person; The label shall be marked as at 4℃, at -20℃ or at -80℃.

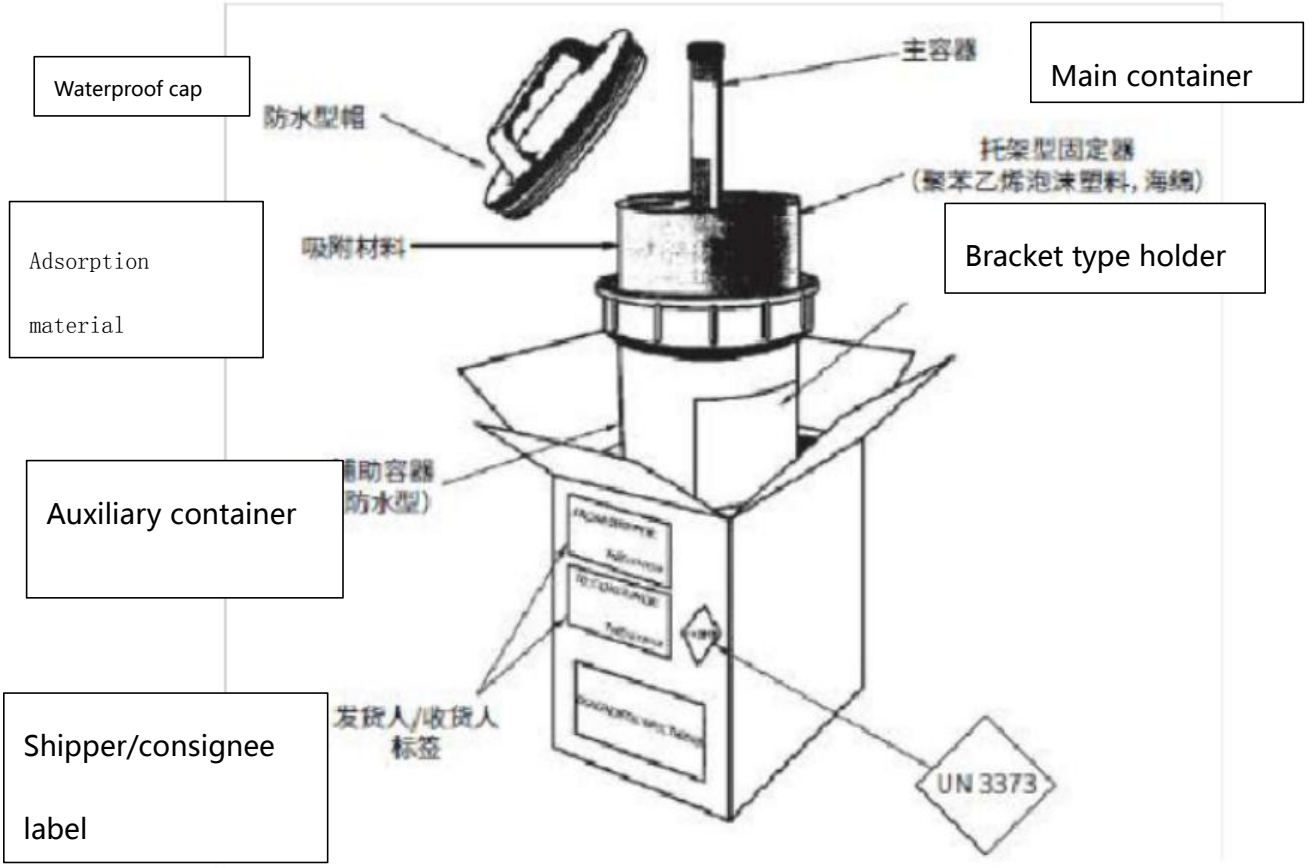


图 5 B 类感染物质的包装标签和三重包装系统的例子

Figure 5 Example of packaging label and triple packaging system for Class B infectious substances.

Note: Reference from the Food and Agriculture Organization of the United Nations (FAO) <<Animal Production and Animal Health Manual>> and the Chinese Center for Animal Disease Prevention and Control’s <<African Swine Fever Field Investigation Manual>>.

3,4,3 Waste disposal

After sampling, do carry out necessary disinfection and harmless treatment of corpses, sites, articles and personal protective equipment. Try to arrange different sampling personnel for different sites to avoid cross-contamination.

4, Laboratory diagnosis and confirmation

4,1 Rapid and accurate detection

Real-time quantitative PCR(qPCR) is a common method for rapid detection of ASFV in laboratory. Large pig-raising enterprises may have their own laboratories for ASFV detection to investigate and monitor the epidemic situation when the surrounding pig farms and their own pig farms are threatened, so as to provide technical support for epidemic situation disposal and accurate elimination. The qPCR detection must be equipped with a special real-time fluorescence PCR instrument and corresponding detection reagents. The laboratory can carry out qPCR detection of ASFV according to technical standards. Because qPCR detection technology is sensitive, any pollution may result in a false positive, and hence, subarea management of the PCR laboratory is advised to avoid any detection error. For example, the PCR laboratory can be divided into reagent preparation area, sample preparation area and product amplification area, etc.

4,2 Diagnosis of epidemic situation

The laboratory of China Animal Health and Epidemiology Center or Provincial Animal Disease Prevention and Control Center can confirm ASF epidemic as required.

Figure 6 Laboratory diagnosis technology of African swine fever.

Note: Reference from the Food and Agriculture Organization of the United Nations (FAO) <<Animal Production and Animal Health Manual>>.

Appendix I: Technical Specifications for Prevention and Control of Classical ASF.

ASF is an acute, thermal and highly contagious animal infectious disease caused by African swine fever virus, which is characterized by high fever, hemorrhage of reticuloendothelial system and high mortality. The World Organization for Animal Health (OIE) listed it as a statutory report of animal diseases, while China listed it as animal disease Category I. In order to prevent, control and extinguish African swine fever, according to <<the Law of the People's Republic of China on Animal Epidemic Prevention>>, <<Major animal epidemic emergency regulations>>, <<National emergency plan for sudden major animal epidemic>> and other laws and regulations, this specification is formulated.

A1 Scope of application: This Code specifies the prevention and control measures for ASF, such as diagnosis, epidemic reporting and confirmation, epidemic disposal and prevention.

This Code is applicable to units and individuals related to African swine fever prevention and control activities within the territory of the People's Republic of China.

A2 Diagnosis:

A2.1 Epidemiology

A2.1.1 Main sources of infection: domestic pigs, wild boars (including sick pigs, rehabilitation pigs and recessive infected pigs) and soft ticks.

A2.1.2 Transmission route: It is mainly transmitted by contacting pigs infected with African swine fever virus or pollutants of African swine fever virus (slops, feed, bedding grass, vehicles, etc.), and digestive tract and respiratory tract are also the main infection routes. It can also be transmitted by insect bites, such as soft ticks.

A2.1.3 Susceptible animals: Domestic pigs and Eurasian wild boars are highly susceptible, with no obvious differences in breed, age and sex. Although warthog and pig can be infected, they do not show obvious clinical symptoms.

A2.1.4 Latent period: The latent periods can be different due to distinct strains, hosts and infection routes. OIE's Hygienic Code for Terrestrial Animals stipulates that the incubation period of domestic pigs infected with African swine fever virus is 15 days.

A2.1.5 Incidence and mortality: Distinct strains have differential pathogenicity. Strong virulent strains can cause 100% death of pigs within 4 ~ 10 days, while moderate virulent strains generally cause 30% ~ 50% mortality, while less virulent strains only cause a small portion of deaths.

A2.1.6 Seasonal change: the disease is not obvious with seasonal changes.

A2.2 Clinical manifestation

A2.2.1 The most acute type: sudden death without obvious clinical symptoms.

A2.2.2 Acute type: The sick pig has a fever with its body temperature of 41℃-42℃, showing anorexia, unwillingness to move around, redness of skin, vomiting, nasal bleeding, bloody stool, constipation, etc. Female pigs will abort pregnancy. The mortality rate can reach 100%. The sick pigs die in 7-20 days.

A2.2.3 Subacute type: similar to acute type. The sick pigs develop moderate fever and decreased appetite, bleeding and swelling of skin, and die in 5-30 days after infection with 30%-70% death rate.

A2.2.4 Chronic type: The sick pigs lose weight and grow poorly, showing intermittent fever, and the skin in ears, abdomen and inner thighs is necrotic or ulcerated, and the joints are enlarged. The infected pigs may also have respiratory symptoms.

A2.3 Pathological changes: Serous surface congestion and bleeding, bleeding spots on kidney and lung surface, large number of bleeding spots on endocardium and epicardium, and diffuse bleeding of stomach and intestinal mucosa. Bleeding of gallbladder and bladder. The lungs are swollen, with foamy fluid flowing out of the section and bloody foamy mucus in the trachea. Spleen is swollen, fragile, dark red to black, with bleeding spots on the surface, blunt net at the edge, and sometimes edge

infarction. Submandibular lymph nodes and abdominal lymph nodes are enlarged, resulting in severe bleeding.

A2.4 Differential diagnosis: The clinical symptoms of African swine fever are similar to classical swine fever and highly pathogenic blue-ear disease of pigs, so laboratory tests must be carried out for differential diagnosis.

A2.5 Detection in the laboratory

A2.5.1 Collection, transportation and preservation of samples.

A2.5.2 Serological detection: Antibodies can be detected by indirect enzyme-linked immunosorbent assay, blocking enzyme-linked immunosorbent assay and indirect fluorescent antibody assay.

Serological testing should be carried out in the laboratories of provincial animal disease prevention and control institutions, China Center for Animal Health and Epidemiology (National Research Center for Exotic Animal Diseases) or the laboratories designated by the Ministry of Agriculture, which meet the relevant biosafety requirements.

A2.5.3 Pathogenic detection

A2.5.3.1 Rapid Pathogenic detection: Double antibody sandwich enzyme-linked immunosorbent assay, polymerase chain reaction and real-time fluorescent polymerase chain reaction can be used.

Samples for rapid pathogenic detection must be inactivated, and the detection should be carried out in the laboratories of provincial animal disease prevention and control institutions, China Center for Animal Health and Epidemiology (National Research Center for Exotic Animal Diseases) or laboratories designated by the Ministry of Agriculture, which meet the requirements of relevant biosafety.

A2.5.3.2 Isolation and identification of the virus: Cell culture and animal regression test can be used. The virus isolation and identification shall be carried out in the laboratory designated by China Animal Health and Epidemiology Center (National Center for Research on Exotic Animal Diseases) or the Ministry of Agriculture, and the biosafety level of the laboratory must reach BSL-3 or ABSL-3.

A2.6 Interpretation of the results

A2.6.1 Clinical suspicious epidemic situation: If the epidemiological characteristics, clinical manifestations and pathological changes conform the African swine fever, a clinically suspicious epidemic situation should be reported.

A2.6.2 Suspicious epidemic situation: A clinical suspected epidemic situation is judged as a suspected epidemic situation if the result is positive by any of the above serological methods or rapid detection methods of etiology.

A2.6.3 Confirmed epidemic situation: If the suspected epidemic situation is double-confirmed by the China Center for Animal Health and Epidemiology (National Center for Animal Disease Research) or the laboratory designated by the Ministry of Agriculture and the result is positive, it will be judged as a confirmed epidemic situation.

A3 Epidemic reporting and confirmation

A3.1 Epidemic reporting

Any unit or individual who finds abnormal death of domestic pigs or wild boars, such

as failure of classical swine fever immunization or large-scale pig death for unknown reasons, shall immediately report to the local veterinary authorities, animal health supervision institutions or animal disease prevention and control institutions.

The local animal disease prevention and control institutions at the county level shall report to the local veterinary authorities within 2 hours and report to the provincial animal disease prevention and control institutions step by step. When the provincial animal disease prevention and control institution judges the suspected epidemic situation of African swine fever, it shall immediately report to the provincial veterinary authorities, China Animal Disease Prevention and Control Center and China Animal Health and Epidemiology Center. The provincial veterinary administrative department shall report to the provincial people's government and the Veterinary Bureau of the Ministry of Agriculture within one hour.

China Center for Animal Health and Epidemiology (National Research Center for Exotic Animal Diseases) or the laboratory designated by the Ministry of Agriculture shall immediately report to Veterinary Bureau of the Ministry of Agriculture and send a copy to China Center for Animal Disease Prevention and Control, and notify the provincial animal disease prevention and control institutions where the epidemic occurred. The provincial animal disease prevention and control institutions shall immediately report to the provincial veterinary authorities, and the provincial veterinary authorities shall immediately report to the provincial people's government.

Appendix II: List of authorized laboratories, specialized laboratories and regional laboratories for ASF.

一、National Reference Laboratory of African Swine Fever

Ceter name: China Animal Health and Epidemiology Center

Address: No.21 Southeast road hongdao economic zone, qingdao city

Code:266032

二、National Professional Laboratory of African Swine Fever (Harbin)

Center name: Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences

Address: NO.678 haping road xiangfang district .harbin city heilongjiang province,china.

Code: 150069

三、National Regional Laboratory of African Swine Fever

四、（一）National Regional Laboratory of African Swine Fever (Lanzhou)

Ceter name： Lanzhou Veterinary Reasearch Institute , Chinese Academy of Agricultural Sciences

Address: No.1 xujiaping yancangbao lanzhou city gansu province.

Code :730046

（二）National Regional Laboratory of African Swine Fever (Wuhan)

Ceter name：Wuhan institute of virology
Address: No.44 xiaohongshan middle district wuchang district wuhan city china
Code:430071
（三）National Regional Laboratory of African Swine Fever (Guangzhou)
Ceter name：SCAU
Address: No. 483 wushan road tianhe district guangzhou city china
Code: 510642