



# National African Swine Fever Prevention and Control Plan - 2021

## Bhutan





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**FOREWORD**

African swine fever (ASF) is a severe viral disease affecting domestic and wild pigs. The disease is one of the most serious transboundary animal diseases because of its high lethality for pigs, crippling socio-economic consequences, and its propensity for rapid and unanticipated international spread.

Historically, outbreaks were reported in Africa and parts of Europe, South America, and the Caribbean. Since 2007, the disease has been reported in multiple countries across Africa, Europe, and Asia, in both domestic and wild pigs. Recently, ASF outbreaks were reported in several Asian and South-East Asian Countries.

After detection of the first case of ASF in Bhutan and considering various risk factors for the incursion and spread of ASF virus in Bhutan, viz., recent outbreaks in neighbouring Indian states, pig farming, production, and pig rearing system in the country, pork import figures, pig population distribution and dynamics across the border areas, etc., the National Centre for Animal Health, Serbithang under the Department of Livestock, MoAF, has come up with the first edition of National African Swine Fever Prevention and Control Plan for Bhutan.

This document has been developed to provide detailed guidelines for the prevention of ASF virus incursion into the country and to ensure rapid mobilization of the required resources during the outbreak(s) to reduce morbidity, mortality and social disruption to the minimum. I am confident that this plan document shall provide ready references for our field professionals and other relevant stakeholders during the implementation of various activities for the prevention and control of African swine fever outbreak(s) in the country.

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## **ABBREVIATIONS AND ACRONYMS**

3-D	Depopulation, Disposal, and Decontamination.
AHD	Animal Health Division, DoL.
ASF	African swine fever.
ASFv	African swine fever virus.
BAFRA	Bhutan Agriculture & Food Regulatory Authority.
BVT	Border vigilance team.
DDM	Department of Disaster Management, MoHCA
DDMC	Dzongkhag Disaster Management Committee
DLS	Dzongkhag Livestock Sector
DNB	Department of National Budget, MoF
DoFPS	Department of Forest and Park Services, MoAF.
DoL	Department of Livestock, MoAF.
DuDMC	Dungkhag Disaster Management Committee.
Dzongkhag	District, an administrative division composing of a group of Gewogs in Bhutan.
FAO	Food & Agriculture Organization of the United Nations.
GDMC	Gewog Disaster Management Committee.
Gewog	Sub-district, an administrative division composing of a group of villages in Bhutan.
IEC	Information, Education and Communication.
IOC	Incident Operation Centre.
LPD	Livestock Production Division, DoL



MoAF	Ministry of Agriculture and Forests.
MoF	Ministry of Finance.
MoHCA	Ministry of Home and Cultural Affairs.
NASFPCP	National African Swine Fever Prevention and Control Plan, 2021.
NCAH	National Centre for Animal Health, DoL.
NCD	Nature Conservation Division, DoFPS.
NDMA	National Disaster Management Authority.
NICC	National Incident Command Committee.
NVL	National Veterinary Laboratory, NCAH.
OIE	World Organisation for Animal Health.
PLQO	Plant and Livestock Quarantine Office.
PPE	Personal Protective Equipment.
PPZ	Provisional Protection Zone.
PZ	Protection Zone.
RLDC	Regional Livestock Development Centre.
RRT	Rapid Response Teams.
SOP	Standard Operating Procedure.
TADinfo	Transboundary Animal Disease Information System.
Tshethar	Practice of freeing animals from imminent slaughter and death.
TWG	Technical Working Group.
VPP	Veterinary paraprofessional.
VVT	Veterinary Vigilance Team.



## 1 BACKGROUND

African swine fever (ASF), first described in the 1920s in Kenya, is a highly contagious haemorrhagic disease of wild and domestic suids with extremely high morbidity and mortality rates. It is an OIE (World Organization for Animal Health) notifiable disease due to its ability to spread rapidly and cause severe illness. ASF does not pose a risk to public health. ASFv is unique, as it is the only known arthropod-borne DNA virus. The disease is one of the most serious transboundary animal diseases because of its high lethality for pigs, its crippling socio-economic consequences, and its propensity for rapid and unanticipated international spread.

### 1.1 Historical Overview

African swine fever (ASF) was discovered by R.E Montgomery in Kenya in 1921, as a new disease, causing high mortalities in imported European pigs. In the following decades, ASF was observed in several Sub-Saharan countries.

The first occurrence of ASF outside the African continent occurred in Portugal in 1957, near Lisbon, where an ASF outbreak caused mortalities of around 100%. Three years later, in 1960, after an epidemiological silence, ASF reappeared in Portugal, rapidly spreading to the whole Iberian Peninsula. Since then, ASF remained present in Spain and Portugal for more than twenty years, until eradication was achieved in 1994 in Portugal and 1995 in Spain as a result of great human and economic efforts.

During these years of ASF in the Iberian Peninsula, several European and American countries suffered outbreaks of ASF, mainly caused by the movement of contaminated meat products. However, these outbreaks were eradicated except on the island of Sardinia, where the disease remains endemic since 1978.

### 1.2 Global situation

Since 2016, a pattern of a significant increase in the number of outbreaks was identified. The disease is present in the African, European, and most recently, the Asian continent. It has never been reported in Oceania, and it was eradicated in the Americas in the '90s. Since 2016, 24% of the reporting countries and territories (48/200) have reported the disease. In Europe, the disease occurred for the first time in Moldova in September 2016, then in June 2017 in the Czech Republic, followed by Romania in July 2017 and more recently in Hungary, and Bulgaria, in April and August 2018, respectively. A recurrence of the disease in wild boars has been reported in Belgium in September 2018. In Asia, the disease was reported for the first time in China (People's Republic of) in August 2018. Later ASF outbreaks were reported from several countries in Asia: Mongolia, Democratic People's Republic of Korea, Republic of Korea, The Philippines, Malaysia, Indonesia, Timor-Leste, Papua New Guinea, Viet Nam, Lao People's Democratic Republic, Cambodia, Myanmar, India and Bhutan.



ASF is present in domestic pigs and wild boars in Europe, while Asia and Africa have notified outbreaks mainly in domestic pigs, and few cases in wild boars (300 cases reported in Asia since August 2018). During this period, Europe accounted for most outbreaks with 96% (9,756) of all outbreaks, but the highest impact in terms of animal losses was reported in Asia (1,711,677 animals lost, which is 68% of the total global reported losses for this period).

### **1.3 ASF situation in Asia (as of 13 May 2021)**

#### ***China***

Since the Ministry of Agriculture and Rural Affairs (MARA) confirmed the first outbreak in Liaoning Province on 3 August 2018, ASF was detected in 32 Provinces/Autonomous Regions/Municipalities/Special Administrative Region. In April 2021, ASF outbreaks were detected in farms in Cocodala City, Xinjiang Uyghur Autonomous Region and Baotou City, Inner Mongolia Autonomous Region. On 1 May 2021, another pig carcass was found on the shore of Wu Sha Jiao, Kinmen County, Taiwan Province, and tested positive for ASF virus. Since December 2018, 16 ASF virus-positive floating pig carcasses have been detected so far in Kinmen County (13), Lianjiang County (2), and New Taipei City (1).

#### ***Mongolia***

Since its first report on 15 January 2019, 11 outbreaks in 6 provinces and Ulaanbaatar have been reported, involving 105 farms/households. More than 3,115 pigs, more than 10 percent of the total pig population in Mongolia, have died/been destroyed due to the ASF outbreaks.

#### ***Viet Nam***

Since the Ministry of Agriculture and Rural Development (MARD) confirmed its first ASF outbreak on 19 February 2019, all 63 provinces/cities experienced outbreaks. MARD reported that ASF caused a loss of about 6 million pigs, but by the end of 2020, the total sow has increased to 3 million, and the total number of pigs reached over 26 million, equalling 85% of the pre-ASF level.

#### ***Cambodia***

Since the Ministry of Agriculture, Forestry and Fisheries (MAFF) confirmed the first ASF outbreak in Ratanakiri Province in April 2019, ASF outbreaks were detected in five provinces. Recently, the media reported that ASF was detected in illegally transported pigs intercepted at two border checkpoints in Banteay Meanchey, and in Takeo Provinces.



### ***Democratic People's Republic of Korea***

The Ministry of Agriculture confirmed the occurrence of the first ASF outbreak in Chagang-do on 23 May 2019.

### ***The Philippines***

Since the Department of Agriculture (DA) confirmed the first outbreak in July 2019, ASF has been reported in Luzon, Mindanao, Leyte and Samar Islands. On 29 March 2021, ASF was confirmed for the first time in Samar Island, on backyard farms in Lope de Vega, Northern Samar Province, a fomite (visitor) is suspected as the route of introduction into the farm. ASF has spread to 12 regions, 46 provinces, 493 cities and municipalities, and 2,561 villages nationwide since it hit the country in 2019. On 4 May 2021, DA confirmed that ASF is still present in 19 districts in Leyte, Southern Leyte, Samar and Northern Samar in Eastern Visayas Region. According to media quoting Boliney Municipal Agriculture Office, ASF reached Remoe Barangays in Boliney, Abra province in mid-April, when a resident brought a wild boar. The media reported that ASF has also been recorded in wild pigs along the forest between Malibcong Municipality and Kalinga Province.

### ***Myanmar***

Since the Ministry of Agriculture, Livestock and Irrigation confirmed the first ASF outbreak on 1 August 2019, a total of six ASF outbreaks were reported in Shan State, two in Sagaing Region and one each in Kachin and Kayah States.

### ***Republic of Korea***

Since the Ministry of Agriculture, Food and Rural Affairs (MAFRA) confirmed the first ASF outbreak on 17 September 2019, ASF was detected in 17 domestic pig farms: Gyeonggi-do (9), Incheon City (5), Gangwon-do (3). On 5 May 2021, MAFRA announced a new outbreak on a pig farm in Yeongwol County, Gangwon-do. This was the first ASF in domestic pigs since last reported in October 2020. Since 9 October 2019, and as of 11 May 2021, a total of 1,414 ASFv infected wild boars were confirmed in Gyonggi-do: Paju (100), Yeoncheon (409), Pocheon (83), Gapyeong (30); Gangwon-do: Cheorwon (36), Hwacheon (419), Chuncheon (160), Yanggu (70), Goseong (4), Inje (78), Yeongwol (13), Yangyang (8), Gangneung (3) and Hongcheon (1).

### ***Timor-Leste***

The Ministry of Agriculture and Fisheries announced the confirmation of the ASF outbreak on 27 September 2019. The disease started on 9 September and spread to all districts of the country by December 2019.





## ***Indonesia***

The Ministry of Agriculture (MoA) confirmed an ASF outbreak in North Sumatra Province in 2019 and reported 443 outbreaks ranging from West Sumatra Province (December 2019) through Nusa Tenggara Timur Province on 19 February 2021. According to the report, ASF was confirmed on Java and Bali as well as Eastern Nusa Tenggara islands, spread to 10 out of 34 provinces in Indonesia. In Manokwari Regency, West Papua Province, ASF caused more than 1000 death of pigs and spread across seven districts, namely West Manokwari, East Manokwari, North Manokwari, South Manokwari, Tanah Rubuh, Warmare and Prafi.

## ***Papua New Guinea***

The National Agriculture Quarantine and Inspection Authority (NAQIA) confirmed four ASF outbreaks in Mendi Munihi District, Southern Highlands Province (SHP) in March 2020. Surveillance conducted in May 2020 showed that ASF has spread within SHP, Hela and Enga Provinces. In January 2021, ASF was confirmed in Western Highlands Province (WHP) and Jiwaka Province, and a point infection was confirmed in Simbu Province.

## ***Lao People's Democratic Republic***

Since the Ministry of Agriculture and Forestry confirmed the first ASF outbreak in Salavan Province on 20 June 2019, ASF outbreaks were reported in all 18 provinces between May and November 2019. The second round started in June 2020, ASF was detected in 15 districts in 7 provinces.

## ***India***

Department of Animal Husbandry and Dairying, Ministry of Fisheries, Animal Husbandry and Dairying announced the confirmation of ASF outbreaks (Genotype II) in Assam State that occurred in January-April 2020. Meghalaya State confirmed ASF cases in 2020. On 29 April 2021, Meghalaya State declared some villages in East Khasi Hills, West Khasi Hills and Ri Bhoi districts as epicentres, after samples dated 13 April tested positive for ASF. In Manipur State, ASF has been reported in Thoubal and Imphal East District, according to the media. Mizoram State reported outbreaks resulted in over 1,700 pig deaths in Aizawl, Lunglei, Mamit, Serchhip and Siaha districts according to media quoting the State Animal Husbandry and Veterinary Department. Some ASF-affected districts in Mizoram were declared as epicentres. Nagaland State also detected an ASF outbreak, samples from Phek and Kiphire Districts tested positive for ASF. In Arunachal Pradesh, four ASF outbreaks that occurred between 26 January and 23 April 2020 in domestic pigs were reported in East Siang and Papum Pare Districts. According to the media, wild boars also died in



East Siang and Upper Siang Districts and a publication reported that local people saw dead wild boars in the rivulets in Pasighat region.

### Malaysia

Malaysia confirmed the first ASF outbreaks in four backyard farms and five wild boar carcasses in February 2021 in three districts in Sabah State. Media quoted the Minister of Agriculture and Fisheries saying that ASF has affected 10 districts in Sabah State including Pitas, Kota Marudu, Beluran, Telupid, Kinabatangan, Sandakan, Lahad Datu, Tawau and Nabawan. The latest case was detected in Nabawan on 23 April 2021. An article wrote that ASF is wiping out populations of the wild bearded pig (*Sus barbatus*); field sites in the east of the Sabah region reporting a complete absence of live pigs in forests.

### Bhutan

The first case of African Swine Fever was confirmed in a stray female pig from a point-of-entry (POE) area, between Bhutan and India, in Phuentshogling thromde, Chhukha district on 13 May 2021 by Real-time PCR at the National Veterinary Laboratory, National Centre for Animal Health, Serbithang, Thimphu.

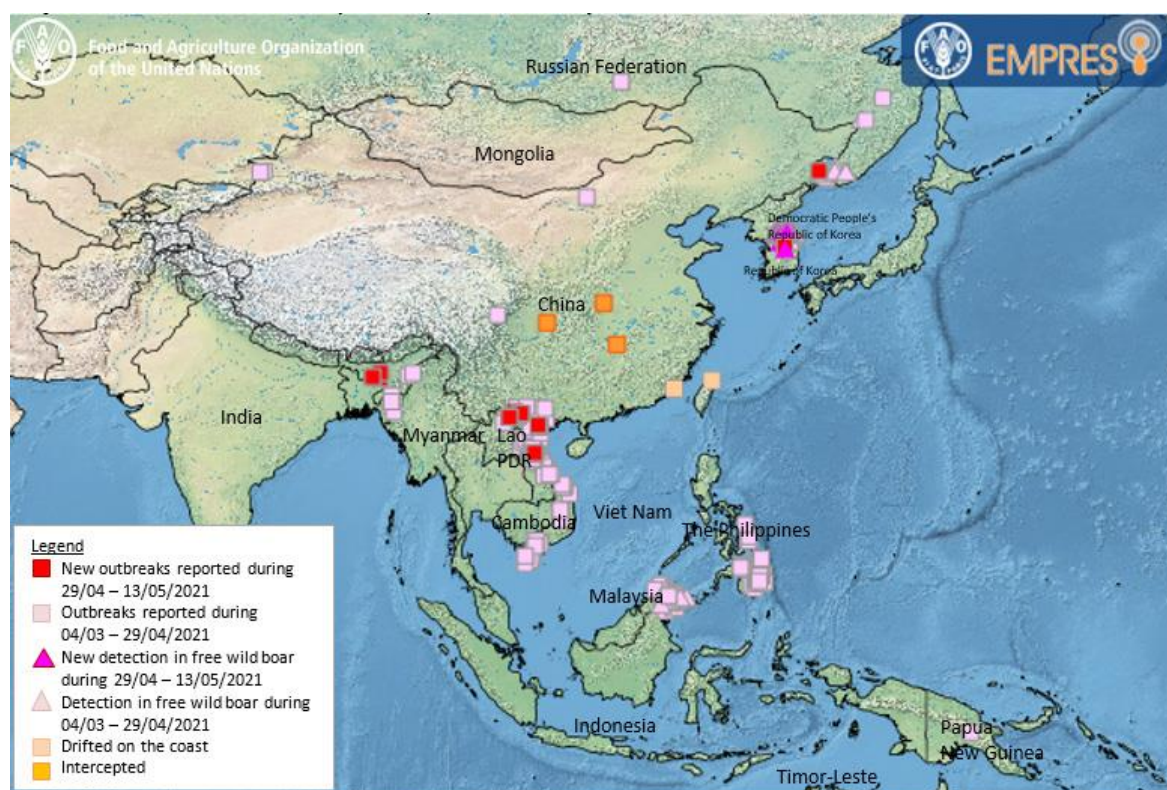


Figure 1: ASF outbreaks in Asia, March – May 2021

(Source: China: MARA, Viet Nam: WAHIS & media information, Republic of Korea, the Philippines, Indonesia, Timor-Leste: WAHIS and government websites, Other: WAHIS.)



## 1.4 Pig population, pork production and import in Bhutan

As of 2020, there are 18,253 pigs in the country and most of the pig population is concentrated in the southern districts, sharing a border with highly pig populated north-east states of India (See Figure 2).

Annual livestock statistics of Bhutan, 2019, show that 113.382 MT of pork was produced in Bhutan during the calendar year 2019. A free-range scavenging system of pig rearing is predominant in many villages throughout the country and swill feeding is a common practice to decrease the cost of production. Many exotic breeds of pigs are imported annually to improve rural livelihood and to meet the protein requirement in the country.

Except for the state-owned piggery farms and a few private farms, most farms or pig sheds in the country are built from locally available materials, thus the adoption of biosecurity measures is a challenge. As per the Bhutan Trade statistics, 2019, Bhutan has imported 1684.014 MT of fresh, chilled, and frozen swine meat worth 285.95M Ngultrum.

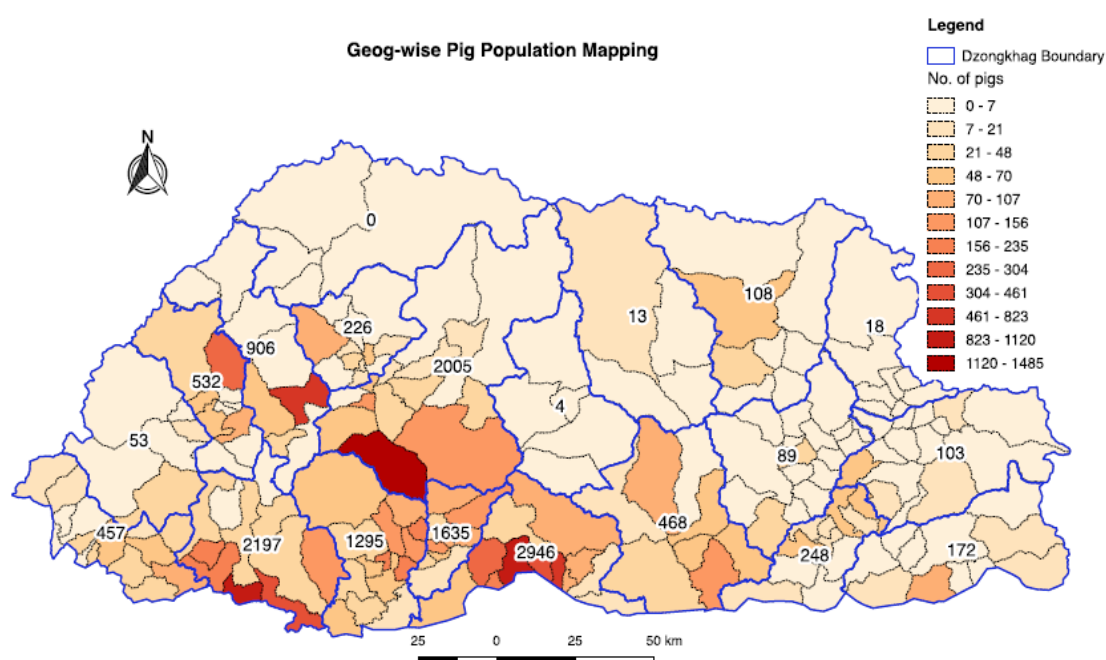


Figure 2: Pig population distribution in Bhutan, 2020

## 1.5 The rationale for the development of NASFPCP

The document is prepared after recording the first outbreak of ASF in Bhutan and considering the risk factors for the incursion of ASF virus in the country, viz., recent outbreaks in North-East states of India: Assam and Arunachal Pradesh, pig farming



and production in neighbouring Indian states, pig rearing system in the country, pork import figures, the threat of sylvatic transmissions, etc.

This NASFPCP is developed to ensure that all the required resources, expertise, and services are mobilized and deployed immediately to respond to ASF outbreaks in the country and to decrease the consequent morbidity, mortality, and social disruption to the minimum. This plan is also applicable for the prevention of ASFv incursion and preparedness for emergency response.

## 1.6 Objectives

The main objectives of this NASFPCP are:

- ✓ To prevent the incursion of the ASF virus into Bhutan.
- ✓ Rapid containment of ASF outbreaks in the country.



## 2 NATURE OF THE DISEASE

### 2.1 Definition

ASF is a highly contagious viral disease of domestic pigs, manifesting itself as haemorrhagic fever with mortalities that can approach 100 percent. The catastrophic effect of this disease on pig production, from the household to the commercial level, has serious socio-economic consequences and implications for food security. ASF is a serious transboundary animal disease (TAD) with the potential for rapid international spread.

### 2.2 Aetiology

ASF is caused by a unique DNA virus that was formerly classified in the family *Iridoviridae* because of morphological similarities. Now considered to be more akin to members of the *Poxviridae*, it is currently the sole member of a family of ASF-like viruses, the *Asfaviridae*. It is unusual among the DNA viruses in behaving like a true arbovirus, able to multiply in both vertebrate and invertebrate hosts.

### 2.3 Pathogenesis in pigs

Acute infections with highly virulent virus strains lead to a clinical course that resembles a viral haemorrhagic fever that is characterized by pronounced depletion of lymphoid tissues, apoptosis of lymphocyte subsets, and impairment of haemostasis and immune functions. It is generally accepted that most lesions can be attributed to cytokine-mediated interactions triggered by infected and activated monocytes and macrophages, rather than by virus-induced direct cell damage. Nevertheless, most pathogenic mechanisms are far from being understood.

### 2.4 Epidemiological Features

#### 2.4.1 Susceptible species

Only species of the pig family (*Suidae*) are susceptible to infection with the ASF virus. Domestic pigs are highly susceptible to ASF, which shows no breed, age, or sexual preference. Warthogs are the major host for the ASF virus. Bushpigs (*Potamochoerus porcus* and *P. larvatus*) and giant forest hogs (*Hylochoerus meinertzhageni*) are infected with the ASF virus but the extent of infection and their role in the epidemiology of the disease are unknown. European wild boar (*Sus scrofa*) is fully susceptible to ASF, with a mortality rate like that of domestic pigs. Human beings are not susceptible to ASF.





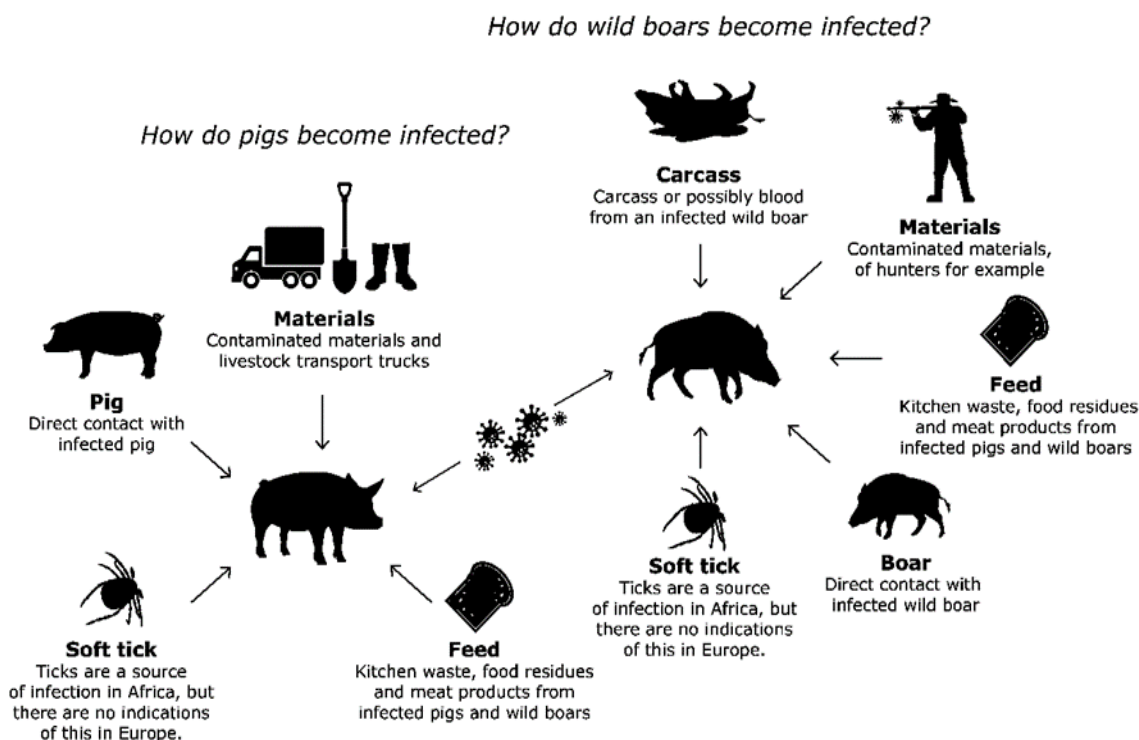
## 2.4.2 Persistence of ASFv

### *In the environment*

ASF virus, in a suitable protein environment, is stable over a wide range of temperatures and pH. It has been shown to survive in serum at room temperature for 18 months, in refrigerated blood for six years, and in blood at 37°C for a month. Heating at 60°C for 30 minutes will inactivate the virus. In the laboratory, the ASF virus remains infective indefinitely at – 70°C but may be inactivated if stored at – 20°C. In the absence of a protein medium, viability is greatly reduced. Putrefaction does not necessarily inactivate the virus, which may remain viable in faeces for at least 11 days, in decomposed serum for 15 weeks and in bone marrow for months. As a result of its tolerance to a wide range of environmental factors, only certain disinfectants are effective in the control of ASF.

### *In the host*

After infection with the ASF virus, domestic pigs may shed infective amounts of the virus for 24 – 48 hours before clinical signs appear. Pigs that survive the acute disease remain infected for several months but do not readily shed the virus for more than 30 days. As in wild suids, infective levels of virus are found only in lymph nodes; other tissues are unlikely to contain infective levels of virus for more than two months after infection.



**Figure 3: ASFv transmission mechanisms**



### ***In animal products***

The ability of the ASF virus to remain infective in edible products such as chilled meat (at least 15 weeks) and three to six months in processed hams and sausages that have not been cooked or smoked at a high temperature has important implications for the spread of ASF. Undercooked pork, dried and smoked pork, and carcass meal derived from pigs must be regarded as potentially dangerous if fed to pigs.

#### **2.4.3 Disease transmissions**

There are three modes of transmission for ASF: direct contact, indirect contact (fomites), and vector-borne. Direct transmission occurs when infected animals come into contact with healthy animals through contact with infected saliva, respiratory secretions, urine, and faeces. Indirect transmission can occur through contaminated fomites, an example of which is the practice of “garbage-feeding” in which domestic swine become infected when fed food waste contaminated with uncooked pork products. Argasid ticks (*Ornithodoros* spp.) serve as a vector for transmission, passing the virus to swine hosts when taking their blood meal. Infected ticks are also able to transmit ASFv to other ticks (sexual), to their offspring (transovarial), and from one life cycle to another (trans-stadial). Detail transmission mechanisms are described in Figure 3.

#### **2.4.4 Incubation period**

The incubation period varies by route of transmission, ranging from 4 – 19 days. For OIE’s purpose, the incubation period in *Sus scrofa domesticus* (domestic swine) is 15 days.

### **2.5 Clinical Signs**

#### **2.5.1 Per-acute ASF**

Pigs are usually found dead without premonitory signs. Recumbence, accompanied by high fever, indicated by flushing of the ventral area and extremities in white-skinned pigs, shade seeking, huddling together and rapid shallow breathing may be observed in some animals before death (See Figure 4).

#### **2.5.2 Acute ASF**

Pigs develop a persistent fever of up to 107°F. White-skinned pigs become flushed to cyanotic, particularly the ears, lower legs, and ventral abdomen. Mucopurulent ocular and nasal discharges may be evident (See Figure 4). Difficult breathing, sometimes with froth that may be bloody at the mouth and nostrils, often occurs and is indicative of the lung oedema that is often the primary cause of death. Pinpoint to larger haemorrhages may be visible on the mucosa and skin. Abortions may occur at any



stage of pregnancy. The duration of clinical signs is generally short (2 – 7 days) but maybe longer and apparent recovery may be followed by relapse and death.

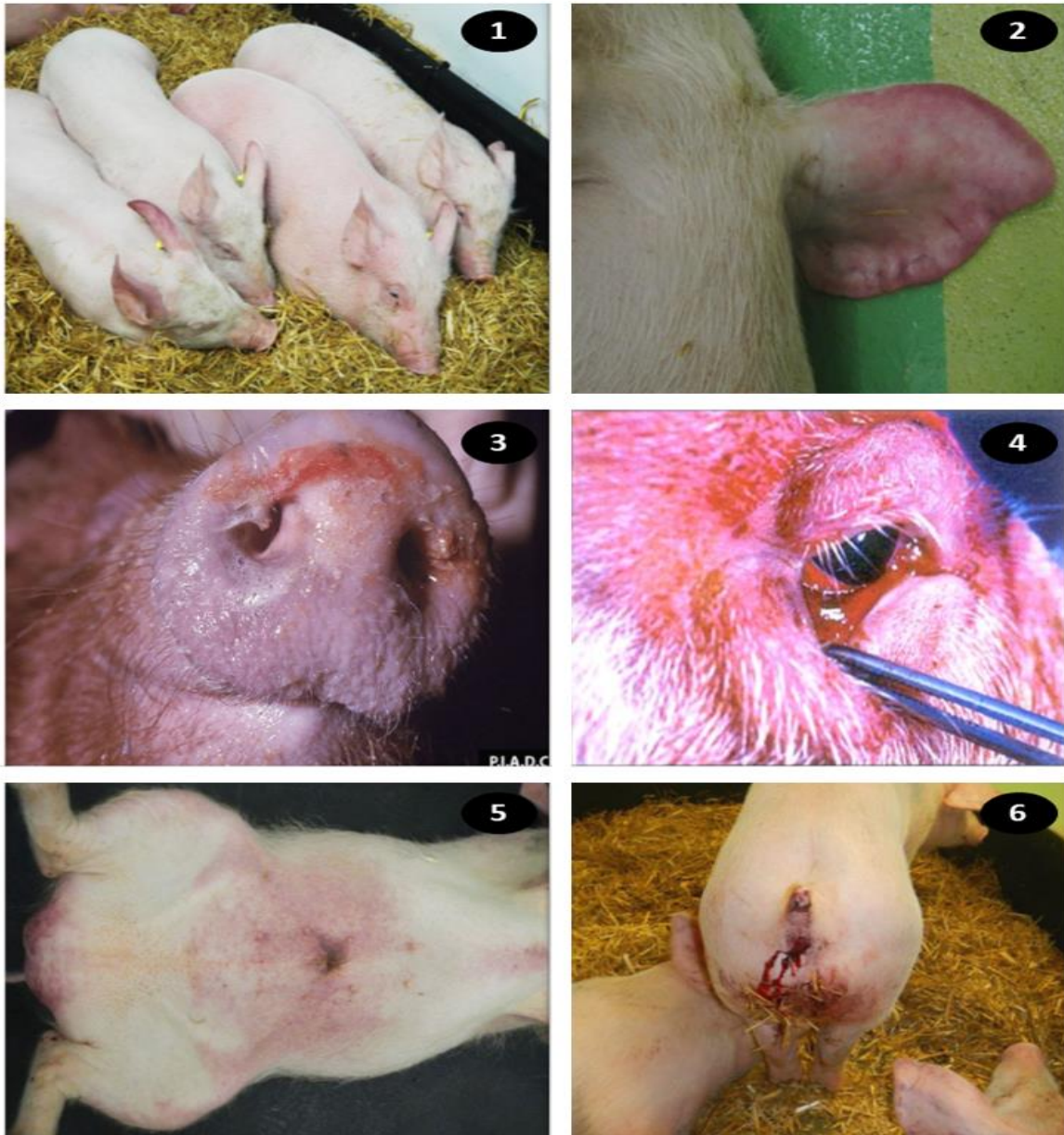
### **2.5.3 Sub-acute ASF**

Pigs that survive longer, usually after infection with less virulent strains, may have a fluctuant fever and usually lose condition. Interstitial pneumonia is usually present, which may result in respiratory distress and moist coughing. Cardiac damage may result in death from acute or congestive heart failure.

### **2.5.4 Chronic ASF**

Chronically infected pigs are usually severely emaciated and stunted, with a long dull hair coat. Signs of pneumonia may be present, as well as lameness and ulcers over bony points. These pigs are subject to secondary bacterial infections. They may survive for several months but recovery is unlikely.





**Figure 4: Clinical signs of ASF**

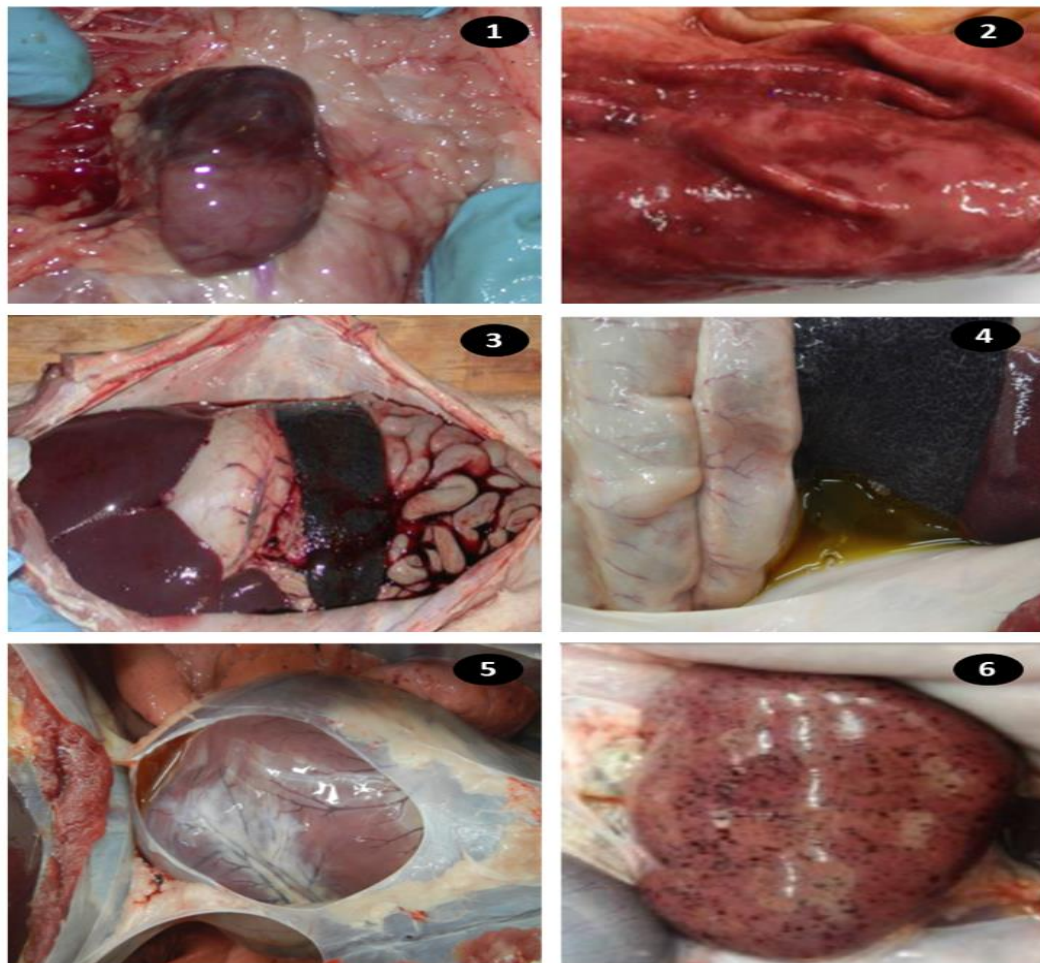
Picture courtesy: The Pirbright Institute

- 1- Reddening of conjunctival mucosa.
- 2- Reddening of the skin of ventral body and scrotum.
- 3- Bloody diarrhoea (dysentery).
- 4- Pigs huddling together and body temperature of 41°C.
- 5- Reddening of the ear pinna.
- 6- Nasal frothing.



## 2.6 Lesions

### 2.6.1. Gross Pathology



**Figure 5: PM lesions for ASF**

*Picture courtesy: The Pirbright Institute*

- 1- Enlarged and hemorrhagic gastro-hepatic lymph node.
- 2- Hemorrhagic gastritis.
- 3- Enlarged and hemorrhagic spleen.
- 4- Excess yellow peritoneal and other body cavity fluid.
- 5- Excess yellowish pericardial fluid.
- 6- Marked multiple hemorrhages over kidney cortex.



In white-skinned pigs, the extremities and the ventral surface may be cyanotic and subcutaneous haemorrhage. The spleen is slight to considerably enlarged, soft, and dark, with rounded edges. Lymph nodes, particularly the gastro hepatic, mesenteric, renal, and submandibular lymph nodes, are enlarged and severely haemorrhagic; they often resemble blood clots. The main features of sub-acute and chronic ASF are loss of condition to emaciation, interstitial pneumonia, and enlarged lymph nodes, which may be firm and fibrous in the chronic form of the disease.

**Table 1: Main clinical signs and post-mortem findings in different forms of ASF**

<b>Signs</b>	<b>Per-acute</b>	<b>Acute</b>	<b>Subacute</b>	<b>Chronic</b>
Fever	High	High	Moderate	Irregular or absent
Thrombocytopenia	Absent	Absent or slight (late)	Transient	Absent
Skin	Erythema	Erythema	Erythema	Necrotic areas
Lymph nodes	-	Gastro-hepatic and renal with a marbled aspect	Most lymph nodes resemble a blood clot	Swollen
Spleen	-	Hyperaemic splenomegaly	Partial hyperaemic splenomegaly or focal infection	Enlarged with normal colour
Kidney	-	Petechial haemorrhages mainly in the cortex	Petechial haemorrhages in cortex, medulla, and pelvis; peri-renal oedema	-
Lung	-	Severe alveolar oedema	-	Pleuritis and pneumonia
Gall bladder	-	Petechial haemorrhages	Wall oedema	-
Heart	-	Haemorrhages in epicardium and endocardium	Haemorrhages in epicardium and endocardium; hydropericardium	Fibrinous pericarditis
Tonsils	-	-	-	Necrotic foci
Reproductive alteration	-	-	Abortion	Abortion

Source: FAO Manual



### **2.6.2. Histopathology**

The most striking histopathological feature of ASF is massive karyorrhexis in lymphoid tissues, often accompanied by haemorrhage. The S-S (Schweiger-Seidel) sheaths of the spleen are virtually obliterated. Blood vessel walls, especially in the lymphoid tissues, often exhibit fibrinoid change resulting from necrosis of the endothelium and leakage of inflammatory mediators.

## **2.7 Immunity**

Antibodies against ASF are detectable in serum 7 – 12 days after clinical signs appear and persist for long periods, possibly for life, in both warthogs and domestic pigs. Serologically positive sows transmit antibodies to piglets in the colostrum. In sub-acutely and chronically infected pigs, virus replication continues in the presence of antibodies. Since no vaccine is available for ASF, the detection of antibodies in pigs can be confidently attributed to exposure to natural infection. There are no known serological cross-reactions with other viruses.

## **2.8 Diagnosis**

### **2.8.1 Field diagnosis**

Unusually high mortality among pigs of all age groups should lead to a strong suspicion of ASF. Additional indicators are the typical clinical signs and lesions of ASF, failure to respond to antibiotic treatment, and the fact that no other livestock species are involved.

### **2.8.2 Laboratory diagnosis**

#### ***Virological tests:***

- a. Polymerase Chain Reaction (See Annexure 13.9)
- b. Hemadsorption test
- c. Fluorescent antibody test

#### ***Serological tests:***

- a. Enzyme-linked immunosorbent assay
- b. Indirect fluorescent antibody test
- c. Immunoblotting test

### **2.8.3 Differential diagnosis**

African swine fever does not always manifest itself with the entire set of clinical signs described in the previous section. Clinical diagnosis can be difficult during the early stages of the disease, or when small numbers of animals are affected. Diagnosing ASF is often speculative, for symptoms may be confused with those of other diseases



and/or conditions. Moreover, many other pig (and wild boar) diseases can cause mortality at the rate observed in an acute ASF outbreak.

Therefore, the following swine diseases should be considered for a differential diagnosis:

- |  |  |
|--|--|
| a. Classical Swine Fever.                              | d. Erysipelas.                                       |
| b. Porcine reproductive and respiratory syndrome.      | e. Aujeszky's disease.                               |
| c. Porcine dermatitis and nephropathy syndrome (PDNS). | f. Salmonellosis (and other bacterial septicaemias). |
|  | g. Poisonings.                                       |





### 3 RISK ANALYSIS

#### 3.1 Purpose

The principal aim of the risk analysis is to provide an objective and defensible method of assessing the disease risk, determine strategies for ASF emergency preparedness, planning, and response operations. The strategies for disease prevention and response when supported by risk analysis provide fair judgments and transparent reasons for the imposition of import conditions or refusal to import. Transparency is essential because data are often uncertain or incomplete and, without full documentation, the distinction between facts and the analyst's value judgments may blur.

#### 3.2 Risk Assessment

Follow the OIE guidelines for risk analysis as described in the Terrestrial Animal Health Code. Risk analysis comprises four components: risk identification, risk assessment, risk management, and risk communication.

Bhutan Agriculture and Food Regulatory Authority and the Department of Livestock of the Ministry of Agriculture and Forests shall conduct a risk assessment to identify risk pathways of introduction, release, exposure, and consequences. Import risk analysis for pig and related products needs to be carried out from time to time and at short notice based on changing disease situations in trading partners, to develop contingency and risk management strategies for minimizing the risk of introduction of ASF virus into the country. Department of Forests and Park Services should be included in the assessment of risk in wild pigs. It is recommended to conduct qualitative risk assessments and describe the risks on a simple scale for the level of potential consequences.

The following disease epidemiologic factors should be considered for conducting risk assessments:

- Disease outbreak(s) and epidemiology in the country.
- Outbreaks occurring in neighbouring countries.
- Sharing border with the north-eastern Indian states: Assam, Arunachal Pradesh and West Bengal.
- Unpredictable outbreak pattern of the disease in the region.
- Stray pig population and their uncontrolled movement across the border.
- Existence of a traditional extensive system of pig rearing in the country.
- Farm bio-security status in the country
- Animal identification and traceability system in the country
- Very high pork import figure in comparison to in-country production.
- The pig population in the country is concentrated in the southern frontier.
- Lack of data on the distribution of wild boars and soft ticks (vector) in the country.



- The yearly increase in the number of tourists visiting the country.
- Waste disposal system from airlines.

### 3.3 Risk Communication

The findings of the risk analysis should be communicated to the relevant stakeholders (all those who could be affected by the consequences of the risk/threats including farmers and politicians). It is important that the risk assessment and risk management strategies be fully discussed with the stakeholders to give assurance that the risk management costs are worthwhile.

The risk analysis findings should be used for determining the rank of ASF in the national disease priority list and deciding the resources needed. Similarly, it can also be used in determining and strengthening the laboratory diagnostic capabilities, readjusting disease response, training, planning awareness, and publicity campaigns.

### 3.4 Risk Assessment Findings

A qualitative risk assessment was conducted in May 2020 to estimate the risk of ASF entry into Bhutan. A survey questionnaire was designed to collect information from government and private pig farms, feed manufacturers, field livestock officials, forestry officials, and BAFRA.

Simultaneously, on-farm biosecurity risk assessment and advocacy for ASF awareness were carried out. Based on the information collected, the possible pathways for entry of ASF were analysed using a risk analysis decision tree. A detailed descriptive scenario for each of the pathways was developed and the probable likelihood was estimated using the risk matrix. The list of most probable pathways identified for the introduction and spread of ASFv in Bhutan are as follows:

- The legal import of live pigs for breeding and processing.
- Illegal import of live pigs for breeding.
- The legal import of pig semen for insemination
- The legal import of pork and pork products for wholesale and retail (consumption)
- Illegal import of pork and pork products for consumption and retailing
- The legal import of pig feed for private & government farms
- Illegal import of feed ingredients for feed formulation
- Domestic (owned scavenging and stray) and wild pigs' movement across the international border.
- Poor farm biosecurity practices in the backyard and semi-commercial farms.



**Table 2: Potential risk pathways for African swine fever**

Identified Pathways	Risk pathways assessment criteria (Yes/No)					Retain for RA (Yes/No)
	Agent survives pathway	Potential for transmission <sup>1</sup>	Capable of adverse consequences <sup>2</sup>	ASFv at source (India)	Pathway occurrence in Bhutan <sup>3</sup>	
Pathways	(Entry)	(Spread)	(Establishment)			
1 Legal import live pigs	Yes	Yes	Yes	Yes	No	No
2 Illegal import of live pigs	Yes	Yes	Yes	Yes	Yes	Yes
3 Legal import pig semen	Yes	Yes	Yes	Yes	No	No
4 Legal import of pork/ products	Yes	Yes	Yes	Yes	No	No
5 Illegal import of Pork / products	Yes	Yes	Yes	Yes	Yes	Yes
6 Legal import of feed	Yes	Yes	Yes	Yes	Yes	Yes
7 Illegal import of feed ingredients	Yes	Yes	Yes	Yes	Yes	Yes
8 Domestic and wild pig movement across intl. border	Yes	Yes	Yes	Yes	Yes	Yes
9 Poor farm biosecurity practices in the backyard and semi-commercial farms.	Yes	Yes	Yes	No	Yes	Yes

**Legend:**

1. Potential for transmission - The commodity in the identified pathway is capable of African Swine Fever transmission.
2. Capable of adverse consequences - The African Swine Fever could potentially produce





adverse consequences in the pig population in Bhutan.

3. *Pathway occurrence in Bhutan: The probability of identified pathway occurring in Bhutan. Where sufficient evidence does not exist, the pathway is considered absent until it is further confirmed.*

**Table 3: Summary of risk assessment findings**

<b>Sl. No.</b>	<b>Risk pathways</b>	<b>Risk estimation</b>
1.	The legal import of live pigs, pig semen, pork, and pork products	<i>Low (Event would be unlikely to occur)</i>
2.	Illegal import of live pigs	<i>Moderate (Event would be nearly as likely to occur as not to occur)</i>
3.	Illegal import of pork and pork products	<i>High (Event would be likely to occur)</i>
4.	The legal import of pig feed	<i>Low (Event would be unlikely to occur)</i>
5.	Illegal import of pig feed & ingredients	<i>Moderate (Event would be nearly as likely to occur as not to occur)</i>
6.	Cross-border movement of domestic and wild pigs	<i>Very high (The event is almost certain to occur)</i>
7.	Poor farm biosecurity practices in the backyard and semi-commercial farms.	<i>High (Event would be likely to occur)</i>

The highest risk for ASF entry to Bhutan is associated with cross-border movement of domestic and wild pigs followed by illegal import of pork and pork products. Illegal import of pig feed, feed ingredients, and live pigs for breeding possess a moderate risk of ASF entry. Considering the current pig farming practices in the country, the risk of transmission/spread of ASF in the country due to poor farm biosecurity practices remains high.

Based on the risk assessment, the following risk mitigation measures are recommended:

- Active surveillance for ASF in the wild, scavenging, stray, and tshethar pigs in the bordering areas.
- Strengthen the collaboration with the Department of Forests and Park services for organizing and conducting surveillance in wild pigs.



- Strengthen border control and sanitary inspection at border posts by BAFRA, especially for control of the illegal entry of pork, pork products, illegal feed and ingredients.
- Strengthen on-farm biosecurity and management practices of the private commercial and backyard farms immediately.
- Removal of stray pigs in high-risk areas.
- Restrict scavenging pig rearing system and impose stronger control on tshethar animals.



## 4 PREVENTION STRATEGIES FOR ASF

### 4.1 Surveillance

During the normal time when there is no report of ASF outbreaks in the neighbouring countries, as a preventive measure for early detection, passive clinical and laboratory surveillance must be undertaken on both domestic and wild pigs.

#### 4.1.1 In Domestic pigs

##### *Clinical surveillance*

Clinical surveillance is aimed at the detection of clinical signs of ASF at the farm level and it should be carried out regularly in the high-risk areas, targeting commercial, free scavenging farms, stray and tshethar pigs. The concerned livestock officials should immediately report any suspect cases of pig morbidity and mortality within their jurisdiction to the National Centre for Animal Health.

##### *Laboratory surveillance*

Along with clinical surveillance, laboratory surveillance should also be carried out as and when required using appropriate laboratory diagnostic tests. Histopathological findings such as massive karyorrhexis in lymphoid tissues, often accompanied by haemorrhage and the S-S (Schweiger-Seidel) sheaths of the spleen with virtual obliteration are indicative of ASF infection.

#### 4.1.2 In Wild boars

Observation of unusual morbidity or mortality in the wild boars should be reported to the nearest livestock centres, and the livestock officials in collaboration with the officials from the Department of Forests and Park Services (DoFPS) and other relevant stakeholders such as the Royal Society for Protection of Nature (RSPN) shall investigate to rule out ASF. Spatial and temporal distributions of wild boars and their overlap with domestic pigs should be studied to develop risk-based surveillance in wild pigs.

### 4.2 Swill feeding control

Every effort should be made to prevent the feeding of food and kitchen waste containing pig products, including from international aircraft, as this constitutes a high risk for the introduction of ASFv into the country. If domestically generated swill is fed to the pigs, it should be done only after boiling at a temperature above 70°C for more than 30 minutes. Animal owners should be educated on the risk of feeding swill to the pigs.



### **4.3 Containment of pigs**

The presence of stray and poorly controlled pigs constitutes a high risk for the entry and rapid spread of ASF. There may be significant delays in recognition of the disease, and elimination will be more difficult. Perhaps the greatest danger is that these pigs have access to the carcasses of dead pigs in the bush or on garbage dumps and the offal of pigs that have died of ASF and been prepared for human consumption. Measures should be taken to encourage the development of properly constructed pig pens and to control the population of stray and scavenging pigs.

### **4.4 Farm biosecurity**

The Ministry has developed an on-farm biosecurity standard for different categories of pig farms. The standard constitutes the biosecurity measures to be implemented by each farm based on the farm categorization (backyard, semi-commercial and commercial). The regulatory authority should visit farms regularly and strengthen the biosecurity measures to protect pigs at the farm from ASFv introduction. Biosecurity in the free scavenging system shall be focused on segregation and feed control.

### **4.5 Regulatory measures**

All live pigs imported into Bhutan should follow strict quarantine and test procedures in line with Bhutan Health Code for Import of Animals, 2018. The imports of the live pig, pig meat and meat products, pig semen, embryos and ova, and other products incorporating pig tissues, such as pharmaceuticals should follow strict quarantine and test procedures in line with Bhutan Health Code for Import of Animals, 2018.

The pigs rescued for Tshethar purposes should strictly abide by the “Guidelines on Animal Tshethar Practices 2018”.

### **4.6 Awareness and education**

Pig owners, pig production input suppliers and importers dealing with live pigs and their products should be made aware of the disease and its nature, economic impact, and various preventive measures to avoid ASFv incursion into their farm/ locality and for early detection and response. Through education and awareness, people involved in the pig industry at all levels should be able to weigh the positive and negative aspects of swill feeding and waste management.

### **4.7 Capacity building**

Since ASF is an exotic and emerging disease, the laboratory capacity and human resource competence in disease investigation, laboratory testing, and 3-D operations should be built to enhance the early detection of ASF cases and response to the outbreaks in the country.



## **5 EARLY WARNING AND CONTINGENCY MEASURES**

During ASF outbreaks in the neighbouring countries or the region, surveillance and prevention measures should be heightened as it presents a high risk of ASFv incursion into the country. The heightened measures include activation of VVT and BVT, imposing import bans, instituting risk-based active surveillance, enhanced capacity building, farm biosecurity upgrading, awareness and education, etc.

### **5.1 Activation of Veterinary Vigilance Teams (VVT)**

When there is a report of ASF outbreak in the neighbouring countries and the region which pose an imminent risk of disease introduction, the Department of Livestock should activate Veterinary Vigilance Teams (VVT). The teams comprise veterinarians and veterinary paraprofessionals (including laboratory officials) from the concerned RLDC/ TVH&SL and Dzongkhag Livestock Sector, and the NCAH if required.

The main role of this team is for early detection of the disease so that disease response and control measures can be implemented in time to prevent its spread to wider areas. The team is also responsible for coordinating the report of their activities and its submission to the NCAH and DoL HQ every week.

### **5.2 Activation of Border Vigilance Teams (BVT)**

During the alert phase, the BAFRA should activate BVTs as and when there is a report of a confirmed ASF outbreak in the neighbouring countries. The BVTs should monitor the illegal movement of pig and pig products at the point of entry to prevent ASF introduction incursion into the country. In addition, the BVTs will visit the pig farms along with the border areas and ensure strict implementation of on-farm biosecurity measures as per the standards. Any suspected ASF cases in the farms or intercepted during the inspection and monitoring must be reported to the nearest Animal Health Centres for investigation. Regulatory officials shall be regularly updated with the latest information about ASF to pick up the suspect case of ASF.

### **5.3 Ban on import of pigs and related products**

BAFRA should ensure that all regulatory measures -- including the import regulations and import ban on pig and pig products originating from ASF-affected countries -- are implemented. BAFRA should also enforce any ban imposed within the country through regular inspection and monitoring. BAFRA shall also implement inspection of all the imports of pig and pig products including other risk goods to prevent virus incursions.



#### **5.4 Enhancement of field staff competence**

The Veterinarians, Veterinary paraprofessionals, laboratory officials, and regulatory officials shall be trained on prevention and response to ASF outbreaks and on their roles and responsibilities to implement NASFPCP through either field simulation or desktop exercise. They should be updated on the current knowledge of ASF and its situation around the world. Also, they should be trained on how to carry out surveillance and the use of rapid diagnostic techniques and test kits.

#### **5.5 Upgrading piggery farm biosecurity**

All government and commercial private farms should enhance farm biosecurity with proper boundary fencing to avoid unauthorized entry of people and vehicles and to prevent contact with stray and wild pigs. All entry and exit points should be well guarded, foot-dips shall be made available for daily workers, and effective zoo-sanitary and other control measures must be put in place. This should include changing footwear and the use of gloves, masks, and overcoats while entering and exiting pigsties. BAFRA shall ensure the implementation of the bio-security standards by these piggery farms. Where the farms are beyond the reach of BAFRA, it shall be the responsibility of the concerned livestock extension office to undertake this task.

#### **5.6 Strengthen the disease reporting system**

Reporting of any suspicious cases of ASF should be done immediately by the fastest means of communication. All pig owners are mandated to report any suspected cases of ASF. Besides, the village *Tshogpa/Chipon/reliable person* should be identified as the focal person to report any suspected cases of ASF to the nearest animal health centre. Farmers/ pig owners and the regulatory authorities are mandated to report any suspected cases/trigger signs of ASF to the nearest animal health centres for immediate investigation and response.

#### **5.7 Awareness and education**

Public awareness and education of field staff on ASF and its risks are essential components of this plan. The public shall be sensitized and educated about the disease to obtain their full cooperation for prevention, early detection, and effective response during ASF outbreaks. Similarly, field extension agents and regulatory inspectors of BAFRA, meat vendors and farmers shall be made conversant with the disease. Television and radio programmes, leaflets, brochures, newspapers insert, and mobile SMS should be used for public awareness.

#### **5.8 Strengthen the diagnostic capacity**

It is consequently impractical and excessively costly for most countries to maintain a national veterinary diagnostic laboratory with full capability for confirmatory diagnosis



of all transboundary and other emerging diseases, many of which will be exotic. The diagnostic laboratory must be equipped and should be competent to undertake standard techniques in pathology, virology and serology to the level where preliminary identification of aetiological agents for emergency livestock diseases could be attempted.

During ASF outbreaks in the region and neighbouring countries, ASF is deemed to be a high-threat disease to the livelihood of pig rearing farmers and the nation's economy; therefore, consideration should be given to developing capacities for some primary key diagnostic tests, such as RT-PCR, or some rapid diagnostic tests.



## 6 ACTION/RESPONSE DURING OUTBREAK(S)

The action plan is aimed to address the situation during the outbreak of ASF. All the response actions should be directed towards rapid containment of the disease to the primary focus or zone of infection and elimination within the shortest possible time to avoid spread and possible progression to endemic status.

### 6.1 Strategies for ASF control

In the absence of vaccines and effective treatments, the only available option for ASF control and eventual elimination is stamping out and disposal of all infected and potentially infected pigs. The main elements of a stamping-out policy for ASF are:

- ✓ Zoning of the area into the infected zone, protection zone, and free zone.
- ✓ Quarantine procedures to contain the disease, including pig-movement controls and prohibitions on the sale of potentially infected pig products.
- ✓ Enhanced epidemiological surveillance for ASF.
- ✓ The immediate slaughter of infected and potentially infected pigs, and safe burial or burning of carcasses and other infected materials.
- ✓ Cleansing and disinfection of infected premises.
- ✓ Provide prompt and fair compensation to affected owners.
- ✓ Keeping infected premises/villages without pigs for a safe period.

### 6.2 Case Definitions

During the outbreak investigation, a case definition shall be established aligning to the following definitions.

*Table 4: ASF case definitions*

Case definition	Description
Suspect case	The sudden death of pigs of all ages with haemorrhagic fever-like symptoms affecting only pig species and the disease spreading very rapidly in the area or other clinical signs consistent with ASF or epidemiological link to ASFv.
Presumptive positive case	A suspect case or any animal that is positive for ASFv antigen and/or antibodies by rapid diagnostic tests at NVL or any other approved animal health laboratories in the country.
Confirmed positive case	A suspect/ presumptive positive case or any other animal with PCR test positive to ASFv at NVL or any other approved animal health laboratories in the country.
Negative	Any suspect/ presumptive positive case with a negative PCR test result.





**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 based on additional information or the changing needs of the elimination effort.

### 6.3 Zoning

Zoning is the proclamation of geographical areas in which specific disease-control actions are to be carried out. The zones are concentric areas around known or suspected foci of infection, with the most intensive disease-control activities in the inner zones. Zoning is one of the early actions to be taken when there is an incursion of ASF into a country. The size and shape of the zones may be determined by administrative or geographical boundaries or by epidemiological or resource considerations based on the assessment conducted by the disease outbreak investigation and risk assessment team. Pig farms with poor biosecurity measures and away from the zone of infection may certainly be at greater risk than farms with high biosecurity measures within the infected zone.

#### 6.3.1 Declaration of provisional infection and protection zones

When ASF is suspected, gewogs/ dzongkhags shall immediately inform the RLDC and BAFRA officials in the respective areas. The affected farm or village and the immediate surroundings shall be identified as a provisional infection zone. Based on the epidemiological assessment of risk from the point of suspected place of infection, the areas surrounding the provisional infection zone shall be declared as the provisional protection zone. All places with pigs within the provisional protection zone shall be considered at-risk and visited to establish their infection status.

Dzongkhag should issue an official order for the detention of the suspected pig and pig products and in-contact materials within the declared provisional areas which shall be regulated by BAFRA. These zones would be re-demarcated after proper outbreak investigation by the Disease Outbreak Investigation Team of the Rapid Response Teams.

These provisional zones shall be declared through official order by the concerned local authority office based on the recommendation of the disease investigating officer or team.

#### 6.3.2 Declaration of Infection Zone

If the confirmed positive case definition for ASF is met, an Infection Zone as defined by the risk assessment team will be demarcated. Its size and shape will be influenced by topographical features, physical barriers, administrative borders, and epidemiological considerations. OIE recommends that it should have a radius of at least 10 km around disease foci in areas with intense livestock raising and 50 km in



areas of extensive livestock raising; however, it is impractical in areas/villages where the proportion of pigs is poorly controlled. To determine infection zones, the extent of the focus of infection must be determined and well-managed farms that have escaped infection must not be regarded as infected. On the other hand, strict vigilance must be maintained over a much wider area, depending upon known patterns of pig movements determined by marketing and other considerations.

In the initial stages of an outbreak, when its extent is not well known, it would be wise to declare larger infection zones and then progressively reduce them as active disease surveillance reveals the true extent of the outbreak.

Stamping-out procedures (3-D: depopulation, disposal and decontamination) should be carried out based on contact tracing and scientific risk assessment. In farms with very good bio-security practices, the culling may only be initiated based on the test results and thorough risk assessment.

Based on the recommendation of the disease outbreak investigation team, the Dzongkhag administration should issue the disease outbreak declaration order with information to the Gewog authority, Thromde authority, BAFRA, RLDC, NCAH, DoFPS and the DoL.

### **6.3.3 Declaration of Protection Zone**

This zone is much larger and surrounds the infection zone. It may cover multiple villages, gewogs, or dzongkhags depending on the infection zone delineated. The size and shape of the zone shall be determined by the risk assessment team and will be used for enhanced surveillance activities in this zone to monitor the possible spread of infection. Inspection, movement control, surveillance, screening, and sanitary measures will be the main tasks in the Protection Zone. In some cases, where the infection spreads uncontrollably, it may cover the whole country.

### **6.3.4 Declaration ASF-free zone**

This encompasses the rest of the country. Because of the potential of ASFv for widespread transmission, it is recommended that all parts of a country experiencing a first outbreak are placed under a high level of surveillance. Regulations preventing the movement of domestic or wild pigs and their products into the free zone from an infected country or zone must be rigorously implemented. Regular inspection and monitoring movement of pigs and their products should be carried out in the free zone.

## **6.4 Control by stamping out**

Containment and elimination process by stamping out shall be implemented promptly following confirmation of the disease. These general principles apply to the 3-D Team (depopulation, disposal, and decontamination).



### 6.4.1 Depopulation

Humane killing should be conducted with due consideration to religious sentiments and social obligations (See Annexure 13.5).

### 6.4.2 Disposal

#### *Carcass*

Burial may be the best means of disposal under certain conditions and a pit should be prepared as soon as the diagnosis is confirmed. Materials that cannot be disinfected such as wood and cardboard must be burnt (See Annexure 13.5).

#### *Infected Materials*

Litter and straw, depending on the amount present and the characteristic of the farm, can be either burnt or buried in a pit with the carcasses. Contaminated animal feed on the site must be disposed of by burial or incineration.

### 6.4.3 Decontamination/ Disinfection

All units which are physically or functionally connected to the establishment such as vehicles used for transporting live animals should be decontaminated with appropriate disinfectants; it may be simpler to burn poorly constructed animal housings where there is a danger of *Ornithodoros* ticks. If ticks are absent, spraying with a disinfectant effective against ASF should be sufficient, as the virus does not remain viable for long outside a protein environment. Appropriate disinfectants for ASF include 2 percent sodium hydroxide, phenol substitutes, sodium or calcium hypochlorite (2 – 3 percent available chlorine), potassium peroxymonosulfate + sodium dodecyl benzenesulfonate (Virkon-S®) and iodine compounds. After washing and disinfecting, all units must be fumigated twice with at least two weeks intervals between fumigations. A detailed guideline for decontamination is given in Annexure 13.6.

## 6.5 Movement control for pigs and their products

To prevent the further spread of infection from the infected zone, the movement of live pigs and pig products out of the designated infected zone must be strictly restricted. An official ban notification must be sent out through the local authority, depending on the location of the outbreak.

Considering the high transmissibility of ASFv through fomites, potentially contaminated farm machinery and equipment must not be allowed to be moved out of the infected farm(s).

In addition, the movement of live animals and their products originating from the integrated farm within the infected area shall be prohibited until further notification from the veterinary authority.



## 6.6 Awareness and education

Public awareness and education campaigns are integral elements of any disease prevention, control and elimination strategy. It should be mainly targeted in places where piggery farming is intensive, and communities are affected by the disease and ASF control actions. The DoL and BAFRA shall jointly coordinate radio and television programmes and carry out advocacy programmes as a means of getting the message across to these people. Meetings are particularly suitable, as there will be community involvement and the opportunity to ask questions and disseminate material such as pamphlets and posters that will reinforce the information.

## 6.7 Tick control

Elimination of *Ornithodoros* ticks from infected premises is a challenge, particularly when involving old buildings, because of the tick's longevity, endurance, and ability to hide in cracks that cannot be reached by acaricides. The destruction of tick habitat (e.g., covering over cracks where ticks can hide and/or building new facilities with materials that leave no cracks) helps to lower their numbers and transmission potential. Infested buildings should not be used as pigsties. Acaricides and other pesticides may be used on bedding or, depending on the product, applied directly to the skin of pigs. Since blood-sucking insects can mechanically spread the ASF virus within herds, insect-control programmes are advisable on infected premises.

## 6.8 Wildlife control

No realistic measures can be taken to prevent sylvatic transmission of ASFv. The only option is to implement prevention measures to protect domestic pigs from being infected. In areas where the sylvatic cycle occurs, adequate fencing, or permanent housing of domestic pigs has been demonstrated to provide complete protection. The fencing or wall must extend below the surface for at least 0.5 metres to prevent burrowing by wild pigs and the recommended height is 1.8 metres.

## 6.9 Human health and safety

Though ASF is not a threat to public health, responders may be exposed to other health hazards. Prevention of adverse human health events related to emergency response efforts is very important, therefore, personal protective equipment should be used always while carrying out various activities in the infected or suspected premises.

## 6.10 Compensation

It is essential that farm owners in Bhutan who have had their pigs slaughtered, pig meat products confiscated, or property destroyed as part of an ASF control programme should be fairly compensated with the current market value of the animals



and goods. Compensation should be paid without delay. Under some circumstances, the replacement of stock may be offered in place of monetary compensation.

A compensation scheme has been incorporated in the ASF control plan to encourage timely and positive reporting of any cases and to compensate for losses due to disease or culling. The Livestock Act of Bhutan 2001, under sub-section 9.3, clearly states that the government has the authority to compulsorily destroy animals, animal products or feed or other at-risk goods that it considers to be risky and pays compensation as prescribed by the Ministry of Agriculture and Forests. The “Guidelines for compensation mechanism” outline the management of the compensation fund (See Annexure 13.11).

### **6.11 Surveillance**

After the successful containment programme, regular monitoring through surveillance and awareness programmes, along with the implementation of other prevention and control measures shall be conducted to eliminate the disease.

### **6.12 Destocking**

After depopulation, disposal, and decontamination procedures are completed; the farm/premises should be left empty for a period determined by the estimated survival time of the pathogen. As a general rule, this would be shorter in hot climates than in cold or temperate climates. In line with the OIE, a minimum of 40-day downtime (destocking period) is recommended.



## **7 RECOVERY STRATEGIES**

### **7.1 Withdrawal of zones declared and bans imposed**

Bans imposed on the movement of live animals and their products in the delineated infected zone shall be lifted based on the findings of the risk assessment conducted. For potentially contaminated farm machinery and equipment, bans imposed shall be lifted after the completion of a downtime period of 6 weeks, however, these items must undergo a thorough disinfection process.

An official ban lifting notification must be sent out through the local authority, depending on the location of the outbreak.

### **7.2 Restocking/Repopulation**

At the end of the recommended destocking period (40 days), piglets may be reintroduced to previously infected farms or villages. Restocking to full capacity should only take place after sentinel piglets have been introduced at approximately 5 percent of the normal stocking rate on each previously infected farm, or at least a piglet in a farm. These piglets must be observed closely for six weeks to ensure they stay free of ASF before full repopulation.

Restocking/repopulation shall be approved by the local veterinary authority upon fulfilling the following criteria:

- a. Registration of farm
- b. Meeting the minimum farm biosecurity standards
- c. Evidence of complete disinfection of the farm

During the period of 6 weeks following the restocking of the sentinel pig population, if an ASF outbreak occurs in the sentinel stock, standard outbreak response measures will be undertaken as per the NASPCP; however, the farm owners shall not be eligible for compensation of the culled piglets.

### **7.3 Surveillance and awareness**

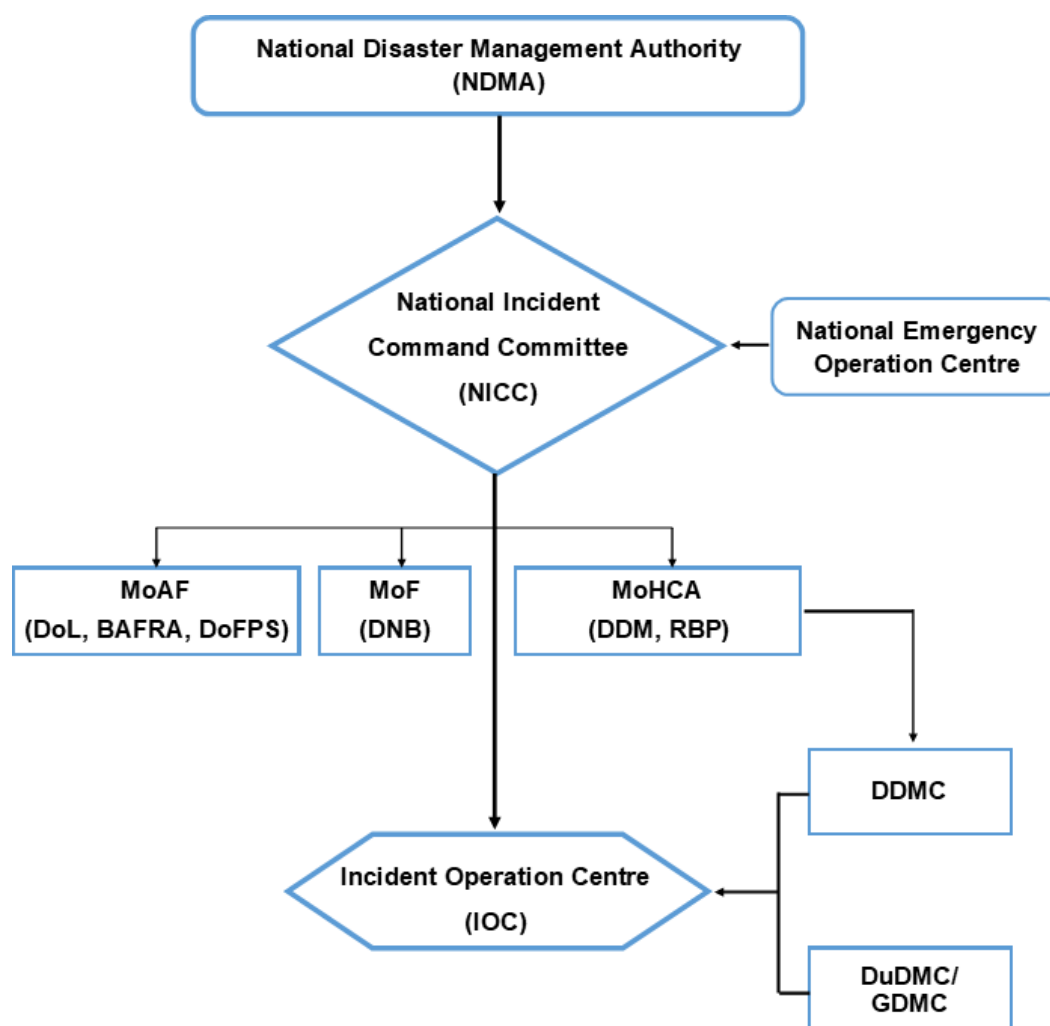
During the 6-week period of sentinel restocking, the designated area must be monitored for morbidity/mortality every week by the respective gewog in-charge and report accordingly.

Whenever possible, information about the disease and its impact and important prevention and control measures at the farm level must be advocated to the farmers in the area.



## 8 INCIDENT COMMAND STRUCTURE AND INSTITUTIONAL ARRANGEMENTS

The Incident Command Structure for the veterinary response to ASF outbreak in animals has been developed in line with the NIPPP&SOP 2020 which further is developed in line with the Disaster Management Act of Bhutan 2013 and considering the requirement of highly technical and sector-based response for disease outbreaks.



**Figure 6: Generic incident command structure during ASF outbreak(s)**

In the event where control of ASF outbreak(s) requires involvement of the National Disaster Management Authority (NDMA), ASF outbreak would be considered a national disaster and therefore activation of the (NDMA) chaired by the Hon'ble Prime Minister and members comprising of high-level decision-makers from key sectoral agencies is essential for smooth implementation of this plan. In such cases, in line with the Disaster Act 2013, the NDMA shall function as the highest policy-making body for NASFPCP (Figure 6).

Figure 7 shows the flow of directives and reporting between NICC, TWG and IOC during the implementation of ASF outbreak containment/response activities.

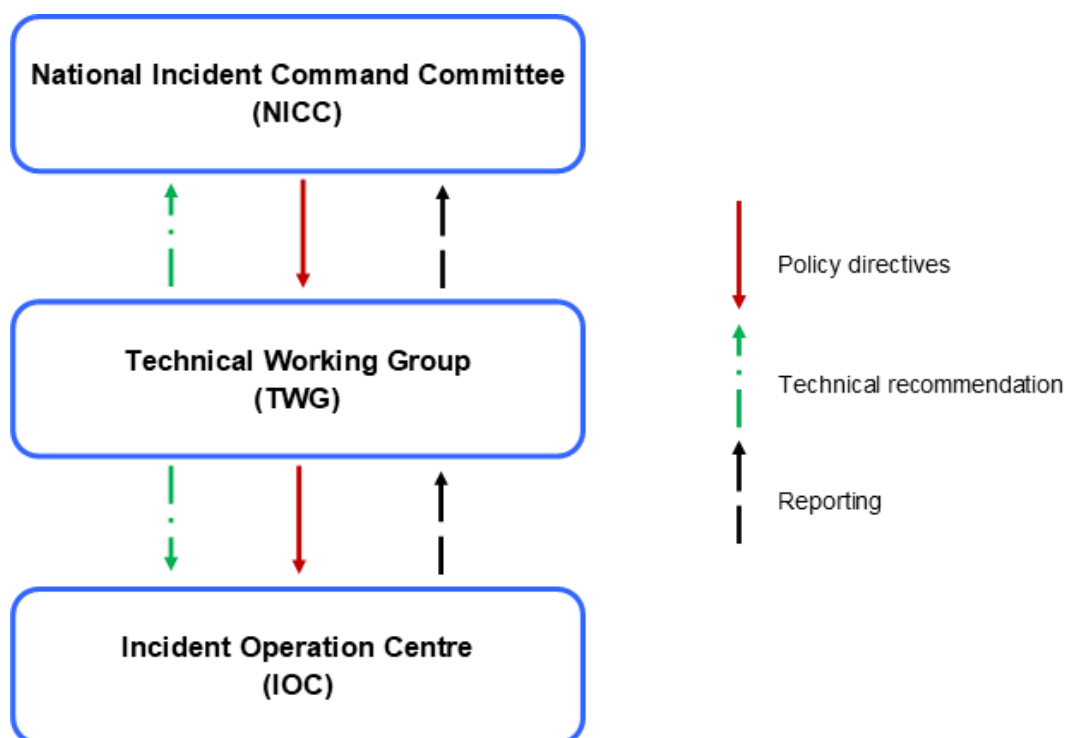


Figure 7: Flow of directives and information (reports)

## 8.1 National Disaster Management Authority (NDMA) for the NASFPCP

The NDMA is the highest authority for the implementation of this plan and will facilitate the implementation as advised by the National Incident Command Committee (NICC). The NDMA shall be chaired by the Prime Minister and shall include members as stipulated in sections 7 and 8 of Chapter 2 of the Disaster Act 2013. The NDMA will make policy decisions during the outbreak containment phase. The members of the NDMA will be as prescribed in the National Disaster Act 2013.

### 8.1.1 Roles and Responsibilities of NDMA

- Make policy decisions for the implementation of the NASFPCP as recommended by the NICC
- Mobilize required resources for the implementation of the plan as recommended by NICC
- Approve inter-sectoral lead agencies and ensure coordination amongst all relevant sectors which would be involved in effective implementation of the NASFPCP when outbreak is considered a national disaster
- Declare ASF outbreak as a national disaster
- Direct NICC to form committees and task forces as necessary to deal with ASF outbreak
- Ensure inter-sectoral collaboration and partnership with international, regional and bilateral agencies including the the Food and Agriculture Organization of the





United Nations (FAO), the World Organization for Animal Health (OIE), the World Bank (WB), the Asian Development Bank (ADB), and the South Asian Association for Regional Cooperation (SAARC).

### **8.1.2 Meeting and procedures**

The NDMA shall meet once a year and as and when required. The Chairperson of the NDMA may call a special meeting, if:

- The ASF outbreak becomes widespread and NDMA's intervention is required
- A written request is made by the NICC; or
- The Chairperson of NICC considers necessary

A simple majority shall constitute the quorum for convening the meeting. The NDMA shall seek technical recommendations from experts within MoAF for decision-making processes. The Incident Command Structures have been adopted for proper coordination of the key stakeholders during the operation. The incident command structure will allow a smooth flow of information from the national level to the incident area and vice-versa.

## **8.2 National Incident Command Committee (NICC)**

The NICC is the highest policy decision-making committee for ASF prevention and containment activities in the country under the guidance of NDMA. It shall be responsible for providing overall guidance in the implementation of the NASFPCP both in the prevention as well as during the outbreak phase. The NICC shall liaise closely with the National Emergency Operations Centre (NEOC) for necessary support especially in the event of the national disaster and beyond. The National Centre for Animal Health (NCAH), DoL will be the secretariat to the NICC.

### **8.2.1 Members of NICC**

The following members constitute the NICC:

- Secretary, Ministry of Agriculture and Forests - Chairperson
- Head, Department of Livestock, MoAF, Deputy Chairperson
- Head, Bhutan Agriculture and Food Regulatory Authority, MoAF
- Head, Department of Public Health, Ministry of Health
- Head, Department of Disaster Management, Ministry of Home & Cultural Affairs
- Head, Department of Forests and Park Services
- Head, Department of National Budget, Ministry of Finance
- Head, Animal Health Division, DoL
- Head, Quality Control and Quarantine Division, BAFRA
- Head, National Centre for Animal Health (Member secretary)



### ***8.2.2 Roles and responsibilities in the event of an outbreak of ASF in Bhutan***

- Declare the outbreak of ASF and issue official/public notification on recommendation of TWG.
- Authorize issuance of notice to the OIE and other trading partners.
- Take policy decisions on response and control measures based on the advice and recommendation of the TWG.
- Command response to ASF outbreak upon request from the TWG.
- Provide policy direction for response activities.
- Issue executive orders, public notifications and press releases on disease outbreak and its containment activities.
- Facilitate and mobilize all the logistics required for responding to ASF outbreaks.
- Designate media spokesperson(s) for providing disease status update, response policies and strategies, press release, etc.
- Ensure the adequacy, timeliness and relevance of communications activities.
- Maintain liaison with relevant sectors such as the Department of Disaster Management (DDM), Ministry of Economic Affairs (MoEA), Ministry of Finance (MoF), Ministry of Foreign Affairs (MoFA), Gross National Happiness Commission (GNHC), Bhutan Chamber of Commerce and Industries (BCCI), Ministry of Education (MoE), International organizations, non-governmental organizations, etc.
- Provide updates to the NDMA when required.

### ***8.2.3 Frequency of meetings and quorum***

The NICC meeting shall be convened within 24 hours of laboratory confirmation of the disease outbreak by the NCAH. During normal times, NICC shall be convened at least once a year or as and when deemed necessary by the Chairperson. The committee will also meet as and when there are outbreaks in neighbouring/trading countries to decide on regulatory measures and upscale preventive measures. A simple majority shall constitute the quorum and as far as possible all the members shall be present. In addition, technical experts shall be invited to the meeting.



### 8.3 Technical Working Group for ASF

The Technical Working Group members will comprise of experts from different sectors to advise and provide technical recommendations to the NICC and IOC

#### 8.3.1 TWG Members

S.N.	Members	Agency	Responsibilities
1	Chief, Animal Health Division	Animal Health Division-DoL	Chair
2	Specialist, Animal Health Division	Animal Health Division-DoL	Member
3	Representative from Animal Health Division	Animal Health Division-DoL	Member
4	Head, Disease Prevention and Control Unit, NCAH	NCAH-Serbithang	Member secretary
5	Head, Laboratory Service Unit, NCAH	NCAH-Serbithang	Member
6	Quality control and quarantine division focal	BAFRA, MoAF	Member
7	Wildlife health focal	NCD, DoFPS, MoAF	Co-opt Member

#### 8.3.2 Terms of Reference for Technical Working Group

- Review and provide technical recommendation to the NICC on the emergencies related to ASF prevention and control activities;
- Apprise NICC and advise IOC on the scale of rapid containment activities including manpower requirement, movement restriction, timeline and allocation of resources depending on the magnitude of the disease outbreak;
- Review the IOC reports and provide timely advice to IOC on outbreak containment activities during outbreak;
- Analyse the IOC report and update on timely basis to the NICC;
- Recommend review of NASPCP, including the SOPs for ASF prevention and control activities;
- Facilitate mobilization of resources for prevention and control activities;
- Fix compensation rate in consultation with the Livestock Production Division and IOC for endorsement by the NICC;
- Carry out any specific tasks assigned by the NICC.

#### 8.3.3 Meeting and Procedures

The committee will meet as and when required or when called by the TWG chair.



## 8.4 Incident Operation Centre (IOC)

The IOC is the field level coordination and implementation unit for rapid response and control measures. The unit will be responsible for providing field level information and updates on the disease status, progress on the response, and control activities to the TWG for ASF outbreak. Besides, it will ensure that all policy decisions and directives for response and control activities are conveyed to the different RRTs. The IOC shall submit daily updates including the minutes of the meeting to the TWG about the status of the ASF outbreak and containment activities.

### 8.4.1 Role and responsibilities of IOC

- Overall coordination of outbreak containment activities in the respective region
- Submit activity report to TWG
- Mobilization of all logistics, supplies and human resource required for rapid response activities
- Liaison with relevant agencies within Dzongkhag/ Dungkha/ Thromde/ Gewog level
- Submit budget proposals and settle all the bills
- Fully responsible and accountable for expenditures incurred during the containment of outbreak and auditing of the expenditures
- Liaise with TWG in fixing compensation rate and advocate to the farmers
- Scaling of manpower based on the scale of the outbreak
- Any other tasks assigned by TWG and NICC

### 8.4.2 IOC Team Composition

Generic team composition of IOC is as shown in the following table

Sl. No.	Member	Agency	Main Tasks
1	Regional Director/ Head of Animal Health Section under RLDC	Concerned RLDC	Incident Commander/ overall coordination including reporting to higher authorities
2	Regulatory and Quarantine Officer (Livestock)	Concerned PLQO / Dzongkhag BAFRA	Deputy Incident Commander - Assist Incident Commander
3	Veterinary Epidemiologist/ VO	NCAH/ RLDC/ DVH	Investigation/ Epidemiological Surveillance
4	Veterinary Officer	DVH/ RLDC	Clinical and lab. Surveillance



5	Regulatory and Quarantine Officer	Concerned BAFRA Office	Oversee enforcement of quarantine and movement control
6	Regulatory and Quarantine Officer	Concerned BAFRA Office	Oversee depopulation, decontamination, and disposal measures and disposal (3D)
7	Dzongkhag Livestock Officer	Concerned Dzongkhag	Logistic support/ risk communication/ Member Secretary for the Compensation Committee
8	Dzongdag/ Dungpa/ Dzongrab/ Thrompon/ Gup	Concerned Dzongkhag (s)	Dzongkhag level logistics support (as Chief Disaster / Emergency Coordinator)  Chair of the Compensation Committee
9	SP or OC or representative	RBP concerned Dzongkhag	Law and Order
10	Dzongkhag Disaster Management Officer	DDM/Concerned Dzongkhag	Coordinate between IOC and local government in the implementation of IOC activities
11	Forest and Park Official	Concerned division/Park	Coordinate disease control in wildlife

The generic team composition of the IOC is as listed in the table above; however, depending on the scale of outbreak, response measures to be implemented and considering other inevitable factors, the TWG, in consultation with the Incident Commander, shall decide on the actual team composition to operate the IOC.

## 8.5 Reporting and monitoring mechanisms during ASF outbreak

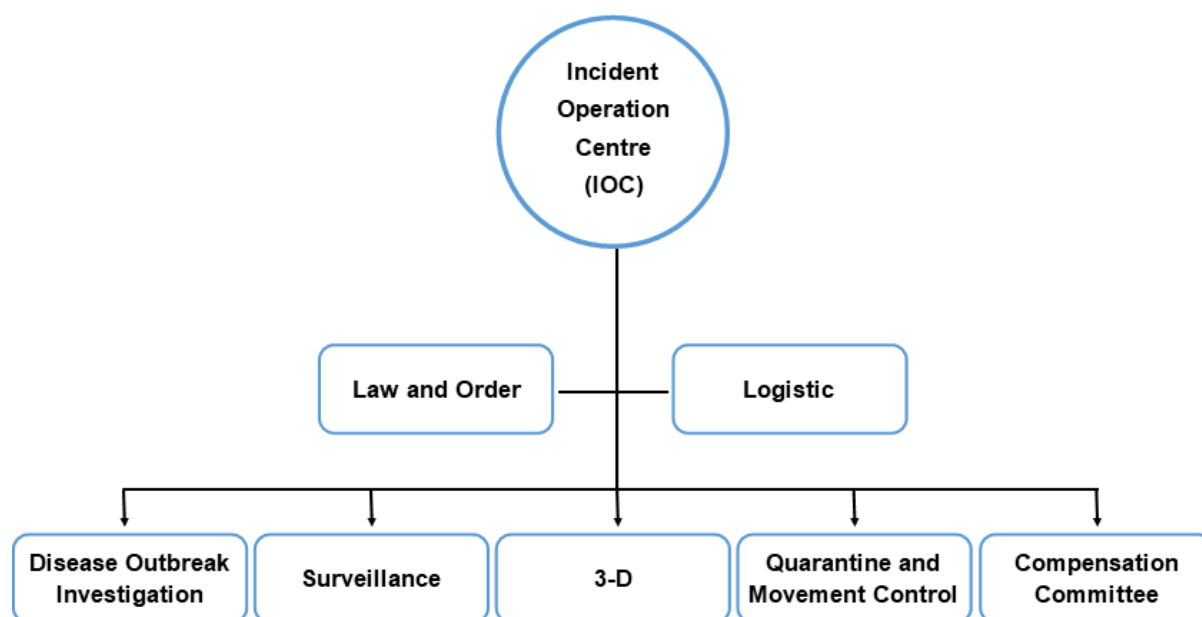
For uniform and consistent reporting, the TWG will design and provide reporting format to the IOC during outbreak response. The IOC shall submit daily updates including the minutes of meeting to the TWG about the status of containment activities. The TWG shall further share information with the NICC. The TWG should monitor the functioning of outbreak response either through a report or by convening meeting.

## 8.6 Rapid Response Teams (RRT)

The RRTs will be divided into different groups as per the mandate of the respective technical sectors involved in the disease control measures. The DoL will be mainly responsible for disease outbreak investigation, surveillance and logistic supply. BAFRA will be responsible for the 3-D operations, quarantine and movement control. The RBP will be responsible for the maintenance of law and order and providing



support for different operations. The Dzongkhag Disaster Management Committee will be responsible for providing necessary logistic support. However, all these team will be formed only if required. As a lesson from the past experience of implementing 3-D operation, rapid response teams shall be formed in ensuring rapid and effective response upon detection of brucellosis positive animals. The requirement of different rapid response teams and mobilization of human resource among the different teams under RRT will be decided by the Technical Working Group in consultation with the IOC. The following figure shows the RRTs required in responding to ASF outbreak(s) in the field.



*Figure 8: RRTs for ASF outbreak containment*

### **8.6.1 Disease Outbreak Investigation Team (DOIT)**

The DOIT shall be responsible for disease investigation and confirmation of the outbreak, and they shall be responsible for the identification and establishment of infected premises, dangerous contact premises, suspected premises, protected and surveillance zones. The DOIT shall also be responsible to undertake risk assessment to establish the zones and to decide on other disease control measures to be applied. However, based on the requirement in the field, the TWG shall deploy an independent risk assessment team before the implementation of 3-D operation.

The DOIT shall consist of the following members:

- Veterinary Officer (Team Leader)
- Laboratory technician

### **8.6.2 Surveillance Team**

The surveillance team (clean team) shall be involved in carrying out all necessary surveillance activities in the protection zone. The team will also provide risk



communication on ASF to the communities. The Surveillance Team shall be composed of the following members:

- Veterinary Officer/ Veterinary paraprofessional
- Laboratory Technicians
- Forestry official during wild animal surveillance (as and when required)

To avoid risk of transmission of ASFv from infected to the protection zone, the clean team shall operate from a separate facility, stationed in the protection zone, away from other RRTs operating in the infected zone. Activities implemented and progress should be reported to the IOC's Incident Commander on a daily basis.

### **8.6.3 3-D Team (Depopulation, Disposal, Decontamination)**

The 3-D team shall be responsible for carrying out culling, disposal and decontamination of animals that has been confirmed positive to ASFv as per the SOP provided in Annexure 13.5 and 13.6. All animals will be subjected to stamping out once a clinical disease or evidence of active infection is confirmed. During the implementation of pre-emptive culling, based on the risk assessment results, 3-D operations shall be implemented in the susceptible pig population – apparently healthy but poses a very high risk of infection and further transmission.

The 3-D Team shall be composed of the following members:

- Team leader: Regional Director or Animal Health In-charge (RLDC)
- One BAFRA Livestock Inspectors who will act as welfare officer, assist the team leader and coordinate decontamination
- Concerned Livestock Extension Officers, for Record keeping
- One or two hired and trained personnel as a culler depending on number of animals to be culled
- Hired labourers for disposal of culled animals (in places where excavator hiring is not possible) and to assist in decontamination process
- One police personnel may be engaged, if farmers are resistant to 3-D implementation despite the best effort by 3-D team, in advocating the importance of 3-D operations

### **8.6.4 Quarantine and Movement Control Team**

The Quarantine and Movement Control Team shall be responsible for enforcement of quarantine and movement control of animal and animal products from the infected farm or areas to control and prevent the spread of the disease and contain the outbreak as soon as possible. The Quarantine and Movement Control Team shall be composed of the following members

- Team leader: Regulatory and Quarantine Officer/Inspector (Livestock)
- Village Tshogpa or Mangmi





### **8.6.5 Compensation Committee**

The main role and responsibility of the Compensation Committee is to ensure provision of compensation in a fair, transparent and timely manner to all eligible owners/farmers. The committee shall strictly adhere to compensation guidelines given in the document (Annexure13.11).

The Compensation Committee shall be composed of the following members:

*A. Dzongkhag level:*

- Dzongdag/Gup / or a representative – Chairperson
- DLO/Gewog Extension Officer – Member Secretary
- BAFRA RQO/RQI Livestock– Member
- Representative from IOC – Member

*B. Thromde level:*

- Thrompon/ Thromde Theumi/Representative – Chairman
- DLO/Gewog Extension Officer – Member Secretary
- BAFRA RQO/RQI Livestock– Member
- Representative from IOC – Member

### **8.6.6 Logistic Team**

The main responsibilities of the logistic team are to ensure that all necessary logistical facilities like PPE, materials and equipment, food and lodge, and transport are available to all RRTs and to reinforce all essential supplies.

The generic logistic team shall be composed of the following members; however, the composition should be determined by the scale of the outbreak and HR deployed in implementing various ASF outbreak control measures:

- Incident Commander.
- Concerned PLQO/ Dzongkhag BAFRA Office.
- Procurement Officer/ Administrative Officer/ Accounts Officer, RLDC.
- Dzongkhag Livestock Officer.
- Dzongkhag Disaster Management Officer.

### **8.6.7 Law and Order Team**

The main roles and responsibilities of the law-and-order team are to ensure compliance and smooth operation of all disease control measures implemented through RRTs, especially the 3-D operations. The team shall support all technical RRTs involved in the disease outbreak control measures.

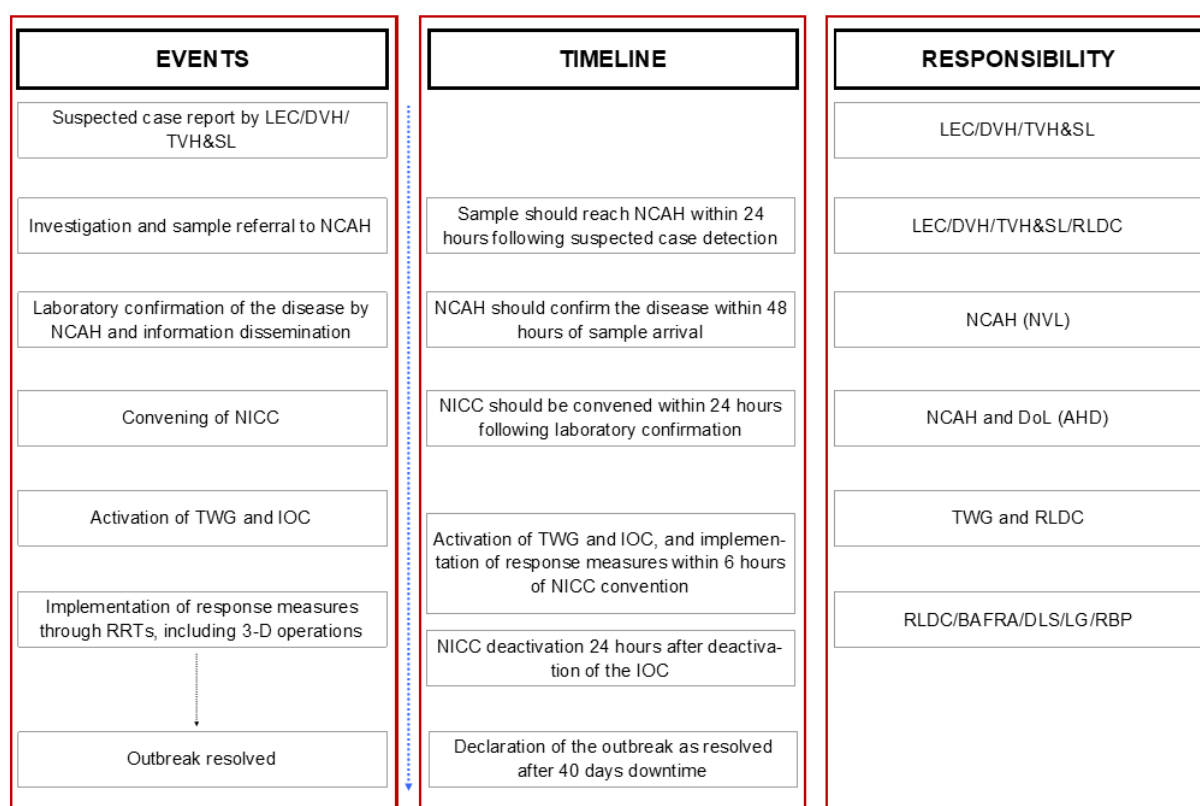




## 8.7 Outbreak response modus operandi

When a suspected case of ASF, as per the case definition, is reported and laboratory sample reaches the National Veterinary Laboratory of NCAH, necessary test shall be performed immediately. Upon confirmation of the disease, NICC shall be convened within 24 hours and recommend activation of TWG and establishment of IOC through an executive order. Required RRTs shall be activated through the IOC. Various ASF control measures, as described in this document, shall be implemented by the respective RRTs. The ASF outbreak shall be considered as resolved only after 40 days downtime, counting from the last day of 3-D operations completion.

Following figure describes the series of events and timeline to follow after suspecting and confirmation ASF outbreak in the country.



**Figure 9: Major events and timeline following confirmation of ASF outbreak**

## 8.8 Role of Relevant Agencies/ Organizations

### 8.8.1 Department of Livestock

The Animal Health Division (AHD) of the Department of livestock shall oversee policy formulation related to NASFPCP in the country. The specific roles include the following:

- Oversee the implementation of the ASF prevention and control programme in the country.



- Mobilize resources including fund for the ASF control programme in the country.
- Collaborate with BAFRA to enable better enforcement of the Livestock Acts, Rules and Regulations and By-laws of the country.
- Collaborate with relevant national/international agencies for ensuring and mobilization of support required for ASF prevention and control.
- Coordinate border harmonization meetings with the Indian counterparts at the state and Central levels.

### ***National Level***

The National Centre for Animal Health shall be the National coordinating centre for all preparedness and response activities during ASF outbreak(s). The NCAH shall provide necessary technical and logistic support to the RLDC while undertaking disease control measures. The NCAH shall also work as secretariat to NICC and TWG and facilitate the organization of NICC and TWG meetings as and when instructed by the Chair of NICC and TWG.

The responsibilities of NCAH, the national focal agency for ASF prevention and control are to:

- Coordinate the overall implementation of the ASF prevention and control programme in the country.
- Liaise with different stakeholders/agencies for facilitating better implementation and ensuring the success of the prevention and control programme.
- Coordinate the conduct of epidemiological research in collaboration with national and international diagnostic and research institutions.
- Production of education (IEC) materials for awareness and education
- Ensure maintenance of a database on ASF prevention and control programme, analysis, and dissemination of information/progress report to the Department/Ministry/other stakeholders, regarding the progress of the programme.
- Conduct ASF coordination workshops at the national level to review and realign the prevention and control programme.
- Monitor and evaluate the prevention and control programmes implemented by the field units.
- Capacity building of RRT members
- Declaration of risk zones/compartments for ASF and assess the status of these zones/compartments by regular surveillance and monitoring.
- Regular update of information about ASF outbreaks in neighbouring countries to all relevant stakeholders.



### ***Regional Level***

The Regional Livestock Development Centre (RLDCs) shall function as a regional focal agency for the ASF prevention and control programme.

The main roles of the regional focal agency should be to:

- Coordinate the overall implementation of the ASF prevention and control programme at the regional level.
- Coordinate the activation of a rapid response teams (RRT) following IOC establishment
- Coordinate surveillance programmes for prevention and control of the ASF outbreak.
- Provide support and coordinate logistics arrangement at the regional level.
- Liaise with the BAFRA for facilitating enforcement of the Livestock Act, Rules and Regulations and By-laws.
- Monitoring and evaluation of the ASF prevention and control activities.
- Ensure prompt reporting of suspect cases and updating the disease status in the existing database.
- Ensure maintenance of a database on ASF prevention and control and submit a progress report to the NCAH.

### ***Thromde Veterinary Hospital and Satellite Laboratory***

The Satellite Veterinary Laboratory shall play an important role in the prevention and control of ASF in border areas. The main roles are to:

- Coordinate the cross-border surveillance of ASF in their respective areas.
- Support implementation of the ASF prevention and control activities.
- Provide support and coordinate logistics arrangement.
- Liaise with the BAFRA for facilitating enforcement of the Livestock Act, Livestock Rules and Regulations and By-laws
- Monitoring and evaluation of the ASF prevention and control measures implemented in their respective areas.
- Ensure prompt reporting of the outbreak and updating the disease status in animal disease information systems.
- Ensure maintenance of a database on ASF prevention and control activities and submit progress report to the concerned RLDC and NCAH.

### ***Dzongkhag Level***

At the Dzongkhag level, the Dzongkhag Livestock Sector would function as the focal agency for the implementation of the ASF prevention and control programme. The Dzongkhag should carry out the following tasks:

- Implement NASFPCP activities in the field.



- Report suspected ASF cases immediately to the RLDC and NCAH.
- Support the activation of rapid response teams (RRT) in the event of case detection or outbreak.
- Maintain animal import records and monitor for any ASF suspect cases in pigs.
- Arrange logistics at the Dzongkhag level and assist the Gewog staff with their logistics.
- Liaise with the BAFRA at the Dzongkhag level for facilitating better enforcement of the Livestock Acts and By-laws.

### ***Gewog Level***

The Livestock Extension Centre/RNR Extension Centre in the Gewog should be the focal agency for that Gewog. They would play a very crucial role in the implementation of the ASF prevention and control programme in their respective gewogs.

The main roles of the Gewog focal agency are as follows:

- Implement the ASF prevention and control programme in the field as per the plan.
- Identify suspected cases and report to the Dzongkhag/DVH.
- Support RRT activities.
- Liaise with the BAFRA at the Gewog level for facilitating better enforcement of the Livestock Act, Rules and Regulations and By-laws.
- Liaise with the Gewog administration and farmers for facilitating the proper implementation of the programme in the field.
- Conduct regular disease awareness campaigns for the farmers and other relevant stakeholders in the Gewog.

### ***8.8.2 Bhutan Agriculture and Food Regulatory Authority***

BAFRA shall be responsible for/to:

- Enforcement of Livestock Act of Bhutan, Livestock Rules and Regulations and relevant By-laws.
- Enforcement of movement ban of pig/pig products and other identified livestock products out of the designated infected zone or from susceptible premises.
- Participate in 3-D operations and implement movement control and quarantine measures during outbreak responses.
- Monitor movement of pigs and pig products within and between Dzongkhags
- Inspection and certification of suspected livestock products.
- Carry out bio-security measures during the outbreaks (segregation, disposal, cleaning, and disinfection).
- Border vigilance on the illegal movement of livestock & livestock products in the peace period.



### **8.8.3 Other stakeholders**

#### ***Department of Forests and Park Services***

The large-scale ASF epidemic travelled thousands of kilometres away from its original incursion point in Georgia and, in addition to endemic establishment in domestic pigs, the disease eventually invaded populations of wild boar. In Europe, from 2014 to 2015 the circulation of this virus in the natural ecosystems developed into a self-sustained epidemiological cycle. Currently, the disease is endemic in wild boar populations in several countries and continues to expand its range in Europe, causing very serious concern. Controlling this sylvatic epidemic of ASF is a very challenging task for the veterinary authorities, given the complexity of the disease epidemiology, the lack of previous experience, the unprecedented geographical scope of the problem, and its transboundary and multi-sectorial nature.

Therefore, the DoFPS could play a critical role in preventing spread of ASFv into the wild boar population of the country through collaborative surveillance, passive and active, with the Department of Livestock.

#### ***Dzongkhag Administration***

The Dzongkhag, Dungkhag, and Gewog administration supports are important for coordinating ASF prevention and control activities including implementation of rapid response activities during ASF outbreaks(s). Local government support is crucial for strict implementation at the village and community levels.

#### ***Ministry of Home and Cultural Affairs***

The ministry shall support, coordinate, and manage activities as per the National Disaster Management Act 2013. The DDM shall facilitate and support the timely release of funds for the implementation of response activities in the event of an outbreak of ASF.

The Royal Bhutan Police support shall be sought, if necessary, during the implementation of control measures for containment of ASF outbreak in the field.

#### ***Ministry of Finance***

The Ministry of Finance (MoF) shall provide adequate funds for the implementation of NASFPMP in the country upon recommendations of the NICC.

The Department of Revenue and Custom under the ministry shall support BAFRA in the examination and inspection of imported livestock and livestock products including other risk goods to ensure their safety in the event of an outbreak of ASF.



### ***International Organizations***

Linkages with international organizations such as the Food and Agriculture Organization (FAO), World Organization for Animal Health (OIE), and other partner organizations must be built for seeking technical and fund support, particularly, human resource and laboratory capacity development and referring of samples for laboratory diagnostic purposes, including molecular characterization of the virus.



## 9 SUPPORT PLANS

Support plans for better coordination and containment of the ASF outbreak require lots of resources both in terms of financial and human resources with strong policy support and legislation.

### 9.1 Financial Plan

Experience has shown that delay in obtaining finances is a major constraint to rapid response to any disease outbreaks in the field. Immediate application of even modest funds can save major expenditure later. Forward financial planning is, therefore, an essential component of preparedness.

The fund mobilization for the preparedness and response activities against ASF will be undertaken as per provisions of the Disaster Management Act of Bhutan 2013. During normal situations, the concerned agencies (DoL and BAFRA) will propose a budget during the annual budgeting exercise for prevention activities such as awareness, disease surveillance, and capacity building activities of relevant stakeholders.

However, in the event of an outbreak, financial plans need to be developed to provide an immediate provision of contingency funds to respond to disease emergencies. These are for expenditure required over and above normal operating costs for the concerned agencies. Plans and budget for response during ASF outbreak(s) must be finalized by the TWG, in consultation with the IOC and should be approved by NICC after review for an immediate disease containment activity as per the provisions of the Disaster Management Act of Bhutan 2013. The NICC will forward the budget requisition to the MoF for final approval and sanction. The MoF shall release the approved budget to the IOC through the Department of Livestock.

The DoL and BAFRA may identify potential international donors, including emergency support from OIE/FAO or other international agencies to support disease prevention and elimination programme.

### 9.2 Legislation

The plan is in line with Livestock Act 2001 Chapter IV, Section 9, wherein, the powers to carry out ASF response actions are authorized to:

- Seal and claim the infected areas and disease-control zones.
- Quarantine farms or other livestock enterprises.
- Ban movements of pig and pig products or potentially contaminated materials.
- Destruction and safe disposal of infected or potentially infected animals and contaminated products and materials, subject to fair compensation.



### 9.3 Research and Extension

The proper study shall be carried out on the following relevant subjects which would benefit in preparing for prevention and control of ASF outbreak in the country and reviewing the present document:

- Pork value chain system in the country.
- Biosecurity of piggery farms in the country.
- Geographical distribution of wild boars.
- Pig management system in the country.
- Prevalence and distribution of the ASFv vector in the country.
- Cross-border movement of live pigs and pork.
- Other risk factors for ASF disease in the country.
- Pig population dynamics in the country.





## **10 CAPACITY BUILDING**

### **10.1 Training**

Staff should be trained in their roles, duties, and responsibilities in an ASF disease outbreak response and enhance their knowledge. The training can be provided in-country through hands-on training in laboratory diagnosis, surveillance, and other control strategies. Further, some training can also be availed through the support of OIE/FAO and liaising with regional and international ASF epi-network aimed at enhancing the capacity of staff and relevant stakeholders.

### **10.2 Simulation Exercises**

The NCAH shall lead in validating and testing NASFPCP through desktop simulation exercises by simulating a real-time disease outbreak scenario, or a drill to test the whole document or important components such as response actions during outbreaks. This provides valuable means for building teams for disease outbreak responses and for training individual staff.

After each simulation exercise and training, there should be an assessment of the results. This review should identify areas where plans need to be modified and further training required.



## 11 REVIEW AND UPDATE OF THE PLAN DOCUMENT

This strategic plan should not be treated as static but as a document that regularly needs reviewing and updating to reflect changing circumstances. The NASFPCP implementation shall be reviewed and validated by the M&E team at the national, regional, and dzongkhag level.

A team comprising officials from DoL and NCAH shall oversee the overall implementation of the NASFPCP. Preparedness and response to ASF outbreak(s) by relevant stakeholders at dzongkhag and regional levels shall be evaluated by respective RLDCs.

M&E must be done at the time of disease outbreak and peace time to see how well the plan works. The M&E team should provide recommendations and feedback for further improvement of the disease response and preparedness plan.



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## 13 ANNEXURE

### 13.1 Guideline for ASF outbreak investigation.

This outlines the general principles and steps for the investigation of the ASF outbreak in the field.

#### Team composition

- Veterinary Officer (Team Leader)
- Laboratory technician

#### Steps for Investigation

- Pre-investigation preparation.
  - Includes briefing each person's roles and responsibilities and packaging necessary materials required in the field.
- Gather preliminary information.
  - Collect necessary information like outbreak details and inform about the team's visit to the area.
- Field investigation - Includes a collection of detailed information about the outbreak, suspected source, prevalence of disease vector, and collection of geo-coordinates. The following information should also be collected:
  - Baseline mortality and clinical signs - General information about affected and at-risk populations, daily morbidity and mortality and detailed clinical signs and symptoms.
  - Bio-security arrangements - Describe the bio-security arrangement of the farm e.g., disinfectant foot wash, perimeter wall/fence, wild pig control, etc.
  - Feed source - Describe feed sources and assess visually for the possibility of wild pig accessibility.
  - Water source - Assess the water source for the possibility of wild pig contamination.
  - Wild pigs - Determine the presence of wild pigs in the area.
  - Scavenging pig - Determine the presence of scavenging pigs in the area
- Laboratory investigation.
  - Includes a collection of samples from both affected (sick and dead) and non-affected populations, environment suspected to have contaminated, packaging, and transportation to the laboratory.
- Characterize the outbreak - Establish or verify the outbreak and describe in terms of time and space.
- Develop a hypothesis.
- Implement prevention and control strategies.
- Communicate the findings.



## 13.2 Guideline for ASF surveillance

### Introduction

Strong surveillance systems are essential to provide decision-makers with quality and timely information on the status of animal diseases in a country, enabling them to develop effective disease control programmes and respond rapidly to emerging threats to livestock and public health.

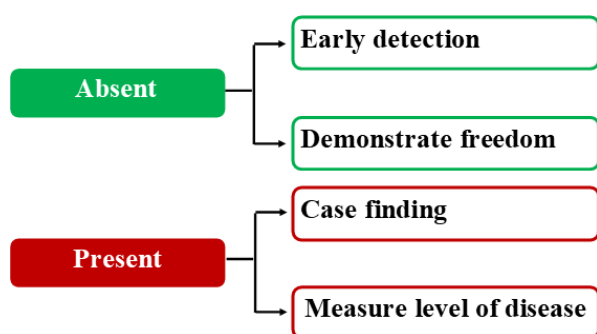
The NCAH, in collaboration with relevant stakeholders, has developed this guideline to support veterinary services in designing comprehensive animal disease surveillance plans by highlighting some of the most important components. The ASF surveillance guideline should be tested, approved and actively communicated and disseminated to all stakeholders of the surveillance system within the country. The National Centre for Animal Health, the steering and/or technical body in charge of national animal health surveillance, should review and update this guideline regularly to ensure their effectiveness and relevance to the purpose.

ASF surveillance implementation plan in the field shall be developed in alignment with this guideline.

### Purposes

There are many reasons why veterinary authorities undertake surveillance activities, but these can be summarised into four general purposes:

- Demonstrating freedom from disease.
- Early detection of disease.
- Measuring the level of disease.
- Finding cases of the disease.



### Materials and equipment

- Data recording sheet.
- Notepad and pen.
- Mobility.
- Mobile phones.



- Sampling kits.
- Field diagnostic kits.
- Extension gears.

## A. Phases of surveillance

The timeline for the implementation of different types of surveillance activities in the field is dependent on the current situation of ASF outbreaks in the country and the region. ASF surveillance shall be conducted during three different phases: prevention, early warning and contingency, and during the outbreak(s).



### I. During Prevention Phase

During peacetime, the regional centres and dzongkhag livestock sectors shall regularly report pig morbidity and mortality cases from their respective jurisdictions to the National Centre for Animal Health (NCAH). Livestock field officials shall liaise with Forest and Park officials for reporting morbidity and mortality in wild pigs.

Passive farmer disease reporting systems are the best tool for early detection because they have comprehensive coverage of the population and are continuous.

#### **Team composition**

- Veterinary Officer/ Veterinary paraprofessional
- Laboratory Technicians
- Forestry official during wild animal surveillance (as and when required)

#### **Activities**

- Passive syndromic surveillance shall be conducted on daily basis in their respective jurisdictions.





- Any suspected ASF case(s) must be reported by the LEC/ DVH to the concerned RLDC and NCAH through the flash report or other fastest means of communication.
- Information on unusual wild pig die-offs must be shared by DoFPS field offices to the nearest animal health centres.

## ***II. Early warning and contingency phase***

Upon the confirmation of the ASF outbreak in neighbouring countries and unusual pig mortality reports from passive surveillance, early warning and contingency surveillance activities shall be conducted. Based on the risk assessment for the incursion of ASFv into the country, areas shall be identified for the implementation of heightened surveillance through activation of the Border Vigilance Team and Veterinary Vigilance team.

### ***Border Vigilance Team (BVT)***

#### ***Team composition***

- OIC/ RQO-Livestock of concerned PLQO/ Dzongkhag BAFRA office.
- Livestock Regulatory and Quarantine Inspectors.
- Temporary recruits.

#### ***Activities***

- Keep strict vigilance at the point of entry to curb illegal movements of pig and their products. Effective targeted surveillance at commercial and government piggery farms including backyard farms.
- Strictly monitor and regulate the bio-security practices in the commercial farms in the high-risk areas.
- Collect samples from intercepted and quarantined pigs that are suspected of ASF and submit them for testing at referral laboratories.

### ***Veterinary Vigilance Team (VVT)***

#### ***Team composition***

- Veterinary Officer/ Veterinary paraprofessional
- Laboratory Technicians
- Forestry official during wild animal surveillance (as and when required)

#### ***Activities***

- Keep vigilance on any ASF outbreak-related events in the immediate border areas and report to DoL, NCAH, BAFRA and other stakeholders.
- Identify local leaders/ relevant people as the focal point to report any suspicious cases at the village level to any VVT member.



- Carry out surveillance in wild pigs in collaboration with DoFPS.
- Submit an immediate report to the NCAH and DoL of any suspicious case.
- Submit weekly reports to the DoL, HQ and NCAH.
- Submit or make weekly briefings to the concerned Dzongdags.

### ***III. During outbreak phase***

The Disease outbreak investigation team, based on risk assessments, shall delineate infection and protection zones. These zones shall determine the surveillance activities to be implemented. The protection zone may include the farm within the infection zone with the practice of high-level farm biosecurity and other ASF prevention measures. Active surveillance shall be conducted in the protection zone.

#### ***Team composition***

- Veterinary Officer/ Veterinary paraprofessional
- Laboratory Technicians
- Forestry official during wild animal surveillance (as and when required)

#### ***Activities***

- Active clinical and laboratory, virological and serological, surveillance should be conducted in the protection zone: for clinical surveillance, refer to clinical signs and lesions and case definition parts of the National ASF Prevention and Control Plan and for laboratory surveillance, refer to the SOP for laboratory sample collection for diagnosis of ASF.
- Survey methodology and sample size shall be determined by the RRTs based on the disease risk assessment.
- Surveillance reports must be submitted to the NCAH and AHD, DoL on daily basis.
- The surveillance programme should be carried out until 6 weeks from the last detected case of the outbreak.

### **B. Data collection**

During the peace period, suspected ASF case(s) or outbreak(s) must be reported with the details as per the flash report format. Refer to the laboratory sample submission form to gather information required during laboratory surveillance.

For clinical surveillance, the following data items must be recorded:

Particulars	Information
Date	
Owner details	
Locality	



Geocoordinates (Lat., Long.)	
Breed	
Farming system	
No. of cases (with age and sex)	
Date of first case	
No. of deaths (with age and sex)	
No. of animals at risk (with age and sex)	
History (introduction of new pigs - from where and when)	
History (Sale of pigs - to where and when)	
Clinical signs	
Post-mortem lesions observed	
Sample details (if any)	
Actions taken	
Reporting Officer	



### 13.3 Biosecurity procedures to be followed when visiting the infected farms.

Equipment needed to ensure good biosecurity when entering a farm:

- One pair of good-quality gumboots that are easy to clean and disinfect.
- Disposable coverall.
- Boot covers.
- Hand gloves (make sure they are the right size).
- Plastic mat.
- Buckets.
- Appropriate detergent.
- Disinfectant (approved for ASFV).
- Scrubbing brushes (two).
- Biohazard bags (including biohazard bags).
- Ziplock bags (for transporting phones or other equipment).
- Disinfectant wipes for the face.
- Water (1-litre minimum).
- Sealing tape.
- Scissors.
- Sampling and recording equipment.

#### **Before departing:**

- Remove all unnecessary equipment from the car.
- Make sure you bring all the necessary equipment with you.

#### **On arrival:**

- The car should not be driven onto the premises (leave it near the farm entrance).
- Choose a suitable location for your disinfection site on a clean and dry surface (preferably concrete), using a clear demarcation between the clean and dirty sides (the gate usually).
- Remove all unnecessary clothes and items (e.g., jacket, tie, watch) and empty your pockets.
- Electronic equipment (e.g., mobile phones) needed on the farm should be placed in sealed plastic bags to facilitate subsequent cleaning and disinfection.
- The phone should never be removed from bags while on the farm and should only be used through the plastic bag.
- Remove from the car all the items needed for disinfection that are to be taken onto the farm.
- Lay down a plastic sheet on the clean side of the disinfection site.
- Place the items you will be taking with you to the farm on the dirty side of the disinfection site (e.g., black plastic bags and sample container).
- Don the PPE.



### **On leaving the farm:**

- Doffing of the PPE should be done at the dirty site.
- Before leaving the premises, use the farm's facilities to clean very dirty areas.
- Wash off and disinfect the bag containing the phone and any similar items taken to the farm.
- Remove boot covers and dispose of in dirty-side plastic bags including used PPE.
- Lastly, hands and glasses should also be disinfected here, as well as your face with disinfectant wipes.
- Non-disposable equipment and samples should be double-bagged and taped shut.
- Regular shoes can be put back on.
- Any equipment and materials from the farm must stay on the dirty side.
- Leave the farm and immediately take samples/equipment for processing.
- If there are no pigs on your premises you may return home, shower, and thoroughly wash your hair.
- All clothes worn that day should be soaked in disinfectant for 30 minutes and washed with water over 60 °C.
- Alongside the procedures for cleaning and disinfecting yourself, you may also need to clean and disinfect the car.



## 13.4 SOP for quarantine and movement control

### Purpose

The purpose of this SOP is to ensure that the implementation of quarantine and movement control measures in protection zones are carried out smoothly, effectively and successfully to prevent and minimize the spread of the ASF virus from infected areas.

This will not apply to routine movement monitoring at other entry and strategic check posts.

**Target/User:** Quarantine and movement control team

### Team composition

- Team leader: Regulatory and Quarantine Inspector/ Officer (Livestock).
- Technical Assistant: BAFRA Livestock Inspectors (number to be determined based on the place and size of outbreaks and entry and exit points in the infected and protected zones).
- Record keepers: BAFRA Livestock Inspector (one each in all entry and exit points of the infected and protected zone).
- One police personnel.

### Disinfectants

Each quarantine and movement control team should be provided with an adequate quantity of the following disinfectants:

- Soaps and Dettol.
- Sodium hypochlorite or a 5 kg container of Virkon for vehicles and machinery.
- Petrol, Kerosene and other lubricants.

### Equipment

- Power sprayer (3000 PSI) used to dispense Virkon®S or other disinfectants.
- Hoses (5 meters).
- Continuous water supply and water storage tank (500 litres).
- Barrier/security line tape
- Duct tapes.
- Foot bath with tray and mat.
- A large bucket that can hold approximately 20 litres of water.
- Heavy-duty trash bags.
- Small plastic bags.
- Clipboard, notebook and pen.



### **Personal cleaning and disinfection supplies**

- Scrub brushes for removing dirt and other particles before using disinfectants.
- Soap to wash hands and face.

### **Biohazard control materials**

- Alcohol cotton pads, 70% ethanol – these are generally used to wipe your hands after removing your PPE.
- Red biohazard bags for placing used PPE.
- First aid kit.
- Flashlight.

### **Procedures**

- Determine all possible entry and exit points around the periphery of infected and protection zones based on the map of disease outbreak zones declared by the Incident Operation Centre on the recommendation of the disease investigation team.
- Establish only one or two entry and exit points from the infected and protection zone and seal all other entry and exit points.
- Place appropriate signboards to inform the public about the movement control measures in place.
- The movement of live pigs, their products and related items such as feed and equipment must be prohibited and quarantined until six weeks from the date of the last case.
- The vehicle and people coming out of the infected zones must be disinfected thoroughly using appropriate disinfectants.
- All personnel involved in disease investigation, stamping out, decontamination operation must follow complete protocols for entry and exit from these zones as described in the respective SOPs.
- The use of foot dip in the main entry and exit point in the infected areas should be placed and changed daily.
- The PPE coming out of the infected zone must be either buried or incinerated.
- All quarantine and movement control measures shall be lifted upon the recommended duration of the cooling period following the end of the outbreak.
- The record on the movement of vehicle and people must be maintained.
- Identify and establish a proper site outside and close to the periphery of infected and protection zones for putting on PPE, and unloading materials and equipment required for enforcing movement control measures.

### **Steps to be followed for exiting the quarantine and movement control duty:**

- The team members should remove PPE and place them in trash bags, which are to be placed in biohazard plastic bags before exiting the area.



- By the end of each workday, the team members shall dump all the used PPE, and other potentially infectious materials including those seized ones.
- All shall disinfect shoes, thoroughly wash hands at the wash station and sanitize your hands.
- All tools and other equipment used shall be cleaned and disinfected at the end of the day's operation.
- All personnel must disinfect their feet by dipping them in the footbath before leaving the place.
- Similarly, all parts of vehicles (especially tyres) must be disinfected at culling and decontamination lines.





## 13.5 SOP for Culling and Disposal

### Purpose

This SOP is to ensure that the implementation of culling and disposal for the control of ASF outbreak is carried out smoothly, successfully within the shortest possible time, and re-establish Bhutan's ASF-free status.

The stamping-out method of the disease control strategy is to be adopted for the ASF outbreak as it is the recommended and most effective control method for elimination. This control measure needs to be accompanied by decontamination of infectious materials, proper surveillance, enhanced biosecurity measures, strict quarantine, and restriction of movement of pigs and risk-possessing goods.

### Scope

This SOP covers the guidelines and steps for humane culling and safe disposal of pigs (dead and slaughtered), pig products, feeds, litters, dismountable sheds, and other infected materials of the culling and disposal team.

### Target/User: Culling and Disposal Team

The Team shall be composed of the following members:

- Team leader: Regional Director or Animal Health In-charge (RLDC)
- One BAFRA Livestock Inspector who will act as welfare officer, assist the team leader and coordinate decontamination
- Concerned Livestock Extension Officers, for Record keeping
- One or two hired and trained personnel as a culler depending on the number of animals to be culled
- Hired labourers for disposal of culled animals (in places where excavator hiring is not possible) and to assist in the decontamination process
- One police personnel may be engaged, if farmers are resistant to 3-D implementation despite the best effort by the 3-D team, in advocating the importance of 3-D operations

### Materials and Equipment Required

#### ➤ *Personal Protective Equipment*

Each culling member must be provided with Personal Protective Equipment (PPE) which includes:

- A coverall (with hood and boots).
- Face mask.
- Goggles.
- Outer glove – (Nitrile).
- Inner gloves – (Vinyl).



- Shoe covers.
- A plastic apron.
- Utility gloves for the pigsty dismantling and cullers.

Each person should be provided with an adequate number of PPE sets depending upon the area of operation and geographical terrain. These items should be always worn when they are in infected or suspected premises.

➤ *Disinfectants*

Each culling group should be provided with each set of the following disinfectants:

- Adequate quantity of disinfectants.
- Sanitary cloth, disinfectant wipes, or antiseptic wash.

➤ *Personal cleaning and disinfection supplies*

- Scrub brushes for removing dirt and other particles before using disinfectants.
- Sprayers (10 litres capacity) for dispensing disinfectant solution.
- Soap to wash hands, legs and face.
- A plastic basin for footbaths.
- A large bucket to mix the disinfectants.

➤ *Biohazard control materials*

- Alcohol cotton-pads, 70% ethanol – to wipe hands after removing PPE.
- A red biohazard bag for collecting used PPE.
- Antiseptic wipes/sanitizer.
- Eyewash.
- First aid kit.
- Flashlight.

➤ *Culling equipment*

Each culling group should have the following set of equipment:

- Heavy-duty trash bags.
- Small plastic bags.
- Roll of paper towels.
- Clipboard, notebook and pen.
- Duct tapes.
- Restraining and euthanizing equipment (dart gun and accessories, tranquillizing drugs, captive bolt)

➤ *Disposal materials and equipment*

The following general equipment and supplies are required:

- Spades, crowbars, pickaxes and shovels.
- Sodium or calcium hypochlorite (2 – 3 percent available chlorine).



- Waste container bags.
- Rolls of black plastic.
- Heavy-duty trash bags.
- Small plastic bags
- Rolls of duct tape.
- Roll of paper towels.
- Ziplock bags.
- Fire extinguisher – portable.
- Barrier tape.
- Excavator – in case of culling of the larger population of pigs.

## **A. Culling**

### **General consideration**

All domestic, stray or wild pigs in the infected premises will be subjected to stamping out once a clinical disease or evidence of active ASF virus infection is confirmed. If the situation calls for the implementation of pre-emptive culling, based on the risk assessment findings, apparently healthy pigs but at high risk of contracting the infection and spreading further shall be culled. Culling of wild pigs should be carried out in consultation and collaboration with the Department of Forests and Park Services. The plan for culling should be established based on the information and situation of the infected premises by the team leader.

Make sure that the area chosen for culling is not in the view of neighbours or other crowds, and that only individuals involved in culling operations are in the area. Clearing the culling area of unnecessary bystanders not only makes the process more efficient but also limits the number of people exposed to carcasses and potentially contaminated equipment or surface areas.

Identify and establish a proper site outside and close to the periphery of the culling and decontamination line, for putting on PPE and unloading materials and equipment required for culling and decontamination. Where the infected area is accessible by road, a culling and disposal crew vehicle shall be parked at this site.

Before entering the infected premises:

- Assemble the team and organize it into groups as per the specific tasks to be performed in an orderly manner and distribute the materials and equipment to each member.
- The Team Leader shall then provide a necessary briefing to all culling and disposal groups.
- Put on PPE before crossing the culling, cleaning, and disinfection line (protected zone).



- The culling team shall be divided into groups – the first group should start culling in the infected farms/area and other groups shall start culling from the periphery of protected zones and move towards the centre of the infected area.
- Once personnel have entered the premises, they may not cross back over the culling and decontamination line for any reason without removing and properly disposing of all PPE and proper personal disinfection.
- Groups identified for culling the infected farms shall only come out after completing the culling and disposal.
- In the infected premises, it is preferable to cull the infected pigs first followed by pigs in contact with infected pigs.

### **Culling Method**

Euthanasia should take place in such a way as to minimize an animal's pain and stress. To meet this requirement, the animal should be rendered unconscious as quickly as possible. Essential to the fulfilment of this objective is the careful selection of the quickest, most humane euthanasia methods, and skilful use of these methods on the part of the culling team. Euthanasia should be performed under the close supervision of a veterinarian, and each animal should be checked after the procedure to ensure that death has occurred.

To cull pigs during ASF control, pigs should be sufficiently sedated using sedatives either by injection or dart gun before the captive bolt is used for euthanasia. Death is confirmed by the absence of a corneal reflex, failure to detect respiration and absence of a heartbeat for more than 5 minutes.

For the depopulation of wild or feral pigs, an appropriate and practical culling method should be adopted in close consultation with concerned authorities.

### **Handling Considerations**

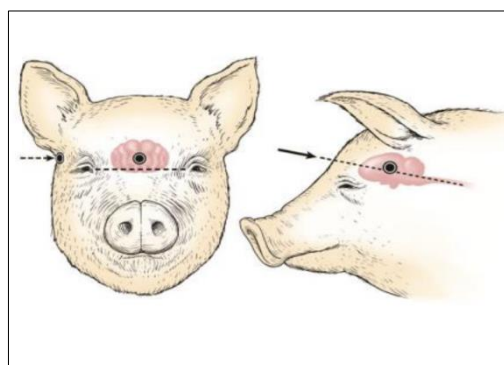
Decreasing stress and excitement during movement and handling will ultimately increase swine welfare as well as human safety and efficiency. From a practical handling point of view, as well as a humane consideration, swine must not be forced to travel faster than normal walking speed. Swine should be handled as quietly as possible on non-slip surfaces; shouting and screaming should be discouraged. Besides, the animals must be restrained in a manner that does not elicit injury or undue pain. Animals that are handled roughly or hurriedly will become excited, making further handling unnecessarily difficult. As a humane consideration, and if human safety will not be compromised, non-ambulatory or disabled animals should be euthanized where they are and moved to the disposal site after death. Euthanasia must be performed by competent personnel trained and experienced in the swine euthanasia method.

## Penetrating Captive Bolt

Euthanasia of swine utilizing a penetrating captive bolt is both humane and efficient. Animals must be properly restrained to minimize the potential for improper stunning or human injury. For suckling and younger nursing pigs, they can be restrained by firmly and securely holding them, using a body sling, or lifting them using two points of contact (such as one hand on their leg and one on their flank). Larger pigs can be restrained using specific restraining systems such as snaring individually or tranquilising the pigs.

### Aiming the Penetrating Captive Bolt

It is essential to aim the penetrating captive bolt correctly. For pigs weighing less than 130 kilograms, the penetrating captive bolt should be placed firmly against the skull and directed at the midline of the forehead and half an inch above the eyes, which is even with the eyebrows. Aim the bolt at the brain and direct the bolt toward the tail. To accommodate the thicker skull structure of more mature pigs, the



targeted point of entry should be adjusted to about an inch above the eyebrow line. It should also be moved just slightly to one side or the other of the skull ridge. If time permits, it is advisable to draw landmarks to increase the efficacy of placement, as illustrated at right. There are significant differences between the skulls of growing and adult swine; therefore, it is important to use an appropriately sized bolt to ensure penetration for larger sows and boars. There are also breed differences that may make proper placement more challenging. Different charges are required depending on bolt length. For example, in sows and boars, the distance to the brain is often 4 inches from the surface; therefore, the charge must be sufficiently large to cause the appropriate length bolt to penetrate the skull of a mature animal. To determine the specific charge, personnel should refer to the operating manual for the captive bolt being utilized. Not all captive bolt weapons use variable charges. A more powerful 0.25 calibre cartridge is now in common use in captive bolt weapons. A new era of penetrating captive bolts has been developed and used correctly, produces enough damage to the brain including the brain stem that it can be used as a single-step euthanasia device. These new captive bolts systems have several interchangeable captive bolts with varying lengths and thicknesses as well as several cartridges of varying strengths. The correct combination of captive bolt size and the cartridge is determined based on pig size, age, and type (suckling, nursery, etc.) because this system is designed to be used as a single-step euthanasia method that causes massive damage to the brain including the brainstem, the target location is moved slightly higher to maximize the destruction of the brain stem.



## **Monitoring effectiveness of the use of penetrating captive bolt**

The use of the captive bolt device, whether penetrating or non-penetrating, typically produces immediate collapse followed by a period of postural rigidity and then gradual relaxation. Often, correctly “bolted” pigs will have a period of involuntary kicking and paddling. Pigs that are rendered insensible should demonstrate signs such as lack of a corneal reflex, no deliberate movements, and no rhythmic breathing. If there is doubt as to whether a pig is insensible, the animal should be immediately re-stunned, or an adjunct euthanasia method should be applied.

## **B. Disposal**

Safety, biosecurity, and compliance with environmental regulations are the primary issues to be kept in mind for the disposal of large volumes of ASF-affected material. Burial is the primary method of disposal for carcasses, litter, discards from cleaning and disinfection activities, and other potentially contaminated material.

Ideally, pigs should be disposed of on-site by burial. Alternatively, if no approved site is identified, they can be transported and disposed of elsewhere. Contaminated feed shall be buried along with other infectious materials at the site. Similarly, manure, litter, and feed must be buried. Equipment and items that cannot be disinfected effectively have to be collected in a disposable bag and must be burned.

### **Precautions to be taken while transporting carcasses to disposal site:**

- To prevent virus spread, you must seal the containers, so they do not leak liquids or release debris such as feeds or litter materials. Large containers like gunny bags or biohazard materials are used to pack carcasses for easy and faster transport.
- Ensure to thoroughly wet the carcasses with disinfectant solution.
- Carefully inspect the container for any breaches, holes, large cracks, or sharp edges.
- Avoid puncturing any plastic bags with your feet or tools. Always inspect the plastic bags to ensure it is not damaged. Small plastic holes can be repaired easily with tape.
- Plastic openings must be sealed using duct tape. Similarly, the container opening must be sealed with plastic and duct tape from the outside of the containers.

## **Disposal by burial**

The first choice, by far, would be an on-site burial. Identify the site for burial such that wild animals or dogs cannot access the carcasses once they are buried. Dig one or more pits to bury all the pigs on the property. Considerations include the amount to bury, site availability, soil type, water table, nearby wells or ponds and digging equipment available.



## Burial site selection

Important considerations include:

- Access to the site – for both equipment to dig the burial pit and for the delivery of livestock, carcasses, or other materials to be buried.
- Environmental – distance to water sources, bores and wells; the height of water-table; proximity to buildings, especially houses; proximity to neighbours or public lands including roads; the slope of the land, drainage to and from the pit; permeability of soil; sufficient space for the temporary storage of overburden; and direction of the prevailing wind.
- Construction considerations-avoid rocky areas (slows digging and increases costs) but select soils with good stability capable of withstanding the weight of the equipment used for the construction of diversion banks if required.

If government land is not available, leasing/compensation for private land for the disposal pit should be considered after negotiation depending on the emergency. If the disposals of the pigs are undertaken in the city area, the selection of the site should be done jointly with the city corporation.

## Burial pit construction

- Following site selection, a pit of 5m deep and 3m wide should be dug for one adult carcass.
- A minimum of 1m distance from the water table should be maintained.
- Carcasses should be covered by about 2m of soil, with an unbroken layer of slaked lime in between. If this lime is applied directly to carcasses the decomposition process will be significantly delayed.
- Disinfectants are needed to be sprayed on equipment used and, on the pathway, used to take carcasses to the pit.

## Disposal by burning

The procedure is as follows:

- Pile up the infected materials to build a pyre.
- Pour fuel like kerosene (but not petrol/ gas) on the fire bed and place rags soaked in kerosene every ten meters along the length of the fire bed.
- Make sure that someone always watches the fire and replaces any infected materials that fall off the fire.
- The ashes can be buried as described in the section on the burial above.

In the case of low-quality pigsties where the scrapping of the litter materials is difficult, the pigsty should be dismantled and burnt on the site on the same day along with the litter materials in the infected areas. The compensation for the pigsty should be provided based on the assessment of the cost by the culling team and compensation committee.





### **Protection of disposal pit**

All the disposal pits should be properly fenced using iron poles and barbed wires depending on the field situation. In the case of remote areas, the use of wooden poles and barbed wires may be explored to reduce the cost of fencing.

### **Steps to follow Culling and Disposal:**

- Culling and disposal team members should remove PPE and place them in a trash bag, which is to be placed in biohazard plastic bags before crossing over the culling and decontamination line.
- By the end of each workday, culling and disposal team members shall dump all the used PPE and other infectious materials.
- All shall scrub and disinfect shoes, thoroughly wash hands at the wash station, and sanitize hands.
- All tools and other equipment must be cleaned and disinfected before being brought across the culling and decontamination line.
- All personnel must disinfect their foot by dipping in a footbath before leaving the place.
- All parts of vehicles (especially tyres) must be disinfected at the culling and decontamination line.
- Once the personnel protective equipment has been removed, designated personnel must disinfect personal footwear.
- Personnel must not re-enter the infected premises without following the requirement for entering the infected premises.





## 13.6 SOP for Decontamination

### Purpose

This SOP is to ensure that all decontamination procedures are carried out successfully, post-disease outbreak. Decontamination means the removal or neutralization of infectious agents (ASF virus) through the process of cleaning and disinfection. The purpose of decontamination is to ensure that the live ASF virus does not remain and re-emerge on the premises after depopulation.

**Users/Target:** Decontamination team

### Composition of the team

- Supervisor: Regulatory and Quarantine Officer (Veterinarian).
- Assistants: BAFRA Livestock Inspectors (one in each decontamination group).
- Hired and trained personnel for cleaning and disinfection

### Materials and Equipment Required

#### ➤ *Personal Protective Equipment (PPE)*

- A coverall.
- Face mask.
- Goggles (chemical splash).
- Outer gloves – (Nitrile).
- Inner gloves – (Vinyl, 4).
- Shoe covers.
- A plastic apron.
- Rubber boots.
- Multiple sets of PPEs will be necessary to allow workers to take breaks.

All the 3-D members involved in the operation should use gumboots and thorough disinfection while moving from one house to the other.

#### ➤ *Disinfectants*

The table below shows different disinfectants effective against ASFV.

**Table 5: Disinfectants effective against ASFV**

<b>Product name</b>	<b>Active ingredient(s)</b>	<b>Use site(s)</b>
Virkon S ®	Sodium chloride  Potassium peroxymonosulfate	Animal feeding/watering equipment, livestock barns, pens, stalls, stables, equipment, hog farrowing pen premises, hog barns/houses/ parlours/pens, animal quarters, animal-



		transportation vehicles, agricultural premises, agricultural equipment, and human footwear.
Pheno Cen Germicidal Detergent	o-Phenylphenol, potassium salt p-tert-Amylphenol, potassium salt Potassium 2-benzyl-4- Chlorophenate	ASFV in livestock pens, manure, equipment (livestock, feeding and watering, farm), hog farrowing house, hog houses, animal quarters, and shoe baths.
Low pH Phenolic 256	o-Phenylphenol 2-Benzyl-4-chlorophenol	ASFV in livestock premises, equipment (feeding and watering, livestock, animal), livestock/animal transportation vehicles, hog farrowing houses,  hog barns/houses/parlours/ pens, farrowing equipment, and shoe baths.

➤ **Decontamination supplies**

- Hand-operated and power sprayer to dispense disinfectants.
- Water tanks (500 litres).
- Rake lawn and Rake gravel.
- Barrier/security line tape.
- Shovel – long handle.
- Scoop shovel.
- Wheelbarrow.
- Trash containers.
- Roll duct tape.
- Ropes.
- Regular brooms, whisk brooms and dustpans.
- Scissors.
- Rolls of paper towels.
- Alcohol wipes.
- Heavy tie-down straps.
- Box of large and small plastic bags.
- Plastic tie-downs.
- Footbath with tray and mat.
- Sharp's container.

➤ **Other supplies required:**

- Maintenance tools (screwdrivers flat and Phillips, hammer, adjustable wrench, crowbar, and scrapers).



- Masking tape.
- ***Personal cleaning and disinfection supplies***
  - A scrub brush for removing dirt and other particles before using disinfectants.
  - Soap to wash legs, hands and face.
  - A plastic basin to create a foot bath.
- ***Biohazard control materials***
  - Alcohol pads, 70% ethanol - these are generally used to wipe hands after removing PPE.
  - A red biohazard bag for placing used PPE.
  - A container with a sprayer nozzle.
  - Antiseptic wipes/sanitisers
  - Eyewash.
  - First-aid Kit.
  - Flashlight.

### **General consideration**

This should be a detailed property assessment starting with making a map and marking the location of electrical and water lines, drains, and effluent runoff.

Cleaning and disinfection activities of infected premises should be limited to areas inhabited by or exposed to pigs. In scavenging pigs, all the surrounding premises of the house including the kitchen garden where the pigs have scavenged should be disinfected adequately.

Materials to be disinfected fall into three categories:

- a. Structures: Pigsty
- b. Clutter: Items that are not structured for housing pigs and require judgment as to whether they can be cleaned and disinfected effectively or must be discarded
- c. Trash: Items that impede the cleaning process should be discarded.

### **Decontamination procedures**

#### ***A. Preparation for decontamination***

- Identify and establish a proper site outside and close to the periphery of the culling and decontamination line for putting on PPE, and unloading materials and equipment required for decontamination. Where the infected area is accessible by road, a decontamination crew vehicle shall be parked at this site.
- Before entering the infected premises, assemble the team and organize into groups as per the specific tasks to be performed in an orderly manner and



distribute the materials and equipment to each member. The team leader shall then provide a necessary briefing to all decontamination groups.

- Put on PPE before crossing the culling and decontamination line (protected zone).
- The decontamination team shall be divided into groups – the first group should start decontamination in the infected farms and other groups shall start decontamination from the periphery of protected zones and move towards the centre of the infected area.
- Once personnel have entered premises, they must not cross back over the culling and decontamination line for any reason, without removing and proper disposal of all PPE and personal disinfection.
- Groups identified for decontamination of the infected farms shall only come out after completing their task.
- The decontamination team should allow the culling and disposal team to complete their task and then only start their operation.
- It is important to wear PPE when mixing disinfectants because it can irritate the skin and eyes.

### ***B. Preliminary disinfection***

It is designed to quickly start and rapidly reduce the amount of virus present on the premises.

Any area known or suspected to be contaminated is sprayed.

The important area, structures, materials and equipment for cleaning and disinfection *inter alia* include:

- Pigsties and the surroundings.
- Feed storage area.
- Culling sites.
- Disposal sites.
- Processing facilities.
- Watering and feeding troughs.
- Access roadways and pathways used for moving pigs and pig products including other risk goods (fomites).
- Vehicles.
- Spraying should be repeated up to 5 times a day. Disinfection with Virkon® S is considered very effective against ASFv.

### ***C. Clean-up***

- The aim is to remove, without using water, all manure, debris, feed, etc., to expose surfaces for second-round disinfection. This is very important as organic material reduces any disinfectant effectiveness.



- All structural surfaces must be cleaned of any litter, dirt, or other contaminated materials.
- The next step is a wash down with a low-pressure sprayer using a detergent or bleaching powder.
- Fences should be thoroughly cleaned and disinfected.
- If the facility has significant evidence of rodent activity, extermination should be done before starting the cleaning and disinfection effort.

#### ***D. Full-scale disinfection***

- Disinfectant to be sprayed in the following order:
  - Roofing → walls and → finally the floor.
- Inspection must be carried out to ensure that everything has been satisfactorily cleaned up.
- Another round of full disinfection shall be carried out one week later.
- Final disinfection before restocking should be done.

#### **Decontamination of equipment used for decontamination.**

- The primary purpose would be to disinfect anything used during stamping out. This would include items like excavators, backhoes, torches, etc.
- Apply the same principles including cleaning first followed by a low-pressure detergent spray, inspection then disinfection spray. Repeat the inspection and disinfectant spray.
- If any trucks, vehicles, motorcycles, etc. are on the contaminated site, they must be decontaminated before leaving the premise.
- Particular attention needs to be paid to mats under the driver's feet.
- Vehicle interiors, including trunks, can be wiped down with clothes soaked in disinfectants.
- All underparts and wheels of cars should be sprayed with water and disinfectant.

#### **Personal decontamination**

The following procedures will apply to ALL personnel before leaving an infected area.

- Culling and disposal team members walk to the cleaning and disinfection line and remove PPE and place them in a trash bag, which is to be placed in a biohazard plastic bag.
- Hands must be scrubbed and washed.
- Warm soapy water is recommended for washing face, hair, skin, etc. Alternatively, the pH of the washing solution can be raised (by adding sodium carbonate) or lowered (by adding citric acid) to enhance antiviral action.
- Hair should be washed/sponged down with shampoo.
- Boots and shoes should be scrubbed down; particular attention being paid to the sole.



- The person then walks across the area, washes feet in a footbath, changes into clean overalls and street shoes, and leaves directly without re-exposure to contaminated areas.
- The plastic bags containing used overalls and other articles are sealed and given a second wash down in disinfectant and then either buried or burnt.
- On returning to home or lodgings, the person should have a long hot bath or shower.



### 13.7 Protocol for Mixing Virkon S ®

Precautions to be taken while mixing:

- Safety or protective gear is required when mixing Virkon S ®.
- Assigned individuals must wear a face shield or safety goggles, a dust mask, and rubber gloves.
- Mix the solution in a separate, well-ventilated room (if possible), or outside.
- Restrict the number of people in the mixing area. Follow the requirements for handling and storage of disinfectant.

#### Equipment and Supplies Needed

##### **A. Safety equipment needed:**

- Face shield or safety goggles.
- Rubber gloves.
- Coveralls.
- Dust mask.

##### **B. Supplies needed:**

- 1.0, 2.5, or 5.0-gallon plastic container with locking lid.
- Funnel.
- Plastic measuring spoon or scoop (included with the Virkon S ®).

#### Procedure

- Reseal the container holding Virkon S ® powder.
- Pour Virkon S ® solution into the 1.0, 2.5, or 5.0-gallon plastic container using a funnel. Close container tightly.
- Dispose of the solution after seven days or when it begins to change from yellow to clear.
- Wash hands and any other areas where the solution or powder may have come in contact with the skin. Clean the mixing area.

#### Handling Virkon S ® Disinfectant

- Store powder tightly in a closed plastic container in a cool, dry place. Ensure that the area where Virkon S ® is stored is secured and cannot be accessed by unauthorized persons.
- Follow instructions on the label for application and disposal.



## 13.8 SOP for sample collection for ASF diagnosis

### Purpose

The purpose of this document is to describe the procedure for sample collection, storage and shipment of samples to the National Veterinary laboratory for the detection of ASFv.

### Scope

This procedure can be applied in any kind of sample collection from porcine for the detection of viral disease by PCR and histopathology.

### Equipment and Materials

- EDTA vacutainers for whole blood for PCR.
- Sterile HP container.
- Sterile 50ml plastic tubes.
- Sterile syringe and needles.
- Cold/Ice packs for transport.
- 10% buffered formalin- histopathological samples only.
- Cool Box.
- Post-mortem set.
- Viral transport medium.
- Biohazard bags.
- Personal protective equipment.
- Sodium/calcium hypochlorite (Bleach): 0.03% - 0.5% active chlorine.

### Procedure for sample collection and storage

#### *Molecular*

Where ASF is suspected, the following samples should be sent to the laboratory:

- Blood – shall be collected from ear vein or cranial vena cava (non-surgical) in EDTA anticoagulant tube.
- Tissues – spleen, lymph nodes, lung, tonsil kidney and bone marrow.

The fresh tissues should be kept as cold as possible, without freezing, and should be shipped to the nearest laboratory at the earliest. After the samples arrive at the laboratory, they should be stored at  $-70^{\circ}\text{C}$  if the processing is going to be delayed.

#### *Histopathology*

Blocks of tissue not more than 0.5 cm thick and 1–2 cm long are cut and placed in neutral buffered 4–10% formalin, which should be at least ten times the volume of the tissue sample. Store and pack formalin-fixed tissues separately from fresh tissues, blood, and smears. Care should be taken to ensure that formalin-fixed tissues are not frozen.





## Shipment of samples

### *Sample Information*

Information and case history should always accompany the samples to the laboratory and should be placed in a plastic envelope on the outside of the shipping container. The sample submission form (See Annexure 13.10) should be filled out and submitted to the receiving laboratory along with the samples.

### *Sample packaging*

The recommended procedure for packing samples is as follows:

- Put the samples in a primary container with screw caps and wrap them with paraffin film or adhesive tape individually to prevent leakage of fluid. The wrapping of primary containers should be carried out in clean surroundings. Put the primary container into a watertight, spill-proof secondary container with absorbent cotton wool sufficient to absorb the entire contents of the primary container (in cases of leakage).
- Place the secondary container in an outer container. This should be a polystyrene foam box covered with a hard box or other appropriate containers (E.g., coolbox).
- It is recommended that a freezer box/ice packs are put outside the secondary packaging to ensure that all materials are kept cool and not frozen during shipment. These packs should be pre-frozen at – 20 degrees centigrade before packaging.

### *Transportation of specimens*

The specimens should be forwarded to the laboratory by the fastest method available. If they can reach the laboratory within 48 hours, samples should be sent refrigerated.



## 13.9 SOP for detection of ASFv by Real-time PCR

### Introduction

African swine fever virus (ASFV) is the causative agent of African swine fever (ASF). ASFV is the only known virus with a double-stranded DNA genome transmitted by arthropods. The virus causes a lethal haemorrhagic disease in domestic pigs. Some isolates can cause the death of animals as quickly as a week after infection. In all other species, the virus causes no obvious disease. ASFV is endemic to sub-Saharan Africa and exists in the wild through a cycle of infection between ticks and wild pigs, bush pigs, and warthogs.

### Purpose

The purpose of this procedure is to rapidly detect the specific presence of African Swine Fever Virus (ASFV) DNA in porcine clinical material by the real-time polymerase chain reaction (PCR) technique using King et. al 2003, procedure.

### Scope

This procedure can be applied in any kind of porcine clinical sample such as EDTA-blood, serum and tissue homogenates and in cell culture supernatants. It is particularly useful for identifying ASFV DNA in porcine tissues that are unsuitable for virus isolation or antigen detection, because they have undergone putrefaction, or when there is good reason to believe that virus may have been inactivated before samples are received in the laboratory. PCR technique is highly sensitive, and its detection limit is below one infectious viral particle.

### Test principles

Polymerase chain reaction (PCR) is a molecular genetic technique that allows the specific detection of ASFV DNA by enzyme-based amplification of a short viral genome fragment defined by a specific primer set. Under controlled conditions, multiple copies of DNA are generated by the action of the DNA polymerase enzyme, which adds complimentary deoxynucleotides (dNTPs) to a piece of DNA known as the "template". Real-time PCR is an advanced amplification method, which allows the automated detection of the amplified product, reducing the risk of carry-over contamination with increased specificity and in most cases, even sensitivity. The PCR method requires the first step of viral DNA extraction from the original material to be analysed, which will be the template for the PCR. In real-time PCR, the appearance of the amplified product is monitored continuously, in special equipment, with the incorporation in the reaction mix of a fluorescent dye that will give a fluorescence signal in a proportional way to the amplicon accumulation. By determination of fluorescence signal intensity in each amplification cycle, a sigmoid-shaped curve, that represents the amplicon appearance along with the PCR, will be obtained. The



described ASFV real-time PCR method uses a primer set and a specific TaqMan probe directed to a highly conserved region of the viral genome, VP72, which ensures the detection of a wide range of ASFV isolates, belonging to all the 24 known virus genotypes. The primers amplify a DNA fragment of 250 bp, from nucleotide position 2041 to 2290 of the complete VP72 gene sequence of the reference strain BA71V (GenBank accession no. ASU18466). TaqMan probe employed for amplified product detection is labelled with a reporter at 5' end [6-carboxy-fluorescein (FAM)] and a quencher at 3' end [6-carboxy-tetramethyl-rhodamine (TAMRA)]. PCR is a rapid method, which can be performed in less than four hours, and is highly sensitive, allowing viral detection even before the appearance of clinical symptoms.

## **Equipment and Materials**

### ***Equipment***

- QuantStudio-5/real time PCR machine.
- MINI spin/ microcentrifuge for Eppendorf tubes.
- Heating block/water bath.
- Freezers -20°C.
- Freezer -80°C.
- Fridge 2-8°C.
- Vortex.
- Bio-Safety Cabinet, Class – II.

### ***Materials***

- Single-channel pipette 1-10µl.
- Single-channel pipette 2-20µl.
- Single-channel pipette 20-200µl.
- Single-channel pipette 100-1000µl.
- Micropipette tips of 1-200 and 200-1000 µl, sterile.
- Micropipette tips with the aerosol resistant filter of 1-10, 2-20, 20-200 and 100-1000 µl, sterile.
- Microcentrifuge tubes of volumes 0.2, 0.5, 1.5, and 2 ml, sterile.
- DNA extraction kit, Qiagen
- Ethanol 100%, Merck
- Forward primer ASFV 5'- CTG CTC ATG GTA TCA ATC TTA TCG A -3'
- Reverse primer ASFV 5- GAT ACC ACA AGA TCR GCC GT - 3'
- Probe ASFV 5' FAM-CCA CGG GAG GAA TAC CAA CCC AGT G-TAMRA
- AgPath-ID, One-Step RT-PCR Reagents, Catalogue number: 4387391
- Distilled H<sub>2</sub>O, sterile, PCR grade.
- Positive control; Known diluted ASF sample.
- negative controls: Nuclease free water.
- Latex or nitrile gloves.
- Biohazard bag.



## Procedure

### *Extraction of DNA (Template DNA)*

- Isolate a suitable piece of tissue and place it in a UV-crosslinked 1.5mL tube.
- Add 180 ml Buffer ATL and 20ml Proteinase K and vortex.
- Place in the 55oC incubator for 3 hours or overnight.
- Remove from incubator, vortex, add 200ml Buffer AL and vortex.
- Place in a heat block at 70oC for 10 minutes.
- Add 200ml 100% Ethanol and transfer the entire volume onto the spin column.
- Centrifuge at 8000 rpm for 1 minute; discard flow-through.
- Add 500ml Buffer AW1 and centrifuge at 8000 rpm for 1 minute, discard flow-through.
- Add 500ml Buffer AW2 and centrifuge at 13000 rpm for 3 minutes, discard flow through.
- Place spin column on UV-crosslinked 1.5mL tube, add 200ml buffer AE. Let sit for 1 minute, then centrifuge at 8000 rpm for 1 minute. Repeat and then combine flowthroughs for a total volume of 400ml.
- Store the extracted DNA at 4oC for immediate use, otherwise at -80oC for the long term.

**Note:** The RNA extraction kit from Qiagen can be used for DNA extraction as well.

### *DNA amplification*

Master mix preparation: In a sterile 1.5 ml Microcentrifuge tube, prepare the PCR reaction mixtures described below for the number of samples to be assayed.

Pipetting steps	Master mix reagents	1x volume (reaction 25ul)
1	Nuclease-Free Water	4.5
2	2X RT-PCR Buffer (Ambion P/N AM1005)	12.5
3	FAM-TAMARA PP MIX (ASFV Risatti PPMIX)	2
4	25X RT-PCR Enzyme MIX	1
Total Volume		20

Add 20 µl of the PCR reaction mix to the required number of 0.2 ml optical PCR tubes including the positive controls and the negative controls, adding at least one additional sample to minimize pipetting mistakes.



### ***Sample addition***

- Add 5µl of DNA template to each PCR tube. Include positive control (5 µl of ASFV DNA) and negative control (5 µl of nuclease-free water).
- After the addition of the template, close the reaction tube and spin down the PCR mix.
- Place all tubes in an automated real-time thermocycler.
- Run the incubation program detailed below.

### ***PCR cycle condition***

- 1X 45°C 10 min, 95°C 10 min
- 45X 95°C 15 sec, 60°C 45 sec
- Program the fluorescence collection in the FAM channel and quencher as TAMRA.

### ***Analysis and interpretation of results***

Interpretation of the results: In a positive sample, a sigmoid-shaped amplification curve will be obtained, indicating the cycles number versus reading fluorescence level, where the Ct value will be under 40. A negative sample will maintain the fluorescence profile under the background fluorescence level and the equipment will not report any Ct value. Therefore, a negative sample will show a Ct value  $\geq 40$ .

### ***Critical points***

Because PCR is a highly sensitive technique, the most critical point along all the analysis procedures is the considerable risk of carry-over contamination, and the false-positive results that could be obtained in this situation. The contamination could be due to the ASFV itself present in the positive analysed samples or the positive controls included in the DNA extraction procedure; also, it could be due to ASFV DNA obtained after amplification of a previous PCR. Personnel working on PCR must follow and carry out some strict work rules to minimize the contamination risk associated with the PCR technique:

- All steps of sample analysis by PCR should be performed in separate locations, using equipment and material specifications for each one: sample preparation, DNA extraction, PCR mix preparation, and removal of PCR products.
- Personnel must work always with clean nitrile or latex gloves in the PCR laboratory.
- Change of gloves whenever personnel go into a different PCR area.
- Tubes containing amplified products should never be opened and manipulated in another laboratory distinct to that exclusively assigned to their analysis by electrophoresis, where they will be discarded.

**Waste disposal** - All the wastes should be discarded after being autoclaved.



### 13.10 Sample submission form

Sl. No.	Sender Details		Farm Details								Animal Details							Sample Details						
	Agency	Name/Designation (Ph. No)	Owner name	Farm type	Farm location (GPS)	Farm size	Domesticated/wild Pigs	Village	Gewog	District	Contact details	Species	Age	Sex	Breed	Health status (Sick/dead/Normal)	Clinical History	Treatment details	Sample ID	Collection date	Type of sample	Pooled sample	Transport media / preservatives	Test requested



## 13.11 Guideline for Compensation

### Background

Early detection and reporting, as well as rapid response to an outbreak of ASF, depend critically on the incentives for pig owners to quickly report any sick and at-risk pigs to the veterinary authority. In confirmed cases of ASF, the government decides on mandatory culling. Without adequate compensation arrangements in place, pig owners will have no incentive to report any sick and at-risk pigs that may result in the loss of all the herds. Therefore, it is essential to establish a fund within the government to compensate the affected pig owners during mandatory culling.

The guideline intends to provide information on the operational aspects of the compensation fund to ensure quick and fair financial compensation to the affected pig owners in the event of an enforced culling during the outbreak of ASF. *The Livestock Act of Bhutan 2001 under its sub-section 9.3 clearly states that the government has the authority to compulsorily destroy animals, animal products or feeds, or any consignments that it considers to be risky and pays compensation as prescribed by the Ministry.*

### Objectives

The main objective of this guideline is to outline the operation and payment procedures for compensation modalities in the event of an ASF outbreak in the country.

### Compensation Committee

The committee will be composed of the following members:

#### *Dzongkhag level:*

- Dzongdag/Gup / or a representative – Chairperson
- DLO/Gewog Extension Officer – Member Secretary
- BAFRA RQO/RQI Livestock– Member
- A representative from IOC – Member

#### *Thromde level:*

- Thrompon/ Thromde Theumi/Representative – Chairman
- DLO/Gewog Extension Officer – Member Secretary
- BAFRA RQO/RQI Livestock– Member
- A representative from IOC – Member



### **Determination of compensation rate**

The Technical Working Group for ASF in consultation with the Livestock Production Division, DoL and the IOC will determine and fix the compensation rate. The following criteria will be assessed for the valuation of the compensation rate.

1. Age
2. Body weight
3. Market value
4. Production stage
5. Cost of production

### **Eligibility for Compensation**

Compensation payments will be made only:

- if mandatory culling measures have been announced and put into effect by the NICC.
- for pigs culled, feed and feed materials disposed of and destroyed under the supervision of the 3-D team (Depopulation, Disposal, and Decontamination); based on completion of all documentation as prescribed in this guideline;
- if the farm is registered with the government and meets the minimum farm biosecurity standards.

Compensation will not be paid for:

- pigs that have died of ASF outside the timeframe of 3-D implementation
- pigs that have died because of diseases other than ASF
- state-owned piggery farms
- pigs with conflicting ownership status, as in the case of stray or uncontrolled pigs
- farms not registered with the government and don't meet the minimum farm biosecurity standards

### **Disbursement mechanisms of the compensation fund**

The NICC shall approve the compensation payments as and when proposed by the Compensation Committee through the Incident Operation Centre.

The IOC under directives from the NICC will implement the compensation procedures. A committee will be instituted to implement the actual compensation calculation and payments of compensation as approved by the IOC. For the commercial farms although the same process will be followed the payment will be released only after approval from the NICC.





The NICC shall approve the compensation payments as and when proposed by the compensation committee through the Incident Operation Centre (IOC) and should make payment to the affected owners within one week of culling.

### **Roles and responsibilities of the compensation committee**

The main role of the compensation committee is to ensure and facilitate the payment of compensation in a fair, transparent and timely manner to the affected household/owners.

- Verify and approve the list of pigs and other products eligible for compensation in the villages/farms.
- To make payments to the eligible farmers in a fair, transparent, and timely manner.
- Compile daily records of the culled pigs, properties/ materials destroyed and owner details (address, main occupation, etc.).
- Review the market value of the pigs and propose the compensation rates.
- Get proof of payment from the recipient of the compensation.
- Submit completed documents to the IOC for payments made.

### **Roles and responsibilities of IOC for compensation**

Concerning compensation, the IOC will be responsible for the following tasks:

- Call for an immediate meeting of the compensation committee in line with the provisions of the NASFPCP.
- Create awareness among the owners on the compensation available for the mandatory culling of pigs before the actual start of the 3-D operation.
- Review and approve the compensation rates proposed by the committee for further submission to the TWG, where the further review will be undertaken before submission to the NICC for final approval.
- Maintain proper books of accounts for all compensation made for future auditing.
- Follow up with NICC for the timely release of the compensation fund for further disbursements.
- Compensation will be paid as soon as funds are received from the Ministry of Finance through the concerned RLDC.

### **Compensation procedures**

Upon official declaration of an outbreak of ASF, the NICC will activate IOC at the outbreak area. The IOC will carry out disease outbreak investigation, 3-D Operations, movement control, surveillance, etc. through concerned RRTs in the infection and protection zones, while the compensation committee will initiate the processing of payment of compensation to the eligible owners.



### **Mode of payment**

The compensation committee will collect copy of slips (forms) from the 3-D team. The details in the slip should be entered in the summary culling record and individual herd owner forms (designed by IOC). The owner should produce slip to the compensation committee and payments will be processed after cross-checking by the committee. The committee will process the payment using two forms and submit completed documents to the RLDC. Payment to the eligible owner shall be done electronically wherever possible.





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