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ROYAL GOVERNMENT OF BHUTAN
Ministry of Agriculture and Forests
Thimphu : Bhutan

SECRETARY

SEC/MOAF/7/ 223

11th October 2018

NOTIFICATION

In accordance with the provisions prescribed in Chapter II, Sections 4.1, 4.2 and 5.5 and Chapter IV, Section 8.2 of the Livestock Act of Bhutan 2001 and Chapter III, Sections 32 to 38 of the Livestock Rules and Regulations of Bhutan 2017, the Ministry of Agriculture and Forests developed Bhutan Health Code for Import of Animals to prevent introduction of animal diseases into the country. The Bhutan Health Code for Import of Animals shall apply to all those persons or Parties responsible for importing animals for personal or commercial purpose. This code shall be applied in conjunction with Chapter III and other relevant Chapters and Sections of Livestock Rules and Regulations of Bhutan 2017, relevant guidelines, and specific disease prevention and control plans.

The concerned agencies as mentioned in this Code shall be responsible for facilitating import of animals in the country.

(Rinzin Dorji)
SECRETARY

Copy to:

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2. Hon'ble Secretary, Ministry of Home and Cultural Affairs, Thimphu
3. Hon'ble Secretary, Ministry of Economic Affairs, Thimphu
4. Hon'ble Secretary, Ministry of Health, Thimphu
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BHUTAN HEALTH CODE FOR IMPORT OF ANIMALS

2018

Royal Government of Bhutan
Ministry of Agriculture and Forests

Prepared jointly by:

Department of Livestock
and
Bhutan Agriculture and Food Regulatory Authority (BAFRA)

Table of Contents

1. Background

The Bhutan Health Code for Import of Animals developed in accordance with the provisions prescribed in Chapter II, Sections 4.1, 4.2 and 5.5 and Chapter IV, Section 8.2 of the Livestock Act of Bhutan 2001 and Chapter III, Sections 32 to 38 of the Livestock Rules and Regulations 2017. The Animal Health Code shall apply to all those persons or Tshogpa responsible for importing animals for personal or commercial purpose. This code shall be applied in conjunction with Chapter III and other relevant Chapters and Sections of Livestock Rules and Regulations 2017, relevant guidelines, and specific disease prevention and control plans.

Growing import of livestock products to the tune of Nu. 2.3 billion (2015) mainly because of inability to produce enough locally has resulted in balance of payment deficits. This has prompted the Royal Government of Bhutan (RGoB) to shift development paradigm and to achieve greater self-sufficiency in livestock products while boosting the economic growth.

The new development model emphasizes on accelerated livestock development, providing economic stimulus support to encourage modernization of dairy, piggery, fishery, poultry and goat production thereby transforming conventional subsistence livestock farming to a commercial venture. In our quest for accelerated economic growth through enhanced livestock production to substitute imports, the Department of Livestock, has been importing large number of animals including dairy cattle, pigs, poultry and goats. For instance, about 10000 live cattle were imported during the 10^{th} and 11^{th} FYP and this poses huge risks for incursion of Transboundary Animal Diseases (TADs) and other animal diseases into the country. Historically, exotic animal diseases such as porcine respiratory and reproductive syndrome (PRRS) in pigs, foot-rot in sheep, Peste des Petits Ruminants (PPR) in goat and Brucellosis in cattle have been introduced into the country through importation of live animals. Due to the geopolitical location of our country, and being primarily an importing country, Bhutan is at very high risk of introduction of exotic diseases unless the animal health services is well prepared. It requires huge amount of resources, both financial and manpower, to manage such diseases once the diseases becomes endemic in the country. Unlike other developed/developing countries, drastic measures such as elimination of infected animals is rarely done due to socio-religious and economic reasons.

Bhutan is endemic to some traditional livestock and poultry diseases. Diseases such as Foot and Mouth Disease (FMD), Rabies, Classical Swine Fever (CSF), Porcine Reproductive and Respiratory Syndrome (PRRS), Haemorrhagic Septicemia (HS), Black Quarter (BQ), Anthrax, Brucellosis, Peste Des Petits Ruminants (PPR) Newcastle Disease (NCD), Infectious Bursal Disease (IBD), Mareks disease (MD) and Highly Pathogenic Avian Influenza (HPAI) are included in the "notifiable disease" list of the Livestock Rules and Regulations of Bhutan, 2017. This list will be updated from time to time based on the emerging needs. Some of these diseases (e.g. FMD, Rabies, BQ, Anthrax, HS, Newcastle disease) are reported frequently in the country.

In addition, other production related diseases that are of importance in dairy sector are Infectious Bovine Rhinotracheitis (IBR), Bovine Brucellosis, Bovine Tuberculosis, Para tuberculosis, Bovine Viral Diarrhea and Enzootic Bovine Leukosis; PRRS in piggery; Fowl Pox and Avian Leucosis Complex (ALC) in poultry. Bhutan is located in South Asia which is the "hub" of emerging and re-emerging infectious diseases and the country being largely an importer of live animals, the risks of disease incursion through live animal imports is real.

Therefore, it is important to develop health code for import of animals so that all animals entering the country are screened against important diseases and necessary measures are put into place by respective authorities to prevent introduction or re-introduction of the animal diseases. The code describes the minimum requirements on animal health that all imported animals must comply with before being allowed entry into the country.

2. Considerations for import of live animals

2.1 Considerations before the import of animals

Following are the important considerations that need to be looked into before deciding to import live animals.

2.1.1 Disease Situation in exporting countries

The disease situation in the exporting countries can be known through information available on OIE's website through the online database called as WAHIS. This is based on the official declaration made by countries on the status of animal diseases through routine reporting made to the OIE. Other sources such as information shared by the FAO, WHO and international reference laboratories can also be referred to.

Among several animal diseases, the following diseases have been found to be of important risk to Bhutan based on the prevalence in the exporting countries.

1. **Bacterial:** bovine tuberculosis, paratuberculosis, brucellosis, anthrax, HS, campylobacteriosis and contagious caprine pleuropneumonia
2. **Viral:** FMD, IBR, Crimean Congo Haemorrhagic Fever, HPAI H5N1, Peste Des Petits Ruminants, Blue Tongue, Classical Swine Fever, PRRS
3. **Protozoan:** babesiosis, anaplasmosis, theileriosis, trichomonosis and trypanosomiasis.
4. **Endoparasite:** nematodes, cestodes and trematodes of major significance.
5. **Ectoparasite:** ticks and lice.

2.1.2 Cattle disease, infection and infestation in India

Following are potential pathogens and parasites of cattle in India. The importers/procurement teams are expected to recognize these diseases, infection and infestation. These are the diseases prevalent in India as per the updated OIE list 2016. Imported animal bringing these diseases and infection into the country must be avoided at all cost.

1. **Bacterial:** bovine tuberculosis, paratuberculosis, brucellosis, anthrax, HS and campylobacteriosis, contagious caprine pleuropneumonia,
2. **Viral:** FMD, IBR, Crimean Congo Haemorrhagic Fever, HPAI H5N1, Peste Des Petits Ruminants, Blue Tongue, Classical Swine Fever, PRRS
3. **Protozoan:** babesiosis, anaplasmosis, theileriosis, trichomonosis and trypanosomiasis.
4. **Endoparasite**: nematodes, cestodes and trematodes of major significance.
5. **Ectoparasite:** ticks and lice.

2.1.3 Veterinary Services in the exporting countries

It is also necessary to review the quality of veterinary services in the exporting countries in order to have confidence and reliability on the veterinary certificates issued by the veterinary authority of that country. Reports on the performance of veterinary services (PVS) conducted by the OIE (if available) may be referred to understand the quality of veterinary services of the exporter country (s).

2.1.4 OIE's standards

The health measures given in the OIE's *Terrestrial Animal Health Code* and *Aquatic Animal Health Code* should be referred to ensure compliance to minimum requirements for early detection of any pathogens/diseases in the imported animals.

2.1.5 Documentations

All necessary documents that provide crucial information on the animal health status of the imported animals should be made available in order for the regulatory authorities in an importing country to consider import of animals. The importers should seek information on the following documents from the veterinary authority of the exporting country/state:

2.1 6 Disease prevalence record

The importer should seek information on disease prevalence, if any, in the animal population at the source, locality and region. This information should provide the probability of purchased animals being infected or free based on the prevalence of particular disease.

2.1.7 Vaccination record

The veterinary authority of the exporting country should provide information on the types of vaccines used and the vaccination schedule followed for important diseases.

2.1.8 Records of recent disease outbreak

An assessment of written evidence of recent outbreaks among the herd needs to be performed. The record should include the name of the disease, time of outbreak and control strategy implemented.

2.1.9 Health certification

An official veterinary certificate certifying the disease-free status of the animals at the time of importation should accompany each batch or individual animal imported. In addition, an official veterinarian must also certify that animals were examined and were free of clinical signs of diseases and that animals are fit for travel. The animals must have been examined not more than 48 hours before the scheduled date of shipment.

2.1.10 Animal identification

All animals need to be permanently identified using appropriate identification methods and all the documentation regarding the particular animal should be linked with the identification number.

2.2 Considerations during the import of animals

The procurement team/importer shall ensure;

1. Thorough disinfection of lorries/trucks before loading the animals.
2. The animals should be thoroughly cleaned prior to shipment.
3. The animal should be cleaned again on arrival at the quarantine.

3. Bovine Diseases

3.1 Infectious Bovine Rhinotracheitis (IBR)/ Infectious Pustular-vulvovaginitis (IPV)

IBR is a highly infectious disease caused by *Bovine Herpes Virus-1* (BoHV-1). The disease is characterised by clinical signs of the upper respiratory tract, such as a (muco) purulent nasal discharge, hyperaemia of the muzzle (red nose disease) and by conjunctivitis. Signs of general illness are fever, depression, in appetence, abortions and reduced milk yield. The virus can also infect the genital tract and cause pustular vulvovaginitis and balanoposthitis. The virus can be isolated from nasal swabs or genital swabs, from animals with vulvovaginitis or balanoposthitis, taken during the acute phase of the infection, and from various organs collected at post-mortem. Antibodies can be detected with an Enzyme-Linked Immuno-Sorbent Assays (ELISA) in the serum.

The disease was reported (detected for the first time in 1997) to be highly prevalent in the bovine population in Bhutan. Although, no clinical cases have been documented, sero-surveillance carried out in cattle, yaks and mithuns in 20 Dzongkhags over the years (1995-1998) revealed the overall prevalence of 33% (404/1224) in the survey group. A sero-prevalance study on all the eight government cattle breeding farm during 2015-16 revealed as high as 37.6%.

3.1.1 Import conditions:

A health certificate from veterinary authority is required attesting that:

1. The animals have originated from an area where no clinical signs or other evidence of IBR/IPV during the past 12 (twelve) months prior to export.
2. The animals showed no clinical signs of IBR/IPV within 48 hours before shipment;
3. The animals were permanently identified and the identification numbers of animals are stated in the certificate.

3.1.2 Procedures to be followed on arrival at the quarantine station:

1. If the animal shows clinical signs or abortions due to IBR/IPV during quarantine period, the animals shall be rejected.
2. In contact animals should also be tested with ELISA and rejected if positive.

3.1.3 After release into the respective farms:

1. Animals should be kept under observation until one pregnancy for signs of abortion or any signs of IBR/IPV.
2. In case of any abortions, it should be reported to the nearest DOL or BAFRA office.
3. Following observation until one pregnancy a regular (routine) disease surveillance and sampling procedures shall apply.

3.1.4 Import conditions for frozen semen:

Import of frozen semen requires a certificate from veterinary authority attesting that:

1. The donor animals were kept in an IBR/IPV free herd at the time of collection of the semen; or
2. The donor animals were kept in isolation during the period of collection and for the 30 days following collection and were subjected to a diagnostic test for IBR/IPV on a blood sample taken at least 21 days after collection of the semen, with negative results; or
3. If the serological status of the bull is unknown or if the bull is serologically positive, an aliquot of each semen collection was subjected to a virus isolation test or PCR, performed in accordance with the Terrestrial Animal Diagnostic Manual of OIE, with negative results; and
4. The semen was collected, processed and stored in conformity with the provisions of OIE standards or Livestock Rules and Regulations.

3.2 Bovine Brucellosis

Bovine brucellosis is usually caused by *Brucella abortus*, less frequently by *B. melitensis*, and occasionally by *B. suis*. Clinically, the disease is characterized by one or more of the following signs: abortion, retained placenta, orchitis, epididymitis and, rarely, arthritis, with excretion of the organisms in uterine discharges and in milk. Diagnosis depends on the isolation of *Brucella* from aborted material, udder secretions or from tissues removed at post-mortem. Presumptive diagnosis can be made by assessing specific cell-mediated or serological responses to Brucella antigens.

B. abortus, B. melitensis and B. suis are highly pathogenic for humans, and all infected tissues, cultures and potentially contaminated materials must be handled under appropriate containment conditions.

Clinical cases of bovine brucellosis have not been detected in Bhutan. Sero-surveillance (using antibody capture ELISA) of bovines found only one positive case out of 20, 000 samples screened in the country in 1997-98 followed by few hundreds samples per annum thereafter. However, a sero-surveillance conducted in six government cattle breeding farm during 2015-16 revealed the overall prevalence of 13.75%. The clinical cases were detected at National Jersey Breeding Centre, Samtse and Calf Rearing Centre, Wangkha during 2016. Surprisingly, one confirmed Brucellosis clinical case was detected in July 2016 in an imported dairy cow in Radhi Geog under Trashigang Dzongkhag.

3.2.1 Import conditions:

For the importation of cattle for breeding or rearing (except castrated males), a health certificate from veterinary authority is required attesting that the animals:

1. Were kept in a herd in which no clinical sign of bovine brucellosis was officially reported during the twelve months prior to shipment;
2. Were subjected to buffered Brucella antigen tests for bovine brucellosis (antibody testing) with negative results not more than 30 days prior to shipment.
3. Were not vaccinated against brucellosis
4. Were permanently identified and the identification number stated in the certificate.
5. Showed no clinical sign within 48 hours prior to shipment.

3.2.2 Procedures to be followed on arrival at the quarantine station:

1. The animals should be screened with RBT and if detected positive, it should be further tested with ELISA using paired sera samples.
2. Clinically suspected animals also shall be tested and confirmed by ELISA.
3. Confirmed positive animals shall be culled/rejected for import. Further, in contact animals should also be tested and if detected positive should be rejected.

3.2.3 After release into the respective farms:

1. Animals should be kept under observation until one pregnancy for signs of abortion.
2. In case of abortion, it shall be reported to the nearest DOL or BAFRA office.
3. Following one pregnancy a regular disease surveillance procedure shall apply.

3.2.4 Import conditions for bovine semen:

Import of bovine semen into the country requires a certificate from veterinary authority attesting that:

1. The semen is from an artificial insemination centre and the animal/bull have been tested negative to brucella by serological tests.
2. The semen is not from an artificial insemination centre, however, the donor animals were kept in a country or zone free from bovine brucellosis; or
3. The animals were kept in a herd officially free from bovine brucellosis, showed no clinical sign of bovine brucellosis on the day of collection of the semen and were negative to brucella by serological tests within 30 days prior to collection.
4. The semen was collected, processed and stored in conformity with the provisions of Chapters OIE standards or Livestock Rules and Regulation of Bhutan 2017.

3.3 Bovine Tuberculosis

Bovine tuberculosis (TB) is a chronic disease of animals caused by a bacteria *Mycobacterium bovis*, which is closely related to the bacteria that cause human and avian tuberculosis. This disease can affect practically all mammals, causing a general state of illness, coughing and eventual death.

In live cattle, tuberculosis is usually diagnosed in the field with the tuberculin skin test. In this test, tuberculin is injected intradermal; a positive test is indicated by a delayed hypersensitivity reaction (swelling). The tuberculin test can be performed using bovine tuberculin alone, or as a comparative test that distinguishes reactions to *M. bovis* from reactions to environmental mycobacteria.

The disease has not been detected in the country so far.

3.3.1 Import conditions:

For the importation of bovines for breeding or rearing, a health certificate from veterinary authority is required attesting that the animals:

1. Originated from a herd free from bovine tuberculosis; or no clinical signs or evidence of TB during the past 12 months prior to shipment.
2. Were permanently identified and the identification number stated in the certificate.
3. Showed no clinical sign within 48 hours prior to shipment.

3.3.2 Procedures to be followed on arrival at the quarantine station:

1. Animals suspected of bovine TB in the quarantine stations shall be subjected to Single Intra-dermal Tests (SIT) using purified protein derivative tuberculin (bovine) or comparative intra-dermal test using avian tuberculin.
2. Confirmed positive animals should be culled/rejected for import.

3.3.3 After release into the respective farms:

1. Animals should be kept under observation for signs of bovine TB for one month.
2. Thereon, follow-up with regular disease surveillance procedures.

3.3.4 Import conditions for the semen of bovines:

A certificate from veterinary authority is required attesting that:

1. The donor animals showed no sign of bovine tuberculosis on the day of collection of the semen and either:
    a. Were kept in an artificial insemination centre free from bovine tuberculosis in a country, zone or compartment free from bovine tuberculosis.
    b. Showed negative results to tuberculin tests carried out 30 days prior to shipment and were kept in a herd free from bovine tuberculosis;
2. The semen was collected, processed and stored in conformity with the provisions of OIE standards or Livestock Rules and Regulations of Bhutan, 2017.

3.4 Bovine Paratuberculosis

Bovine paratuberculosis is caused by an acid fast, fastidious *Mycobacterium avium* subsp *paratuberculosis* and is characterized by diarrhoea, emaciation, reduced body weight and production.
Paratuberculosis can be detected using various tests such as microscopic examination of rectal pinch, DTH to Johnin, serum antibody titre, culture and PCR. A nationwide sero-surveillance conducted during 2014-15 indicated the prevalence of 5% and in some Dzongkhags as high as 12%.

3.4.1 Import conditions:

For the importation of bovines for breeding or rearing, health certificate from veterinary authority is required attesting that the animals:

1. Originated from bovine paratuberculosis free area or where no clinical signs or evidence of paratuberculosis has been found during the past 12 months prior to export.
2. Did not show any clinical signs associated with bovine paratuberculosis within 48 hours prior to shipment.
3. Were permanently identified and the identification number stated in the certificate.

3.4.2 Procedures to be followed on arrival at the quarantine station:

In contravene to the above import requirements except for vaccination record, following screening measures are to be taken at quarantine station and after releasing to extension or farms:

On arrival of animals at the quarantine station, it is required to:

1. Examine the individual animal for diarrhea and poor body condition.
2. Suspected animals shall be subjected to antibody ELISA using serum samples at the quarantine station (Antibody detection ELISA).
3. Clinical and antibody positive animals should be culled/rejected for import.

3.4.3 After release of animals to extension or respective farms:

1. Serum sample to be collected from animals tested negative on first test within 3 months of release from quarantine and re-tested for antibody by ELISA.

3.5 Anthrax

Anthrax is a disease caused by the spore-forming bacteria *Bacillus anthracis*. Anthrax causes acute mortality in ruminants and is a zoonosis (a disease which primarily affects animals, but causes disease in humans also). The bacteria produce extremely potent toxins which are responsible for the ill effects, causing a high mortality rate.

Anthrax is diagnosed by examining blood (or other tissues) for the presence of the bacteria. Samples must be collected carefully to avoid contamination of the environment and to prevent human exposure to the bacteria. Blood samples from relatively fresh carcasses will contain large numbers of *B. anthracis*, which can be seen under a microscope, cultured and isolated in a laboratory, or detected by rapid tests and also can be identified by molecular tests like polymerase chain reaction (PCR). The proper disposal of dead animals is critical; the carcass should not be opened, since exposure to oxygen will allow the bacteria to form spores; premises are to be quarantined until all susceptible animals are vaccinated and all carcasses disposed of preferably by incineration or alternatively by deep burial with quick lime; cleaning and disinfection are important.

Anthrax outbreaks in animals, primarily cattle are being reported sporadically throughout the country on an annual basis with more cases reported from the warmer areas. An outbreak under Zhemgang Dzongkhag during 2010 affected both livestock and humans (NCAH, 2010). Similarly, series of outbreaks are being reported from different areas of Bhutan.

3.5.1 Import conditions:

Importation of ruminants, equines and pigs into Bhutan will require a health certificate from veterinary authority attesting that the animals:

1. Were kept for at least 20 days prior to shipment in an establishment where no case of anthrax was officially declared during that period.
2. Showed no clinical sign within 48 hours prior to shipment.
3. Were permanently identified and the identification number stated in the certificate.

3.5.2 Procedures to be followed on arrival at the quarantine station:

1. If animals show signs of anthrax during the quarantine period, the period should be extended by another 14 days during which a course of therapeutic treatment should be provided to all the animals including in contact animals.
2. Vaccinate and release at the end of quarantine period.
3. In case of mortality due to suspected anthrax, blood smear samples and ear/tail tip samples shall be submitted to the laboratory for confirmation of the *B. anthracis* and the carcass of the dead animal should be disposed appropriately.

3.6 Hemorrhagic Septicemia

Haemorrhagic Septicaemia (HS) is a major disease of cattle and buffaloes characterized by an acute, highly fatal septicaemia with high morbidity and mortality and is caused by bacteria *Pasteurella multocida*. The bacteria is not always found in blood samples before the terminal stage of the disease, and is not consistently present in nasal secretions or body fluids of sick animals; in freshly dead animals, a heparinised blood sample or swab should be collected from the heart within a few hours of death, and a nasal swab. Blood smears from affected animals can be stained with Gram's, Leishman's or methylene blue stains. The organisms appear as Gram-negative, bipolar-staining short bacilli.

It is a sporadic disease in the country and cases are also being reported from yaks and mithuns besides cattle.

3.6.1 Import conditions:

For importation of bovines, a health certificate from veterinary authority is required attesting that the animals:

1. Were vaccinated not less than 30 days or not more than 6 months prior to shipment.
2. Were permanently identified and the identification number stated in the certificate.
3. Showed no clinical sign within 48 hours prior to shipment.

3.6.2 Procedures to be followed on arrival at the quarantine station:

1. Booster vaccination post 7 days upon arrival in quarantine station to all animals.
2. In the suspected animals, blood smear examinations for the presence of bi-polar bacterial organism should be performed and for positive animals, further confirmed by culture & isolation should be performed.
3. Positive animals should be treated with appropriate treatment regime.

3.6.3 After release into the respective farms:

1. Animals should be kept under observation for signs of HS.
2. Thereon, follow-up with regular disease surveillance procedures

3.7 Bovine Genital Campylobacteriosis

Bovine Genital Campylobacteriosis (BGC) is a venereal disease also known as Bovine Venereal Campylobacteriosis (BVC). The causal agent of this sexually transmissible disease is *Campylobacter fetus subsp. venerealis*. The species is divided into two closely related subspecies: *C. fetus* subsp. *venerealis* and *C. fetus* subsp. *fetus*. By definition *C. fetus* subsp. *venerealis* is associated with BGC, causing fertility problems with considerable economic losses, particularly in endemic regions. Bovine infections with *C. fetus* subsp. *fetus* are associated with abortion and have a more sporadic occurrence.
Samples taken from bulls, cows or aborted fetuses can be analyzed for the presence of the causal organism. The organism is a thin Gram-negative curved rod that may form S-shapes, seagull-shapes and spirals, and can be cultured at 37°C for at least 3 days in a micro-aerobic atmosphere. Confirmation of the isolate and discrimination between the subspecies of *C. fetus* can be performed by biochemical or molecular methods.

Although. the disease is suspected to be present in bovine population in the country, no prevalence studies have been conducted so far.

3.7.1 Import conditions:

For importation of bovines for breeding, a health certificate from veterinary authority is required attesting that the animals:

1. Were kept in a herd in which no case of bovine genital campylobacteriosis has been declared during 12 (twelve) months prior to export.
2. In case of bulls for breeding purpose, such bulls have never been used for natural service; or have only mated virgin heifers.
3. Showed no clinical sign within 48 hours prior to shipment.
4. Were permanently identified and the identification number stated in the certificate.

3.7.2 Procedures to be followed on arrival at the quarantine station:

1. For the suspected female animals which have been mated, the culture of vaginal mucus for the presence of the causal agent of bovine genital campylobacteriosis proved negative.
2. For the suspected breeding bulls, the preputial specimen or for cows, vagina specimen cultures and/or the associated tests for the presence of the causal agent of bovine genital campylobacteriosis were negative.
3. All positive animals should be treated therapeutically before release.

3.7.3 Import conditions for bovine semen:

For importation of bovine semen, a certificate is required from veterinary authority attesting that:

1. The donor animals:
    a. Have never been used for natural service; or have only mated virgin heifers or
    b. Were kept in an establishment or artificial insemination centre where no case of bovine genital campylobacteriosis has been reported/detected.
2. The culture of semen and preputial specimens for the presence of the causal agent of bovine genital campylobacteriosis was proved to be negative.

3.8 Contagious Bovine Pleuropneumonia

Contagious Bovine Pleuropneumonia (CBPP) is a disease of ruminants (Bos and Bubalus genuses) caused by *Mycoplasma mycoides* subsp. *mycoides* SC (MmmSC; SC = small colony). It is manifested by anorexia, fever and respiratory signs such as dyspnoea, polypnoea, cough and nasal discharges in bovines.

For Isolation of pathogen from clinical samples (nasal swabs and/or broncho-alveolar washings) and identification, the growth of MmmSC takes can take up to 10 days. The organism is then identified routinely with immunological tests. Based on the facilities and the capacities available at NCAH, Competitive ELISA as prescribed by OIE for international trade will be conducted.

The prevalence study on disease has not been conducted in the country.

3.8.1 Import conditions:

For importation of bovines for breeding requires a health certificate from the veterinary authority attesting that the animals:

1. Originated from herd and area free of CBPP.
2. Were permanently identified and the identification number stated in the certificate.
3. Showed no clinical sign 48 hours prior to shipment.

3.8.2 Procedures to be followed on arrival at the quarantine station:

1. Examine the individual animal for respiratory signs and poor body condition.
2. Serum samples should be collected from suspected CBPP animals and subjected to antibody ELISA.
3. Animals positive to CBPP antibody ELISA shall be rejected for import. Further, in contact animals should also be tested and if detected positive should be rejected.

3.8.3 After release into the respective farms:

1. The animals positive to CBPP antibody should be sero-monitored using CBPP antibody ELISA.

3.8.4 Import conditions for bovine semen:

For importation of bovine semen, a certificate from veterinary authority is required attesting that:

1. The donor animals:
    a. Showed no clinical sign of CBPP on the day of collection of the semen;
    b. Were negative to CBPP by serological tests
    c. Were isolated from other domestic bovidae from the day of the first test until collection;
2. Either have not been vaccinated against CBPP or were vaccinated using a vaccine complying with the OIE standards not more than four months prior to collection.

The semen was collected, processed and stored in conformity with the provisions of OIE standards.

3.9 Foot & Mouth disease

Foot and Mouth Disease (FMD) is caused by a virus of the genus *Aphthovirus*, family *Picornaviridae* and infects cloven hoofed animals. It is characterized by a vesicular condition of the feet, buccal mucosa and, in females, the mammary glands. Clinical signs can vary from mild to severe, and fatalities may occur, especially in young animals. It is the most contagious disease of mammals and has a great potential for causing severe economic loss in susceptible cloven-hoofed animals.

The demonstration of FMD viral antigen or nucleic acid is sufficient for a positive diagnosis. ELISA can be used to detect FMD viral antigens and for serotyping. The demonstration of specific antibodies to structural proteins in non-vaccinated animals is indicative of prior infection with FMDV.

The disease is endemic in the country and the prevalent serotypes include serotypes O, A and Asia1. Serotype C has not been detected since early nineties and serotype O is very common in the country.

3.9.1 Import conditions:

For the importation of domestic ruminants and pigs, a health certificate from veterinary authority is required attesting that:

1. The animals showed no clinical sign of FMD during 48 hours prior to shipment;
2. FMD has not occurred within a ten-kilometre radius of the establishment of origin for the past 3 months.
3. The animals were vaccinated using FMD vaccine (oil adjuvant trivalent vaccine containing serotypes O, A and Asia1) minimum 21 days or not more than 6 months prior to the shipment day.
4. The animals were permanently identified and the identification number stated in the certificate.

3.9.2 Procedures to be followed on arrival at the quarantine station:

1. The clinically suspected animals will be tested for NSP (non-structural protein) antibodies. Laboratory confirmation with sero-typing (sandwich ELISA/PCR) needs to be done to support the clinical diagnosis.
2. If the outbreak occurs during quarantine, animals will be subjected to further quarantine for another 21 days from the date of first case. If affected animals have healed completely on the 21st day of quarantine, the animals can be released.

3. All animals upon completion of quarantine period shall be vaccinated against FMD with trivalent vaccine (serotypes O, A and Asia 1) minimum of 7 days prior to release except in case of confirmed clinical outbreak during quarantine.

3.9.3 After release into the respective farms:

1. Animals should be kept under observation for a period of one month.
2. Follow regular disease surveillance procedure.

3.9.4 Import conditions for semen:

For the importation of semen of domestic ruminants and pigs, a certificate from veterinary authority is required attesting that:

1. The donor animals:
    a. Showed no clinical sign of FMD on the day of collection of the semen;
    b. Were kept in an establishment where no animal had been added 30 days before collection, and that FMD has not occurred within 10 kilometres for the 30 days before and after collection;
    c. Have not been vaccinated and were subjected, not less than 21 days after collection of the semen, to tests for antibodies against FMD virus, with negative results; or
    d. Had been vaccinated at least twice, with the last vaccination not more than 12 and not less than one month prior to collection.
2. The semen:
    a. Was collected, processed and stored in conformity with the provisions of OIE standards;

3.10 Trichomonosis

Bovine venereal trichomonosis is caused by the flagellate protozoan parasite, *Trichomonas foetus* and is characterized by abortion and infertility, especially in dairy cattle. Transmission of the disease is primarily by coitus, but mechanical transmission by insemination instruments or by gynaecological examination can occur.
In infected herds, the most reliable material for diagnosis is either preputial or vaginal washings or scrapings. Confirmation of infection depends on the demonstration of organisms in placental fluid, stomach contents of the aborted fetus, uterine washings, pyometra discharge, vaginal mucus or preputial smegma.
The prevalence study for the study has not been conducted so far in the country.

3.10.1 Import conditions:

For the importation of bovines for breeding, a health certificate from the veterinary authority is required attesting that the animals:

1. Showed no clinical sign of Trichomonosis within 48 hours prior to shipment;
2. Were kept in a herd in which no case of Trichomonosis has been reported during 12 months prior shipment.
3. Have never been used for natural service; or the bulls have only mated virgin heifers.

4. The animals were subjected to a direct microscopic and cultural examination of preputial specimens with negative results.
5. Were permanently identified and the identification number stated in the certificate.

3.10.2 Procedures to be followed on arrival at the quarantine station:

1. All the breeding bulls and animals suspected for Trichomoniasis should be subjected to direct microscopic and culture examination of preputial specimens and vaginal mucus.
2. The infection will persist for animal's lifetime in bulls and hence, the positive bulls should be culled.
3. In female cattle, the infection is usually self-limiting and the positive female animal may be missed for one cycle, treated and released.

3.10.3 After release into the respective farms:

1. Animals should be kept under observation for a period of one month.
2. Follow regular disease surveillance procedure.

3.10.4 Import conditions for bovine semen:

For the importation of semen of bovines, a certificate from veterinary authority is required attesting that:

1. The donor animals have never been used for natural service; or have only mated virgin heifers; or if have mated others, should be tested three times at weekly intervals on a preputial specimen, with a negative result in each case.
2. The donor animals were kept in an establishment or artificial insemination centre where no case of Trichomonosis has been reported;
3. The donor animals were subjected to a direct microscopic and cultural examination of preputial specimens with negative results;

The semen was collected, processed and stored in conformity with the provisions of OIE standards or Livestock Rules and Regulations of Bhutan, 2017.

4. Ovine and Caprine Diseases

4.1 Peste des Petits Ruminants (PPR)

A Peste des petits ruminant (PPR) is a highly infectious viral disease of small wild and domestic ruminants. PPR affects nearly one billion small ruminants around the world and will results in high economic losses due to the high morbidity and mortality rates. The etiologic agent is PPRV belonging to the *Morbillivirus* genus, *Paramyxoviridae* famlily. Serological diagnosis is classically realized using competitive ELISA (cELISA).

In Bhutan, the first outbreak of PPR occurred in Chukha during 2010 with 100% morbidity and 80% mortality followed by second outbreak in the same location in 2013 and 2014. The purposive emergency surveillance conducted during 2013 in goats in Chukha and Sarpang Dzongkhag also detected antibodies to PPR. The disease was also further detected at Lhomizingkha at Dagana Dzongkhag during 2014 and also at Gelegphu at Sarpang Dzongkhag during 2016. The entry of PPR into Bhutan is likely through the import of goats from the neighboring countries since PPR is endemic in these countries. Therefore, the country is at high risk of the introduction of the disease.

4.1.1 Import conditions:

A health certificate from the veterinary authority is required attesting that the animals:

1. Showed no clinical sign suggestive of PPRV infection within 48 hours prior to shipment.
2. Were vaccinated against PPR with live attenuated PPRV vaccines.
3. Originated from the organized farms.

4.1.2 Procedures to be followed on arrival at the quarantine station:

1. Screen all the goats with rapid tests and the positive animals should be tested further with ELISA. PPR confirmed animals shall be further quarantined for 14 days from the day of onset of clinical signs.
2. All the goats shall be vaccinated against PPR 7 days after arrival in the quarantine station with live attenuated PPR vaccine.

4.1.3 After release into the respective farms:

1. Monitor clinical surveillance.
2. Any cases detected should be isolated and treated till recovery.

4.2 Caprine and Ovine Brucellosis

Caprine and ovine brucellosis is caused by *Brucella melitensis* (biovars 1, 2 or 3). Clinically, the disease is characterised by one or more of the following signs: abortion, retained placenta, orchitis, epididymitis and, rarely, arthritis, with excretion of the organisms in uterine discharges and in milk.

The disease is of zoonotic importance affecting primarily sheep, goats and of major public health concerns. There is no evidence of disease in the country.

4.2.1 Import conditions:

For the importation of sheep and goats for breeding or rearing (except castrated males) into Bhutan, a health certificate is required from the veterinary authority attesting that the animals:

1. Showed no clinical sign of caprine and ovine brucellosis within 48 hours prior to shipment;
2. Originate from a sheep or goat flock free from caprine and ovine brucellosis.
3. Testing at the source provide negative to brucella.

4.2.2 Procedures to be followed on arrival at the quarantine station:

1. If the animals show clinical signs accompanied by Rose Bengal Test (RBT) positive the flock shall be culled/rejected for import.
2. If the healthy animals test positive to RBT, it should be confirmed by indirect antibody ELISA. If positive, it should be further re-confirmed through indirect ELISA of paired sera sample. If positive even in paired sera sample testing, the animals shall be culled/rejected for import.

4.2.3 After release into the respective farms:

1. Routine disease monitoring.

4.2.4 Import conditions for Caprine/Ovine semen:

Import of Caprine/Ovine semen into the country requires a certificate from veterinary authority attesting that:

1. The semen is from an artificial insemination centre and the animal has been tested negative to brucella by serological tests.
2. The semen is not from an artificial insemination centre, however, the donor animals were kept in a country or zone free from bovine brucellosis; or
3. The animals were kept in a herd officially free from ovine/caprine brucellosis, showed no clinical sign of caprine/ovine brucellosis on the day of collection of the semen and were negative to brucella by serological tests within 30 days prior to collection.

The semen was collected, processed and stored in conformity with the provisions of Chapters OIE standards or Livestock Rules and Regulation of Bhutan 2017.

4.3 Caprine & Ovine Foot & Mouth Disease (FMD)

FMD is a severe, highly communicable viral disease of cattle and swine. It also affects sheep, goats, deer, and other cloven-hoofed ruminants. The disease in adult sheep and goats is frequently mild or unapparent but can cause high mortality in young animals. The disease is endemic in Bhutan.

4.3.1 Import conditions:

A health certificate from the veterinary authority is required attesting that the animals:

1. Showed no clinical sign of FMD within 48 hours prior to shipment.
2. Were isolated in an establishment for the 30 days prior to shipment, where FMD did not occur within a ten-kilometre radius of the establishment during that period.
3. Were vaccinated within six months prior to shipment.

4.3.2 Procedures to be followed on arrival at the quarantine station:

1. Same as above for cattle.

4.3.3 After release into the respective farms:

1. Regular monitoring.

5. Swine Diseases

5.1 Classical Swine Fever

Classical Swine Fever (CSF), also known as hog cholera, is a contagious viral disease of pigs. It is caused CSF virus of the genus *Pestivirus* and the family *Flaviviridae*. It is closely related to the viruses of Bovine Viral Diarrhea (BVD) and Border disease. Pyrexia, huddling, inappetance, dullness, weakness, conjunctivitis and constipation followed by diarrhoea are the prevailing signs of disease in all age groups. Detection of antibodies can be done only during the third week of illness.

Vaccination is done in all government breeding farms and private commercial farms in the country. Despite, the disease is endemic in the country where the outbreaks occur in backyard farms.

5.1.1 Import conditions:

A health certificate from the veterinary authority is required attesting that the animals:

1. Showed no clinical sign of CSF within 48 hours prior to shipment.
2. Were kept since birth or for the past three months in a CSF free compartment.

5.1.2 Procedures to be followed on arrival at the quarantine station:

1. The animals should be clinically examined for any sickness on a daily basis and the sick animals should be segregated.
2. If found clinically sick and confirmed through antigen ELISA, then it should be culled. Rest of the sounder of swine to be further quarantined for next 15 days.
3. Vaccinate against CSF week prior to release to respective farm.

5.1.3 After release into the respective farms:

1. The animals should be vaccinated on annual basis.
2. Routine disease monitoring.

5.1.4 Import conditions for semen:

For the importation of semen of domestic and captive wild pigs, a certificate from the veterinary authority is required attesting that:

1. The donor animals:
    a. Were kept in a compartment free from CSF since birth or for at least three months prior to collection;
    b. Showed no clinical sign of CSF on the day of collection of the semen and for the following 40 days;
    c. Have been vaccinated against CSF and were subjected to a serological test performed at least 21 days after collection and it has been conclusively demonstrated that any antibody is due to the vaccine; or
    d. Have been vaccinated against CSF and were subjected to a virological test performed on a sample taken on the day of collection and it has been conclusively demonstrated that the boar is negative for virus genome.
2. The semen was collected, processed and stored in conformity with the provisions of OIE standards or Livestock Rules and Regulations of Bhutan, 2017.

5.2 Swine Foot & Mouth disease

FMD is endemic in Bhutan and occasional infection as a result of spillover from infected cattle has been reported. However, no instance of specific outbreaks in pigs has not been reported in Bhutan.

5.2.1 Import conditions:

A health certificate from the veterinary authority is required attesting that:

1. The animals showed no clinical sign of FMD within 48 hours prior to shipment.
2. FMD has not occurred within a ten-kilometre radius of the establishment of origin for the past 3 months.
3. The animals were vaccinated using FMD vaccine (oil adjuvant trivalent vaccine containing serotypes O, A and Asia1) minimum 21 days or not more than 6 months prior to the shipment day.

5.2.2 Procedures to be followed on arrival at the quarantine station:

1. Same requirement as given for cattle above.

5.2.3 After release into the respective farms:

1. Animals should be kept under observation for a period of one month.

2. Follow regular disease surveillance procedure.

5.3 Porcine Respiratory and Reproductive Syndrome (PRRS)

Porcine Reproductive and Respiratory Syndrome (PRRS) is characterized by reproductive failure of sows and respiratory problems of piglets and growing pigs. The disease is caused by the PRRS virus, a virus currently classified as a member of the order *Nidovirales*, family *Arteriviridae*, genus *Arterivirus*. Since isolation of the virus requires porcine cells and is technically challenging, serological testing is done commonly.

The disease has been diagnosed in government pig breeding farms in Bhutan and has been controlled through restriction of breeding cycles (3 cycles). The disease has been introduced in Bhutan through import of pigs from other countries.

5.3.1 Import conditions:

A health certificate from the veterinary authority is required attesting that:

1. The animals showed no clinical signs (history of abortion, still-births, high mortality in piglets) of PRRS within 48 hours prior to shipment.
2. No case of PRRS in farm or establishment for last 2 years.
3. The animals were subjected to serological test against PRRS with negative result.

5.3.2 Procedures to be followed on arrival at the quarantine station:

1. The animals should be clinically examined for any sickness on a daily basis and segregate accordingly.
2. Conduct antibody ELISA to detect antibodies against both type 1 and type 2 PRRV from the suspected animals.
3. Re-sampling and testing of animal after one week to be done and if tests positive, should not be used for breeding purpose. They should be segregated and can be used for fattening.

5.3.3 After release into the respective farms:

1. The animals should be kept separately for one month before mixing with old stocks (acclimatization).
2. The animals must be subjected to routine disease monitoring.

5.3.4 Import conditions for semen:

For the importation of semen of domestic and captive wild pigs, a certificate from veterinary authority is required attesting that:

1. The donor animals:
    a. Were kept in a compartment free from PRRS since birth or for at least three months prior to collection;
    b. Showed no clinical sign of PRRS on the day of collection of the semen and for the following 40 days;

c. Have not been vaccinated against PRRS and were subjected to a serological test performed at least 21 days after collection, with negative results.

2. The semen was collected, processed and stored in conformity with the provisions of OIE standards.

6. Equine Diseases

6.1 Equine strangles

Strangles is caused by *Streptococcus equi*, *a* gram-positive, capsulated bacterium that is easily transmitted and is reported worldwide. The disease is characterized by high temperature, depression, purulent discharges from nose swollen head and neck, which often gets abscessed. Culture of nasal swabs, nasal washes, or pus from abscesses is essential for confirming the presence of *S. equi*. Zoonotic transmission has been reported, but is not very common. Immunocompromised individuals should avoid exposure to Strangles.
The disease is reported frequently in the country.

6.1.1 Import conditions:

A health certificate from the veterinary authority is required with attesting that:

1. The animals showed no clinical signs (nasal discharge) of Equine Strangles on the day of shipment.

6.1.2 Procedures to be followed on arrival at the quarantine station:

1. Monitor temperature twice daily and laboratory test for equine with a temperature greater than 102.5º F or clinical signs consistent with Strangles.
2. Bacterial culture should be conducted for the suspected animals (nasal discharge).
3. The positive animals should be treated before release.

6.2 Glanders

Glanders is a zoonotic disease of equines caused by bacteria *Burkholderia malle*. Causative bacterium was previously known as *Pseudomonas mallei*. Equidae, humans, occasionally felidae, and other species are susceptible and the infections are usually fatal. The most common clinical signs of infection in animals is yellow-green nasal discharge and ulcers on the nose. The animals may have enlarged lymph nodes and nodules on the skin. In some cases, they may look like long, hard ropes, under the skin. Severe coughing can also occur in mules and donkeys can start rapidly (acute) and can lead to death in 1 to 2 weeks after exposure.

The disease is reported from Asian countries including India. However, there are no reports of clinical cases in the country till date.

6.2.1 Import conditions:

A health certificate from the veterinary authority is required with attesting that:

1. The animals showed no clinical signs on the day of shipment.
2. Glanders is a notifiable disease in the country of export and no case has been reported during the past 3 years.
3. OR Glanders is a notifiable disease in the country of export and no case has been reported for a period of at least 6 months and a surveillance programme is in place demonstrating the absence of the disease in accordance with OIE recommendations.
4. OR the horse was tested for glanders by the complement fixation test with negative result(s) within 30 days of export.

6.2.2 Procedures to be followed on arrival at the quarantine station:

1. All the animals should be examined for any clinical signs consistent with glanders.
2. The suspected animals should be screened with ELISA. The positive animals shall be rejected.
3. If the animal dies during the quarantine, affected animal carcasses should be burned and buried. All disposable materials on positive premises (feed and bedding) should be burned or buried and conveyances and equipment should be carefully disinfected.

6.3 Equine Infectious anemia (EIA)

Equine infectious anemia (EIA) is a retroviral disease of equids that may be characterized by acute and/or chronic recurring clinical signs including fever, anemia, edema and cachexia in some animals. If death does not result from one of the acute clinical attacks, a chronic stage develops and the infection tends to become in apparent. The disease is spread by biting insects, such as horse flies and deer flies. Once a horse is infected with EIAV, it remains carrier for the lifelong.
The disease is not detected clinically in Bhutan.

6.3.1 Import conditions:

A health certificate from the veterinary authority is required with attesting that:

1. The animals showed no clinical signs on the day of shipment.
2. The country has been free from equine infectious anaemia for the last 3 months prior to date of export OR
3. The horse was tested for equine infectious anemia by the immunodiffusion (Coggin's) test with negative results within 30 days of export.

6.3.2 Procedures to be followed on arrival at the quarantine station:

1. The animals should be examined for any clinical signs and the suspected animals should be subjected to Agar gel immunodiffusion tests or ELISA.
2. The positive animals shall be rejected.

6.4 Equine Influenza (EI)

Equine influenza is a highly contagious upper respiratory tract infection caused by strains of the influenza virus type A: H7N7 and H3N8, of the family Orthomyxoviridae. They are related to but distinct from the viruses that cause human and avian influenza. Clinical signs include high fever (up to 106°F [41.1°C]), serous nasal discharge, submandibular lymphadenopathy, and dry, harsh cough. In addition, depression, anorexia, and weakness are frequently seen. The disease are generally self-limiting.

The disease is not detected clinically in Bhutan.

6.4.1 Import conditions:

A health certificate from the veterinary authority is required with attesting that:

4. The animals showed no clinical signs on the day of shipment.
5. During the 90 days prior to export, the horse has not suffered from or been exposed to nor been in premises infected with Equine Influenza (EI), and was vaccinated against EI according to manufacturer's recommendation with an inactivated or recombinant vaccine approved by the country of export, between 21 and 90 days before shipment either with a primary course or a booster.

6.4.2 Procedures to be followed on arrival at the quarantine station:

1. The animals should be examined for any clinical signs and the suspected animals should be subjected to HI.
2. The positive animals shall be rejected.

7. Diseases of Poultry and Game Birds

7.1 Infectious Bursal Disease (IBD)

Infectious Bursal Disease (IBD) is caused by a virus of a member of the genus *Avibirnavirus* of the family *Birnaviridae*. Although turkeys, ducks, guinea fowl and ostriches may be infected, clinical disease occurs solely in chickens. Only young birds are clinically affected. Severe acute disease of 3–6-week-old birds is associated with high mortality, but a less acute or subclinical disease is common in 0–3-week-old birds.
Clinical disease also known as Gumboro disease, can usually be diagnosed by a combination of characteristic signs and post-mortem lesions. Laboratory confirmation of disease, or detection of subclinical infection, can be carried out by demonstration of a humoral immune response in unvaccinated chickens or by detecting the presence of viral antigen or viral genome in tissues and also histological examination of bursa.

The disease has developed endemicity in Bhutan through import of infected parent stock in the past.

7.1.1 Import condition:

A health certificate from the veterinary authority is required attesting that the birds:

1. Showed no clinical sign of IBD within 48 hours prior to shipment;
2. Come from an establishment which is free from IBD and regularly inspected by the Veterinary Authority;
3. Were vaccinated against IBD (the nature of the vaccine used and the date of vaccination should also be stated in the certificate) in case of adult bird import; and demonstration of protection against IBD by AGID or antibody detection ELISA.

7.1.2 Screening at quarantine station:

1. Suspected flocks for IBD should be subjected to rapid antigen detection test randomly
2. If positive, the flocks should be culled.

7.1.3 Surveillance/monitoring after release into the farms:

1. Regular vaccination, as per the standard vaccination schedule at the farm.
2. Regular clinical surveillance at farm level.
3. Regular laboratory surveillance by the laboratory personnel.

7.2 Marek's Disease:

Marek's Disease (MD) is a lymphomatous and neuropathic disease of domestic fowl caused by an *alphaherpesvirus*. Chickens may become persistently infected with MD virus (MDV) without developing clinical disease. In chickens, MD occurs at 3–4 weeks of age or older and is most common between 12 and 30 weeks of age. Clinical signs observed are paralysis of the legs and wings, with enlargement of peripheral nerves, but nerve involvement is sometimes not seen, especially in adult birds.

Antibodies to MDV develop within 1–2 weeks of infection and are commonly recognized by the agar gel immuno-diffusion test, the indirect fluorescent antibody test, and sometimes by other serological tests such as enzyme-linked immuno-sorbent assay.

It developed endemicity in Bhutan through import of infected parent stock in the past. It is also endemic in South Asian countries.

7.2.1 Import condition:

A health certificate from the veterinary authority is required attesting that the birds are:

1. Are from disease free flock.
2. Were clinically free of the disease within 48 hours prior to shipment.
3. Vaccinated as per the standard schedule (i.e., 0 day of age).

7.2.2 Screening at quarantine station:

1.Suspected flocks should be tested with immunological tests like AGID and reject if detected positive.

7.2.3 Surveillance/monitoring after release into the farms:

1. Regular clinical surveillance at farm level.
2. Regular laboratory surveillance by the laboratory.

7.3 New Castle Diseases

Newcastle Disease (ND) is caused by virulent strains of *avian paramyxovirus type 1* (APMV-1) serotype of the genus *Avulavirus* belonging to the subfamily *Paramyxovirinae*, family *Paramyxoviridae*. Strains of NDV have been grouped into five pathotypes on the basis of the clinical signs seen in infected chickens. Viscerotropic velogenic: a highly pathogenic form in which haemorrhagic intestinal lesions are frequently seen; Neurotropic velogenic: a form that presents with high mortality, usually following respiratory and nervous signs; Mesogenic: a form that presents with respiratory signs, occasional nervous signs, but low mortality; Lentogenic or respiratory: a form that presents with mild or subclinical respiratory infection; Asymptomatic: a form that usually consists of a subclinical enteric infection. NDV is a human pathogen and the most common sign of infection in humans is conjunctivitis that develops within 24 hours of NDV exposure to the eye.

A wide range of serological tests like enzyme-linked immunosorbent assays (ELISA) and HI for assessing antibody levels is used for diagnosis in birds.

The disease is highly endemic in Bhutan and South Asian countries.

7.3.1 Import condition:

i. Day Old Chicks (DoC)

A health certificate from the veterinary authority is required attesting that the birds are:

1. Derived from parent flocks which had been kept in an ND free country, zone or farm for at least 21 days prior to and at the time of the collection of the eggs;
2. Are transported in new or appropriately sanitized containers.

If the poultry or parent flocks have been vaccinated against ND, the nature of the vaccine used and the date of vaccination have to be attached to the certificate confirming the approved vaccination schedule for B1 & R2B.

ii. Hatching eggs of poultry:

A certificate from the veterinary authority is required with attesting that:

1. The eggs came from an ND free country, zone or farm;
2. The eggs were derived from parent flocks which had been kept in an ND free country, zone or compartment for at least 21 days prior to and at the time of the collection of the eggs;
3. The eggs are transported in new or appropriately sanitized packaging materials.

   If the parent flocks have been vaccinated against ND, the nature of the vaccine used and the date of vaccination have to be attached to the certificate confirming the approved vaccination schedule for B1 and R2B.

7.3.2 Screening at quarantine station:

1. Suspected flocks shall be subjected to rapid antigen detection test.

2. All the positive flocks shall be rejected.

7.3.3 Surveillance/monitoring after release into the farms:

1. Regular vaccination as per the standard vaccination schedule of the farm.
2. Regular clinical surveillance at farm level.
3. Regular laboratory surveillance by the laboratory.

7.4 Avian Leukosis Complex (ALC)

Avian leucosis complex is one of the commercially important neoplastic diseases of poultry caused by certain members of the leukosis/sarcoma group of avian *retroviruses*. Isolates that can induce lymphoid leukosis in chickens are commonly called avian leukosis viruses and are divided into subgroups A, B, C, D, and J, on the basis of differences in their viral envelope glyco-proteins, which determine antigenicity, viral interference patterns with members of the same and different subgroups, and host range.

A subclinical disease syndrome characterized by depressed egg production in the absence of tumor formation is more important economically than are deaths from lymphoid leukosis.
These diseases are responsible for economic loss due to both mortality and depressed performance.

The disease has become endemic in the country due to the importation of the parent flock.

7.4.1 Import condition:

A health certificate from the veterinary authority is required with attesting that the birds are:

1. The birds are from disease free flock.
2. Were clinically free of the disease within 48 hours prior to shipment

7.4.2 Screening at quarantine station:

1.Random testing of birds with anti**g**en ELISA and all the positive flocks shall be rejected.

7.4.3 Surveillance/monitoring after release into the farms:

1. Regular clinical surveillance at farm level.
2. Regular laboratory surveillance by the laboratory.

7.5 Avian Influenza

Avian Influenza (AI), caused by the influenza virus Type'A', can affect several species of food producing birds (chickens, turkeys, quails, guinea fowl, etc.), as well as pet birds and wild birds with some strains resulting in high mortality rates. The virus has also been isolated from mammalian species including humans, rats and mice, weasels and ferrets, pigs, cats, tigers and dogs.The virus belongs to *Orthomyxovirus* type A, its pathogenicity is variable, and isolates are designated sero-type/species/location/reference number/year/subtype designation (H/N).The strains of AI viruses and generally can be classified into two categories: low pathogenic (LPAI) that typically causes little or no clinical signs in birds and highlypathogenic (HPAI) that can cause severe clinical signs and/or high mortality in birds. Highly pathogenic forms are usually of the H groups 5 and 7 and now identified (if H5 or H7) by the presence of a sequence at the haemagglutinin cleavage site that codes for multiple basic amino acids.

Diagnosis is by isolation of the virus or by detection and characterisation of fragments of its genome.

Outbreak of HPAI occurred in Bhutan since 2010.

Note: For further details, NIPPP can be referred

7.5.1 Import condition:

A health certificate from the veterinary authority is required with attesting that the birds are:

1. Originated from disease free flock.
2. Were clinically free of the disease within 48 hours prior to shipment.

Day-Old Chicks (DOC):
A certificate is required from the veterinary authority with attesting that:

1. The poultry were kept in an avian influenza free country, zone or compartment since they were hatched;
2. The poultry were derived from parent flocks which had been kept in an avian influenza free country, zone or compartment for at least 21 days prior to and at the time of the collection of the eggs;
3. The poultry have not been vaccinated ;
4. The poultry are transported in new or appropriately sanitized containers.

If the parent flocks have been vaccinated against avian influenza, the nature of the vaccine used and the date of vaccination have to be attached to the certificate.

7.5.2 Screening at quarantine station:

1. Random testing of birds with rapid antigen detection test.
2. Polymerase Chain Reaction (PCR) in case of suspected outbreak/positive rapid test & if positive cull (as prescribed in NIPPP document).

7.5.3 Surveillance/monitoring after release into the farms:

1. Regular clinical surveillance at farm level.
2.Regular laboratory surveillance by the laboratory.

7.6 Others diseases of poultry and game birds:

Infectious Laryngeotracheitis, Infectious Bronchitis, Avian Tuberculosis and, Avian Mycoplasmosis.
Note: to develop standards based on the epidemiology of these diseases in future and should meet, among others, below given minimum standards.

7.6.1 Import condition:

A health certificate from the veterinary authority is required with attesting that the birds are:

1. Originated from disease free flock.
2. Vaccinated, where applicable, as per the standard schedule of the exporting country.
3. Showed no clinical sign of any diseases within 48 hours prior to shipment.

7.6.2 Screening at quarantine station:

1. Rapid antigen test & Enzyme-linked immunosorbent assay (ELISA); if positive should be rejected.

7.6.3 Surveillance/monitoring after release into the farms:

1. Regular vaccination, where applicable, as per the standard vaccination schedule of the farm.
2. Regular clinical surveillance at farm level.
3. Regular laboratory surveillance by the laboratory.

8. Diseases of Dogs and Cats

8.1 Rabies

Rabies is fatal viral disease which affects all warm blooded animals including humans. Rabies is caused by neurotropic viruses of the genus *Lyssavirus* in the family *Rhabdoviridae*, and is transmissible to all mammals including man. The disease affects the central nervous system of warm-blooded animals. Rabies is transmitted through the saliva of an infected animal. Infection occurs primarily via bite wounds, or infected saliva entering an open cut or wound or mucous membrane, such as those in the mouth, nasal cavity or eyes.

As there are neither gross pathognomonic lesions nor specific and constant clinical signs for rabies, accurate diagnosis can only be made in the laboratory. Agent identification is preferably undertaken using the fluorescent antibody test (FAT).
The disease is reported annually and is endemic in southern parts of the country.

8.1.1 Import conditions for dogs and cats

A health certificate from the veterinary authority is required meeting the requirements mentioned below;

1. The animal showed no clinical sign of any infectious diseases within 24 hours prior to shipment;
2. The animal has been vaccinated against canine distemper, parvovirus, infectious canine hepatitis and Leptospira interrogans Canicola and Leptospira interrogans Icterohaemorrhagiae. The vaccinations should be done at least one month before the date of entry into the country.
3. Animal that has never been vaccinated against rabies must be vaccinated with inactivated rabies vaccine not less than 1 month prior to the date of entry. In the case of booster vaccination, the animal should have been vaccinated not more than 1 year prior to its entry.
4. The animal has been treated with broad-spectrum endo-parasiticidal and ectoparasiticidal drugs at least one week before the date of entry and must be free of ectoparasites upon entry.
5. Were permanently identified and their identification number stated in the certificate; and

8.1.2 Procedures to be followed on arrival at the quarantine station:

1. Animals showing signs of rabies should be isolated and observed.
2. Animals died of suspected rabies shall be subjected to rapid antigen detection test at the quarantine station.
3. Brain samples from animals died of suspected rabies need to be confirmed through FAT/ RIAD.
4. If confirmed rabies, the in-contact animals should be observed for another 10 days before release.
5. People including the staff who had direct contact with the confirmed rabid dogs should be referred to the nearest human health facilities for the post exposure treatment.

8.1.3 After release

1. Animals should be kept under observation for a period of one month.
2. Follow regular disease surveillance procedure.

9. Parasitic diseases

All the animals, poultry and other birds kept at the quarantine station:

1. Must be examined for clinical signs, or other evidence of important blood parasites like Anaplamosis, Babesiosis, Theileriosis and Trypanosomiasis. The suspected animals should be screened for above parasites through blood smear examination and positive, should be treated accordingly.
2. Should also be screened for other helminths through fecal examinations and should be dewormed if detected positive.
3. Must be examined for external parasites and should be sprayed with appropriate ectoparasiticides if detected.

10. Other exotic diseases

Import requirement for other exotic diseases not covered under this document will be dealt in accordance with OIE Terrestrial Animal Health Code and Aquatic Animal Health Code or based on the findings of the risk assessment conducted by Department of Livestock and BAFRA.

11. References:

- OIE, 2012. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals
- OIE, 2017. Terrestrial Animal Health Code
- OIE, 2016. Manual of Diagnostic Tests for Aquatic Animals
- OIE, 2017. Aquatic Animal Health Code
- OIE, 2018. WAHIS

Annexure I. Check list for screening of animals at the quarantine stations

Sl. No.	Diseases/ Test	Tests	Bovine	Ovine/ Caprine	Swine	Equine	Poultry	Dogs & cats	Remarks
1	IBR	Antibody ELISA	X						Vaccination at QS not required
2	Brucellosis	RBT/Antibody ELISA	X	X					RBT for screening, ELISA only if positive
3	Tuberculosis	Intradermal tests	X						Only suspected animals
4	Bovine Paratuberculosis	Antibody ELISA	X						Only suspected animals
5	Anthrax	Microscopy - Smear	X						Only suspected animals
6	Haemorrhagic septicemia (HS)	Blood Smear	X						Only suspected animals
7	Bovine genital campylobacteriosis	Vaginal /Preputial washing culture	X						Only suspected animals
8	Contagious Bovine Pleuropneumonia(CBPP)	Antibody ELISA	X						Only suspected animals
9	Foot & mouth disease	Rapid test/antibody and antigen ELISA/PCR	X	X	X				Only in clinically suspected Animals
10	Trichonomiasis	Direct microscopy & Culture of preputial washing & vaginal mucus	X						Only in breeding bulls and suspected animals

11	PPR	Rapid tests/ ELISA		X					Tests all the goats
12	Classical swine fever	Antigen ELISA			X				Suspected only animals
13	PRRS	Antibody ELISA			X				Suspected only animals
14	Strangles	Culture & identification				X			Suspected animals
15	Glanders	culture & identification or ELISA				X			Suspected animals
16	Equine Infectious anemia (EIA)	Agar gel immunodiffusion tests or ELISA				X			Suspected animals
27	Equine Influenza (EI)	Haemagglutination inhibition				X			Suspected animals
18	Infectious Bursal Disease	Rapid antigen detection test					X		Random testing of birds
19	Mareks Disease								No test required. AGID in future
20	Newcastle Disease	Rapid antigen detection test/PCR					X		Random testing of birds
21	Avian Leucosis Complex	Antigen ELISA					X		Random testing of birds
22	Avian Influenza (HPAI)	Rapid antigen detection test/ PCR					X		Random testing of birds. Not required in DOC
23	Rabies	Rapid antigen detection test, FAT/RIAD						X	Brain samples from animals died of suspected rabies

24	Parasitic Diseases	Blood smear examination/Fecal examination/general examinations	X	X	X		X	X	