



MANUAL DE INVESTIGAÇÃO DE DOENÇA VESICULAR

1ª EDIÇÃO

MINISTÉRIO DA AGRICULTURA, PECUÁRIA E ABASTECIMENTO

Ministério da Agricultura, Pecuária e Abastecimento
Secretaria de Defesa Agropecuária

MANUAL DE INVESTIGAÇÃO DE DOENÇA VESICULAR

MANUAL OF INVESTIGATION OF THE VESICULAR DISEASE

1ST EDITION

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INTRODUCTION

The technical procedure that should be carried out by veterinarians trained for the care and investigation of notification of suspected vesicular disease was described, initially, at the Action plan for foot-and-mouth disease, volume I, attendance to notification of suspected vesicular disease", published in 2009.

This document is an update of this document and contains the procedures for acting in the face of suspected vesicular disease in the investigation and alert phases. Its contents must be known and mastered by all veterinary doctors that act at the official veterinary service (SVO) and use in SVO's routines besides serving as a basis for treatment in this area.

Considering the constant advances in knowledge regarding the dynamics of infectious diseases, the availability of laboratory analysis e methods, and the control and eradication strategies, the annual manuals need revision and update. This version was prepared based on current standards and guidelines on foot-and-mouth disease, national and international, and was preceded by technical discussions with the participation of different sectors of the Ministério da Agricultura, Pecuária e Abastecimento - Mapa, Centro Panamericano de Febre Aftosa - Panaftosa, and Serviços Veterinários Estaduais - SVE.

Definitions for this document

Vesicular disease: a set of communicable diseases characterized by the presence of vesicles or associated vesicular lesions in the regions of the mouth, snout, feet, or udders, associated with clinical and epidemiological conditions that present indications of the previous contact with a causal infectious agent, which should be confirmed or discarded by laboratory diagnosis;

Clinical inspection: Clinical inspection: a procedure performed by a veterinarian, with individual inspection of the animal, detailed observation in the mouth region, snout, interdigital and udder spaces, in the search for clinical signs compatible with vesicular disease

Prohibition: prohibition of entry and egress of animals in a breeding establishment, for any purposes, as well products or by-products or materials that the serum constitutes source and of transmission of the infectious agent, at the discretion of the SVO.

Epidemiological unit: a group of animals with defined epidemiological relationship and with similar probabilities of exposure to a certain pathogen, according to the characterization made by the SVO. It may be constituted by one or more contiguous rural properties, part of a rural property or group of animals susceptible to the disease, sharing the same environment or under common biosafety management practices and conditions;

Epidemiological link: Connection or contact between probable or confirmed cases of disease and other susceptible animals, indicating the possibility of transmission of the infectious agent according to the characterization made by the SVO.

Inspection: observation of animals in the herd, and may make them walk or run, in search of clinical signs compatible with vesicular injury (salivation, gimping, vesicles on the palate, paws, or mouth).

ACRONYMS

e-Sisbravet – ~~das~~ Specific electronic tool for the management of data obtained from passive surveillance in animal health, developed for the recording and monitoring of immediate notifications of suspected diseases and investigations carried out by the Official Veterinary Service.

GTA - Animal Traffic Guide

LEF - Esophageal-pharyngeal fluid

Mapa – Ministry of Agriculture, Livestock, and Supply

MVO - Official veterinarian

Panaftosa - Pan American Foot-and-Mouth Disease Center

Pnefa - National Foot-and-Mouth Disease Surveillance Program

SFA - Federal Superintendence of Agriculture

SVE – State Veterinary Service

SVO – Official Veterinary Service

UVL – Local Veterinary Unit

1. Introduction

This document describes the procedures for investigating suspected cases of the vesicular disease. For didactics and processing, it is grouped into two phases: Research and Alert. The performance and procedures to be performed in these phases constitute necessary knowledge for all veterinarians working in the SVO.

Following this process, when confirming a foot-and-mouth disease outbreak, there are two other phases, called emergency and conclusion, and the procedures are written in the Contingency Plan for Foot-and-Mouth Disease.

In a representative way, it is possible to visualize in Figure 1 the documents where the procedures of each of the stages of the foot-and-mouth disease surveillance system are described in the country.

Figure 1 shows the documents in which the procedures of each of the stages of the foot-and-mouth disease surveillance system are described in the country.

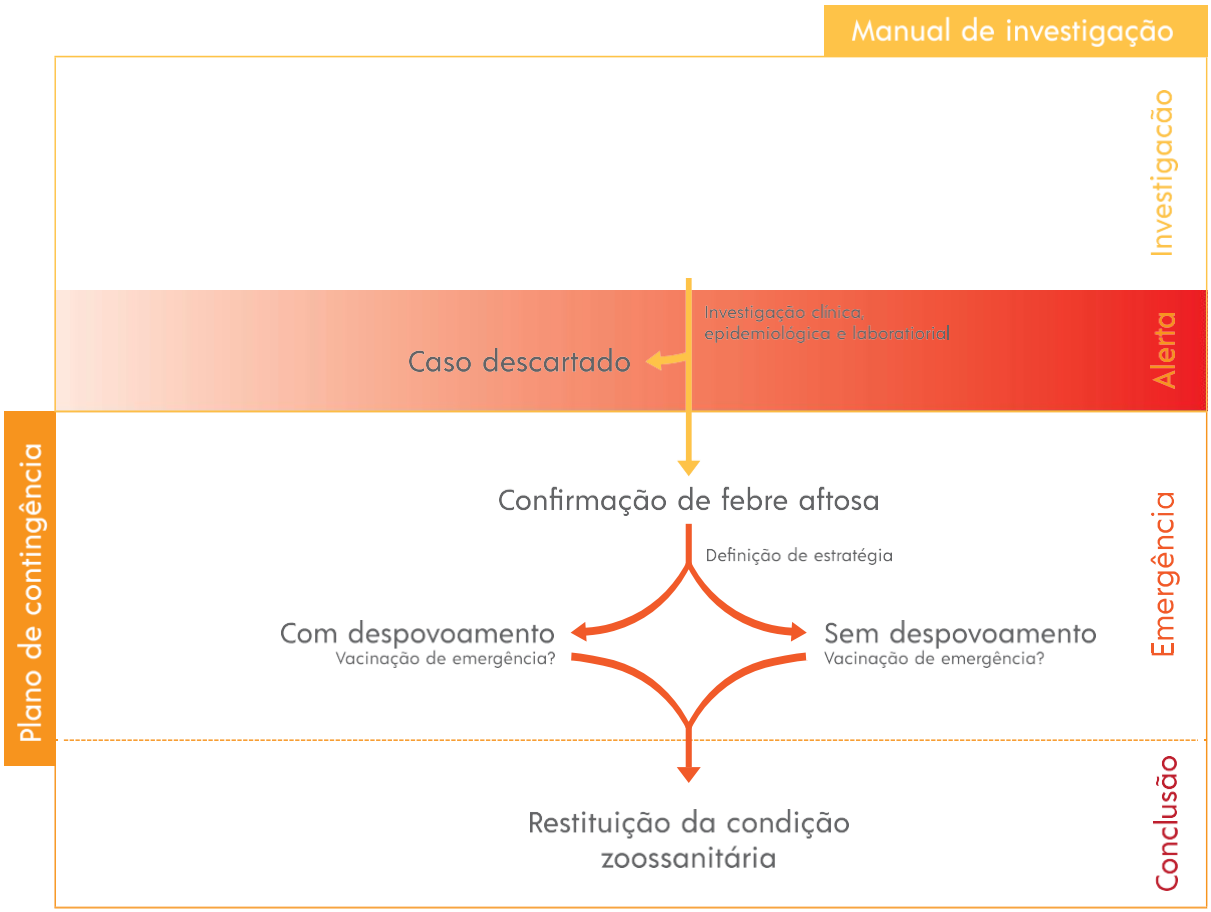


Figure 01. Representation of the main phases of the surveillance system for vesicular disease
Line 1: Research Manual
Line 2: The suspected case of vesicular disease
Linha3: Clinical research and epidemiology
Linha4 (direita): Discard suspicions
Line 4 (esquerda): Confirmation of suspicion
Linha5 (direita): Incompatible clinical signs
Line 5 (esquerda): Probable case of gallbladder disease
Line 6: Clinical research
Line 7: epidemiological and laboratory
Line 8: inconsiderate case
Linha9: Confirmation of foot-and-mouth disease
Line 10: Definition of strategy
Line 11 (direita): With depopulation
Line 11 (esquerda): Without depopulation
Linha 12 (direita): Emergency vaccination?
Linha 12 (esquerda): Emergency vaccination?
Line 13: Restitution of the condition
Line 14: of animal health

1. Initial recommendations

To improve the effectiveness of vesicular disease surveillance

actions and the ability to prompt reaction in emergencies to contain and eliminate dos[illegible]oci of foot-and-mouth disease, in addition to capacitated human resources, basic equipment, and financial resources, there is a need to have some specific information and structures detailed below.

1. Information and database needed for research

1. Database referring to properties, farmers, and herds: The ES, both local and central, must have, in electronic means, the updated list of rural properties and herds existing in the geographical area of their activity, according to the guidelines defined by the Map. Special emphasis should be given to the coding and georeferencing system of rural properties, according to the standards established by the Map. This information assists during research and alert actions, besides being essential in the zoo-sanitary emergency.
2. Animal movement database: The E[illegible], both local and central, must have a computerized system for the control and emission of GTA, with timely access to animal movement data of any rural property.
3. Other information: The SVE, both local and central, must have knowledge and registration, in an electronic and standardized way, of a series of data and information that will be of great importance in the alert phase and also for a timely response in the event of an emergency in foot-and-mouth disease. This data must be updated at least once a year. ~~A breakdown of~~ the necessary information is described in **ANNEX 1**.

1. Equipment, resources, and procedures for surveillance activities

Means of transport and communication: all UVL must have an adequate form for displacement and communication in its area of operation.

~~4. All UVL shall have an adequate way to move and to communicate in their operational area.~~

5. **Material for care and investigation of suspected cases of the vesicular disease:**

The material must be available and in conditions of use. For this, there is a need for discipline and organization on the part of the MVO responsible for UVL, which should systematically check the available equipment. ~~For this, there is a need for discipline and organization on the part of the MVO responsible for UVL, which systematically should check the available equipment.~~ If the material is incomplete, the trader must officially notify his or her superior. The SVE shall also create control and monitoring mechanisms at

the central level of the SVE, regarding the completion of the service kits in all UVLs of the FU. The focal point of the PNEFA in the EUF should implement a methodology of validation by sampling or census of THE UVL to evaluate every six months, the situation of this material in the STATE, taking the necessary measures to maintain 100% of the UVL with adequate material for the care of suspected cases of the vesicular disease. **ANNEX 2** shows the list of materials and instruments needed to perform an adequate service to a suspect case of the vesicular disease. The conference of the availability of the material includes the verification of the shelf life of detergents, disinfectants, antiseptics, and means of storage of samples. Concerning the latter, one should also observe the color and appearance (cloudy and color-altered solutions should be replaced even before the expiry date), besides periodically checking the pH (even for solutions containing pH indicator in its constitution).

Established and described procedures for a quick record and transportation of samples for lab tests: The ES shall maintain contracts with transport companies, for sending samples to the laboratory following the existing biosafety rule for packaging and sending biological samples, as well as have described alternative measures to ensure this logistical support if it has a problem with the contract in force.

~~SVE shall maintain contracts with transport companies, for sending samples to the laboratory following the existing biosafety rules or packaging and sending biological samples, as well as have described alternative measures to ensure such logistical support if it has a problem with the contract in force.~~

The shipment of the material by the SVE must be preceded by contact with the destination laboratory, to agree on details of time and form of delivery, which must be confirmed by phone or e-mail. The records of the investigation in e-Sisbravet must be made before sending the samples to the laboratory so that the PNEFA focal point in the SFA is already aware of the situation and can monitor the progress of the investigation. It is the responsibility of the PNEFA focal point in the SVE and the SFA, the follow-up of the shipment until it arrives in the laboratory.

6. **Financial resources: There must be** in the SVS, both at the UVL as well as in the central level, administrative procedures established and described for ready availability and use of financial resources, in the event of need during the investigation and alert stages.

3. Investigation stage

It is important to be aware that the foot-and-mouth disease surveillance system covers the following categories of diseases :

- **Target disease:** foot-and-mouth disease;

classical vesicular diseases (clinically indistinguishable):

- vesicular stomatitis, Senecavirus A (VAS) infection, vesicular exanthema and the vesicular disease of pigs (the last two exotic in Brazil);

other infectious diseases that, during their course, may present vesicular or ulcerative lesions:

- bovine vaccinia, bovine pseudo-culture, papular stomatitis, contagious ecthyma, bovine herpetic mamilitis, malignant bluetongue, infectious rhinotracheitis, and bovine viral diarrhea;
- **non-infectious diseases that may confuse clinical signs** (ex. claudication, sialorrhea) with infectious vesical diseases: intoxication by plants, fungi, chemical products, trauma, and others.

The definitions of a suspicious case of vesicular disease, a probable case of vesicular disease, a discard suspicion of vesicular disease, a discarded case of foot-and-mouth disease, and a confirmed case of foot-and-mouth disease are shown in the **technical form** at the specific site of MAPA and are according to the criteria of the Sanitary Code of Terrestrial Animals of **OIE**. Figure 2 shows the flow of investigation of a suspicious case of the vesicular disease.

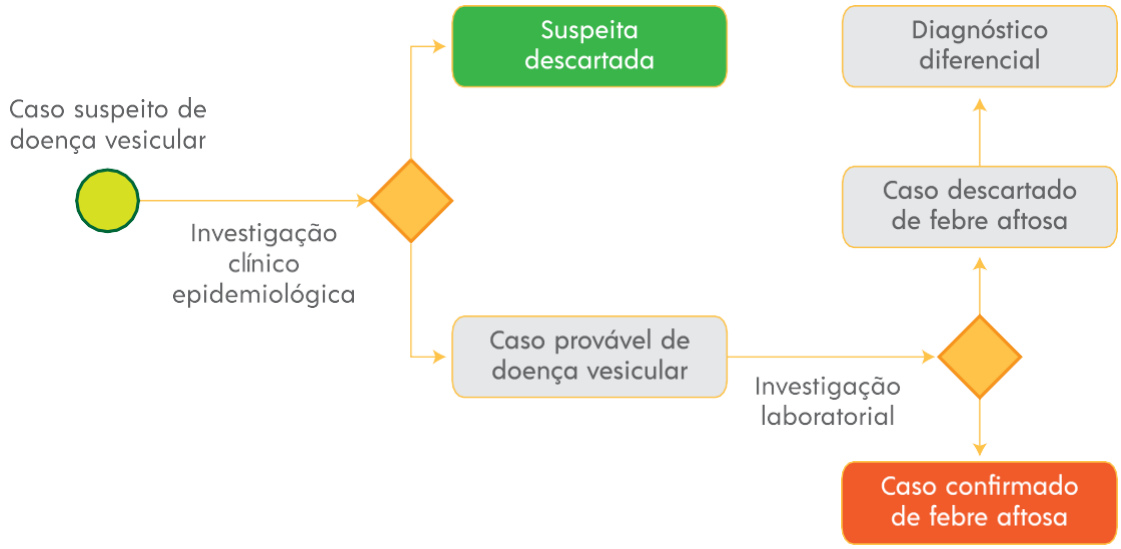


Figure 02. Representation of the investigation flow of suspected cases of vesicular disease

Case of suspicion of vesicular disease Epidemiologic-clinical investigation Discarded suspicion

3.1. General considerations on the investigation of a vesicular disease

The investigation phase begins when the SVO becomes aware of a suspicion of vesicular disease.

The notification of suspected vesicular disease is mandatory for any citizen, as well as for any professional who works in the area of diagnosis, teaching or research in animal health, according to current legislation.

Any suspected case of vesicular disease, regardless of its origin, should be investigated by the SVO within 12 hours. The outcome of the initial investigation may be suspected discarded or probable case of a vesicular disease. Among reasons of suspicion ruled out are the "absence of susceptible animals", "absence of compatible clinical signs" and identification of "non-infectious disease" or other infectious diseases that do not fit the definition of vesicular disease. Probable cases of the vesicular disease require continued investigation, including harvesting of laboratory diagnostic material, and mark the beginning of the alert stage.

The clinical and epidemiological evaluation of the suspect case of vesicular disease represents a decisive phase in the surveillance system. The official veterinarian should be able to technically decide on the progress of the investigation, requiring knowledge about the pathogenesis and epidemiology of vesicular diseases, training for investigation of vesicular disease, including material harvesting, and mastery of semiology techniques.

The clinical and epidemiological evaluation of the suspected case of vesicular disease represents a decisive phase in the surveillance system. The official veterinarian must be able to take a technical decision on the progress of the investigation, requiring knowledge on pathogeny and epidemiology of vesicular diseases, training to investigate a vesicular disease, including the collection of material, and mastery of semiology techniques.

Table 1 shows the main stages of the foot-and-mouth pathogeny, while **Figure 3** shows the theoretical evolution of those reactions expected in a post infected animal with no history of vaccination, highlighting the ideal times for the collections of material for viral insulation. This information was adapted from materials created by the Centro Panamericano de Febre Aftosa – **Panaftosa** – and they are significant so that the veterinary physician, after the clinical evaluation, is able to inform the date of the probable onset of the clinical symptoms in those animals evaluated.

Between the introduction of the virus (intracellular penetration)

and the appearance of the first lesions, there is the so-called incubation period, which lasts for 14 days, and is characterized by two distinct phases: the **eclipsis phase and prodromal phase.** During the eclipsis phase, the virus is not insulated even using sophisticated investigation means. This phase may last for a few hours and corresponds to the intracellular penetration of the agent and the formation of the first complete viral particles. As of the moment that these particles are disseminated throughout the system, through the blood, (viremia) and lymphatic, there is the beginning of the prodromal phase that lasts until the appearance of the typical foot-and-mouth lesions. In the prodromal phase, the animals show non-specific signs (feverish reaction, depression, and anorexia), common to several infectious diseases.

The vesicular diseases considered clinically indistinguishable from the foot-and-mouth disease are vesicular stomatitis, infection by Senecavirus A, swine vesicular disease, and swine vesicular rash, and differentiation between them is possible only through laboratory tests. For complementary knowledge about the main vesicular diseases, in **ANNEX 3** electronic addresses are available that direct to technical files, with information on foot-and-mouth disease and confusing vesicular diseases. In **ANNEX 4** it is possible to find information on the characteristics of the lesions and the susceptibility of domestic species, and of the human species, in the face of the group of vesicular diseases that can be confused with foot-and-mouth disease.

Table 1. Phases and period of development in foot-and-mouth disease pathogenesis

Foot-and-mouth disease pathogeny	Estimated time
a – Virus inhalation b – Infection of cells at the nasal cavity, pharynx, and esophagus c – Replication of the virus and dissemination to adjacent cells d – The virus goes to blood and lymphatic vessels e - Infection of lymph nodes and other glands f - Infection of cells in the oral cavity, paws, udder, and rumen	24 - 72h (1 to 3 days)
g – Fever starts h - Appearance of vesicles in the oral cavity, paws, udder, and rumen i - Salivation, nasal discharge, and claudication	72 - 96h (3 to 4 days)
j - Vesicle rupture and symptoms intensification k – Fever stops l - End of viremia and start of antibody production	120h (5 days)

m - Decreased virus titer in various tissues and liquids	As of the 8th day
n – Lesions heal and the animal starts to eat	As of the 10th day
o - Gradual disappearance of the tissue and liquid virus p - Increased antibody production	As of the 15th day
q - Complete healing (The virus can persist in the nasopharyngeal region for 6 to 24 months in cattle and 4 to 6 months in small ruminants)	15 days

SOURCE: www.paho.org

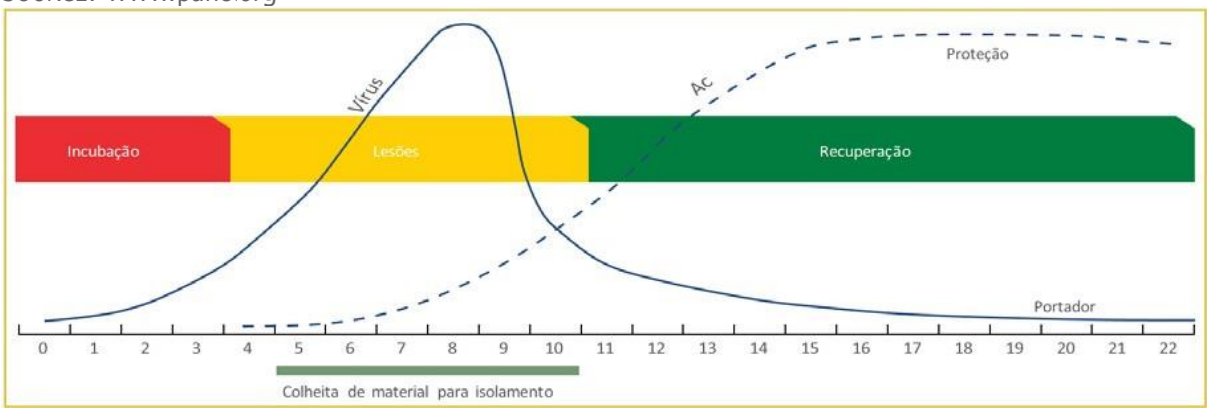


Figure 03. Theoretical evolution of the foot-and-mouth disease in one infected bovine, in days.

Source: PANAFTOSA, 1978.

~~Collection of material for insulation~~

In investigations of diseases indistinguishable from foot-and-mouth disease, it is essential to observe some aspects during the clinical and epidemiological evaluation, with emphasis on:

1) in a region where vaccination against the foot-and-mouth disease is not practiced, the clinical picture in cattle tends to be much more acute and evident, and the rate of infection is much higher;

2) foot-and-mouth disease does not always evolve with all the classic symptoms described, and lesions may appear to a greater or lesser extent depending on the strain of the active virus, the amount of infecting virus, and the immune status of the animals;

3) bovines are more susceptible to the foot-and-mouth disease virus, however, in animals with a certain degree of immunity to foot-and-mouth disease, only lesions in the mouth can occur, without generalization in the

legs, or only in one or two legs, without the appearance of oral lesions. An example of this scenario was the focus recorded in Monte Alegre (PA), in 2004, when in the investigation of the suspicion, the SVO identified only one bovine with a discreet clinical sign in only one paw. In non-vaccinated herds, susceptibility is independent of the age of the bovines;

4) swine are more sensitive to infection and show much more serious signs: the vesicles in the snout can be large and full of bloody fluid; lesions in the mouth are usually dry, with necrotic epithelium; foot injuries are severe and ~~the hoof can be completely released at the level of the coronary band~~. The main route of infection is digestive, which requires a higher infectious dose when compared to cattle. This explains, in part, the presence of uninfected pigs in properties with the occurrence of foot-and-mouth disease in bovines, as observed in the index focus registered in Eldorado (MS), in 2005, and during the occurrence in Rio Grande do Sul, in 2000;

5) in sheep and goats, mainly considering strains present in the South American continent, foot-and-mouth disease occurs more mildly (with mild symptoms), even though the animals are not vaccinated. These animals present lesions in the mouth and vesicles in the region of the ~~crown of the hooves~~ in smaller quantities, smaller and more difficult to be identified;

6) 6) depending on the strain of the foot-and-mouth disease virus, not all susceptible species are always affected, even when living in the affected epidemiological unit. For example, in the outbreaks recorded in 2000 and 2001 in Rio Grande do Sul, although pigs and sheep are living with cattle, only the latter showed clinical signs;

7) 7) vesicular stomatitis, on the other hand, is endemic in some regions of Brazil. Its major difference is the susceptibility of Equidae. However, there are cases in which the disease has been identified in cattle and pigs, not manifesting in equines. In bovines, the morbidity rate related to vesicular stomatitis tends to be higher in adult animals;

8) although rare, foci of vesicular stomatitis and foot-and-mouth disease can occur simultaneously. Thus, even in the concomitant presence of clinical signs in bovine and horses, the possibility of the foot-and-mouth disease cannot be ruled out without laboratory testing in bovine samples;

9) *Senecavirus* A infection affects pigs and is endemic in some regions of Brazil, occurring mainly in farms with technical apparatus. The first records in the country occurred ~~as~~ of 2015. It is commonly detected in slaughter establishments with the detection of healing or healed lesions. ~~For this reason, it is important to raise the awareness of the producer and of the responsible veterinarians, technicians for timely notification, even in the farms, to allow the collection of suitable material for the diagnosis;~~

3.2. swine vesicular disease has a low worldwide incidence, registered in European and Asian countries - it has never been registered in the Americas. It only affects pigs; swine vesicular rash has been diagnosed only in the United States and Iceland. The disease was considered

eradicated in 1959 and, since then, no more cases have been reported anywhere else in the world.

3.3. Clinical inspection of animals and epidemiological evaluation

The priority of the veterinarian responsible for the investigation of the suspected case of vesicular disease, at the time of the first clinical inspection of the animals, is to dismiss the suspicion or confirm the probable case of the vesicular disease. Regardless of the susceptible species involved, the checklist should, among other things, assess the presence of:

1) high fever up to 41 ° C, which declines from the second day;

2) intact vesicles and blisters, noticeable only during the acute phase of the disease, which lasts up to two days (blister is a small survey of the epidermis containing serous fluid, while the blister is a blister larger than 0.5 cm in diameter, usually formed by vesicle coalescence);

3) sharp drop in milk production in dairy herds, preceding the first clinical signs;

4) salivation and claudication (in pigs, greater difficulty in locomotion is observed);

5) bright red, moist, and non-bleeding secondary erosions, with or without fibrin deposit, in the regions of the snout, nostrils, mouth, coronary band (crown) of the hooves, interdigital space, nipples, and udder;

6) sudden death in very young animals, caused by hyperacute myocarditis;

7) distribution of animals with clinical signs:

a) in non-vaccinated species the percentage tends to be high among cattle living in the same pasture, paddock, or sheds, which may not be observed in herds submitted to repeated vaccination steps;

b) in herds with a recent history of vaccination, clinical signs prevalent in animals or age groups with low expectations of immune protection; and

c) list of the probable onset of clinical cases with the entry of susceptible animals into the herd or of cattle trucks for loading or unloading animals. In pig farming, special attention should be paid to the origin of the food.

8) In cases where animals show salivation and claudication simultaneously, with detection or suspicion of vesicular injury, the measures foreseen for probable cases of vesicular disease must be taken. To avoid a lower sensitivity of the diagnosis, ~~one must~~ examine the mouth of every animal with a limp and the paws of animals with lesions in the mouth or nostrils.

9) 9) The identification of a probable case indicates the possibility of the occurrence of foot-and-mouth disease, pointing to another important objective of the investigation phase: the determination of the probable beginning of the infection. For this, in addition to the information obtained during the interview and anamnesis, it is important to ~~thoroughly~~ describe the secondary lesions (which develop after the eruption of vesicles and the

beginning of the healing process) to estimate the beginning of the appearance of clinical signs the likely start of the infection. It is not uncommon for the interviewee to be mistaken when asked about the onset of injuries. For this reason, it is extremely important that the veterinarian uses his knowledge and experience to correctly assess and record the estimated time, whether due to a good anamnesis or to a good clinical inspection and a correct estimate of the age of the lesions. For example, an old lesion, in the healing phase, could not have started two days ago, or else the incompatibility between the report of the presence of signs 20 days ago and all animals present freshly ruptured vesicles. In these cases, the investigation should be expanded to the maximum to clarify all the inconsistencies detected in the information provided. Therefore, the definition of the age of the lesions, particularly the oldest ones, is essential to establish the historical evolution of the outbreak, with emphasis on the definition of the origin of the infection and the period of greatest risk of diffusion of the viral agent. As a source of consultation and estimate of the age of the lesions, the collection of FMD images can be consulted.

10) In general, once the vesicles are broken, the speed of healing it will be influenced by different factors, which allows, in practice, a rough estimate of the age of the injury. Up to the fifth day, good accuracy is still possible, however, as time goes by, the difficulty of estimating the age of the lesion increases. Below are some examples in the estimate of the age of lesions on the tongue of bovines and on paws of pigs:

a) closed vesicles: up to two days;

b) vesicles recently ruptured with pieces of epithelium still adhered to the edges of the lesions: one to three days;

c) ruptured vesicles with loss of epithelium and absence of clear edges of fibrous tissue: between three and seven days;

d) open lesions with fibrous tissue with sharp edges: between seven and ten days.

3.4. Clinical and epidemiological aspects of other diseases that can be confused with foot-and-mouth disease

It is important to emphasize that the dismissal of suspicion must be technically well-founded and when in doubt, the professional should continue the investigation. It is worth remembering that in regions without vaccination the clinical picture in cattle is more evident, while in regions with vaccination it is unlikely that classic clinical pictures will occur in this species, with easily detectable lesions. In this case, the expected is the presence of clinical signs in a reduced number of animals, with less severe lesions, which can be verified indiscriminately in the tongue, mouth, interdigital spaces, or udder.

In the late treatment of suspicions, it is more frequent to notice the presence of secondary lesions, such as erosions, ulcers, and crusts. In these cases, the veterinarian should be aware of some diseases that can confuse the diagnosis of vesicular disease: bovine vaccinia, bovine false

smallpox, papular stomatitis, contagious eczema, bovine herpetic mamilitis, blue tongue, malignant blue tongue, bovine viral diarrhea / mucous membranes, bovine infectious rhinotracheitis/infectious pustular vulvovaginitis, among others. It is important that the MVO access technical materials and recent publications to be aware of the clinical aspects and the course of these other diseases that may have clinical signs considered to be confusing with the vesicular disease. In addition to the confounding infectious diseases, other common cases of discarding suspected vesicular disease involve intoxication and physical or chemical trauma. In the case of intoxications, the substances responsible for photosensitization, the caustic or abrasive chemicals, and the fungi of the genus *Clavaria* and *Phytomyces chartarum* stand out.

Phytomyces chartarum fungi cause a disease called facial eczema, affecting cattle and, more rarely, sheep, characterized by a clinical picture of photosensitization.

Fungi of the genus Clavaria, associated with eucalyptus plantations, in hot seasons, and with high humidity, are important in the south of the country, causing intense sialorrhea and necrosis of the lingual epithelium. Conjunctival congestion and corneal opacity are observed in sheep, leading to blindness, difficulty in walking, and falling of the animals. In cattle, it is possible to observe the detachment of horns, tail hair, or wool strands in sheep.

As for the traumatisms, different elements can lead to salivation and lameness, especially the injuries caused by dry and hard pastures, freshly cut pastures and crops, or even soils with a predominance of gravel. In dairy cattle holdings, foot disorders are common, with the following conditions: interdigital dermatitis; erosion of the horny layer; bead erosion; verrucous dermatitis; vegetative interdigital pododermatitis; digital dermatitis; interdigital phlegmon; diffuse aseptic pododermatitis; circumscribed pododermatitis; necrotizing pododermatitis; fissure of the nail; white line disease; phalanx fractures; sole and heel abscesses; sole ulcer; forceps ulcer; high arthrosis; dislocations; and sole bleeding. Still concerning foot disorders, in sheep farms, it is common to occur the contagious ovine pododermatitis (foot root).

3.4 Step by step in the care and investigation of reports of vesicular disease

The following are the procedures to be adopted by the veterinarians of the SVO before notification of suspected vesicular disease. It should be emphasized that, in addition to the importance of time in attendance, another fundamental issue is the correct and complete record of the activities performed.

Upon receipt of notification at UVL or e-SISBRAVET, The UVL should:

1. Immediately register the notification of the suspicion in e-SISBRAVET According to guidelines available in the e-SISBRAVET Manual.

When the notification is made by telephone, it is recommended, as a precaution, to register the originating telephone number and confirm through a callback.

However, if the person does not want to be identified, confidentiality must be guaranteed. The person who made the notification of the suspicion, if he or she has been in contact with the animals, should be advised on the necessary biosafety procedures to avoid the spread of the possible infectious agent, mainly regarding the non-movement of suspect animals and their direct contacts, and not entering any other property with animals susceptible to foot-and-mouth disease until the end of the investigation that will be carried out by SVO.

If the notification is made directly by e-SISBRAVET, it will be included in the list of pending notifications for classification of the respective UVL linked to the municipality where the animals are located, where the MVO can consult and make its classification.

2nd Initial information survey

Initially, the information available in the property registry (SVE registration system or "Prepare service" tab of e-SISBRAVET) must be evaluated, highlighting: identification of the property and its producers, a survey of the number of livestock farms and the existing herd; the intensity of movement of animals (mainly occurrence of entry and egress in the last 30 days); date of last vaccination; geographic location and access routes. Also, identify the neighboring properties and those with some link (that maintained in the last 30 days some type of relationship - entry/exit - with the property that is under investigation). It is still important to obtain information about other properties belonging to the same owner and other producers who may be involved in the property. This first survey of information must be carried out objectively and quickly so as not to compromise the reaction time. Depending on the result of the initial service, new information must be obtained to carry out additional analyzes.

Cautiously, between the period of notification of a suspicious case and its attendance by the SVO, it is up to the MVO to prevent the issuance of an animal transit document that originates or destines the epidemiological unit where suspected cases of the vesicular disease are.

In the "Prepare Service" tab of e-SISBRAVET, inform when the investigation will be attended, consult support material, if necessary, and print the investigation forms and Annexes.

3rd Displacement to meet the notification

Alongside the collection of initial information, the preparation of the vehicle for displacement and the kit with the material to care for suspected vesicular diseases must be provided. The UVL team and the immediate superior should be informed of the time and reason for departure: attendance to notification of suspected vesicular disease.

The service should preferably be immediate or, at most, within 12 hours. In the case of notifications by third parties or by surveillance, seek to identify and make prior contact with the owner or person responsible for the animals to combine the best and quickest way to carry out the clinical inspection of animals susceptible to foot-and-mouth disease. If the notification was presented at the end of the day and depending on the distance and road conditions and lighting at the location, the most recommended is the service in the early morning hours of the following day. If the veterinarian responsible for the UVL is not present at the time of notification, the server that receives it must perform the initial registration in the system and contact the central unit or the regional unit (if any) to assess and define the care by another SVO veterinarian. If there is resistance on the part of the owner or responsible for the animals, the notification can be attended with the help of the police forces, and all resources must be exhausted before using this action. SVO professionals must carry their functional card or another professional identification document. It is recommended to have a copy or access to the legislation that gives them the competence to take the necessary measures in the area of animal health protection, with emphasis on entering the rural property or any other place to examine animals suspected of having vesicular disease, and to the interdiction of the site, if the risk of the presence and diffusion of an infectious agent is confirmed.

The investigation forms can be printed with the previous information from the e-SISBRAVET, in the tab "Prepare Attendance", facilitating the filling during the investigation in the field and later in the system.

The treatment and resolution of the suspicion must be carried out as quickly as possible. Thus, **the displacement of the professional should be direct to the property with suspected cases**, without stopping at other rural properties during the journey. If the notification was presented on holidays or weekends, those responsible for care must have full autonomy for the use of vehicles and the entire structure of the institution necessary for the work in question.

4º Actions at the property

Upon arrival at the property with suspicious animals, the professional must take all necessary care with biosafety and dedicate himself with attention to the research work, interview, clinical inspection of the animals, and epidemiological investigation. Some important points to consider:

a) go directly to headquarters to conduct an initial interview with those responsible for the animals (carry out a detailed anamnesis, use the questions in the initial investigation form and the specific vesicular disease investigation form) and define the best way to perform the clinical inspection of the animals. In more extensive properties, it is desirable for epidemiological

evaluation, the elaboration of a simplified sketch, indicating the location of the houses or pastures and the distribution of animals susceptible to foot-and-mouth disease;

b) go, with all the necessary material, directly to the batch of animals under suspicion and inspect them, if possible in the same place where they are. If necessary and, as long as the risks for spreading the disease are reduced, animals can be moved within the property to a location that facilitates clinical examination. The inspection must start with the suspected batches, considering that at this stage of the investigation the most important thing is to confirm or rule out the suspicion of infectious vesicular disease;

c) inspect the largest possible number of animals in the batch under investigation. For transmissible diseases such as foot-and-mouth disease, the order of inspection of animals that are living together has no epidemiological importance and may start inspection by healthy animals or by animals with clinical signs. However, given the need to quickly assess the suspicion presented, and especially in situations where the clinical inspection of the animals proves to be complex, it is recommended that the inspection begins with the animals with apparent clinical signs, to collect material for laboratory diagnosis (if necessary). The important thing is to examine the largest number of animals, both those with clinical signs and those that are apparently healthy, to assess the dispersion of the disease and the age of the lesions, in addition to establishing, with the support of the interview, the probable beginning of the sanitary episode; the clinical inspection must be extended to other species susceptible to foot-and-mouth disease and Equidae on the property. The veterinarian responsible for the care must keep in mind that, depending on the clinical and epidemiological picture found, there will be a need for other visits for complementary inspections in the herd. The first visit has the priority of discarding or confirming the suspicion and, when necessary, collecting samples to be sent to the laboratory recommended by the Map. A basic guide for examining animals ~~suspected of having the~~ vesicular disease can be found in ANNEX 5;

d) d) in addition to the clinical inspection, an epidemiological assessment should be carried out, taking into account indicators of animal demography (age group, sex, density, type of breeding, etc.), the expectation of immunity of existing animals, recent entry of animals to the batch, management, simultaneous occurrence in different species, quality of pasture and soil (if there are stumps or stones, for example), among other aspects.

e) The clinical and epidemiological investigations carried out in that first moment (still on the rural property) serve to support the judgment of the health condition of the animals, guiding the veterinarian to establish a definitive or provisional diagnosis and leading him to one of the following possibilities: dismiss the suspicion or confirm the occurrence of a probable case of vesicular disease (Figure 4)

Notification of the suspected case of vesicular disease
Investigation by the SVO
No
Yes
Suspicion discarded
Confirm the suspicion?
Probable case of the vesicular disease

Figure 04. Beginning of the service flow for suspected vesicular disease

a. Discarded suspicion

The suspicion can be dismissed on the property by the MVO, given the following possibilities:

- cases of false reporting or absence of animals susceptible to foot-and-mouth disease; an occurrence of non-infectious disease (intoxications, foreign bodies, injuries); or
- occurrence of another infectious disease, presenting an incompatible clinical picture with the vesicular disease.

The MVO must list all the information that supported its diagnosis, registering it in the investigation form, being able to make photographic records for insertion in the system. To describe the lesions, appropriate technical terms should be used, including location, number, shape, size, depth, color, degree of healing, and age estimate.

In the case of discarded suspicions of vesicular disease, the investigation should be closed, recording the final diagnosis and the information on which it is based, followed by the registration and closing of the occurrence in e-SISBRAVET.

In all cases, take advantage of the trip to the property to update the registration information and existing herds. If the property is not in the register of the state veterinary service, obtain the necessary information for its inclusion in the database and pass on to the owner or responsible for the animals the guidelines and information on the legal and sanitary aspects involved.

b. B. Probable case of vesicular disease

Faced with a probable case of vesicular disease, the MVO should pay special attention to the activities of collecting material for diagnosis, information gathering, and biosafety. Then, some procedures and information about each of these activities shall be highlighted, to be considered in the place where there are

probable cases of the vesicular disease. It is worth emphasizing that, in this alert phase, a very important time interval elapses, in which actions must be conducted, taking into account the possibility of confirming a case of foot-and-mouth disease, and which are described in the Alert Phase.

b.1. Collection of material for diagnosis

The impossibility of making a differential clinical diagnosis of vesicular disease, associated with the frequent lack of epidemiological information at the beginning of the investigations, requires laboratory support to support the confirmation of the diagnosis. The quality of the collection and shipment of samples directly interferes with the time for laboratory processing. At this point, the following guidelines stand out:

1) take a maximum of samples from 10 animals in each visit;

2) all samples must be listed in the form associated with the respective occurrence, previously registered in e-SISBRAVET, thus allowing the preparation of materials and personnel for the execution of the tests and, consequently, greater agility in the processing of the sample;

3) the samples sent to the laboratory must be accompanied only by the specific form (FORM LAB) in physical format; whenever possible, the photographic record of the lesions is recommended, taking all necessary biosafety precautions;

4) every animal submitted to the collection of samples must present permanent or long-term individual identification, unique and unambiguous. The collection of samples must be informed in the "Clinical Investigation" tab of e-SISBRAVET for the animals inspected and submitted to the collection. FORM LAB will be completed by filling in the "Sample" sub-tab, where the animals' identification will be transported.

5) use tubes with screw cap and conical bottom of 15 or 50 ml, sealed with plastic paraffin film, or microtubes, depending on the sample volume;

6) 6) The volume of the preservation medium used must be as small as possible, just enough to cover the harvested material.

b1 . Serology

In the initial phase of the investigation, serum collection should be limited to animals with clinical signs, and it is recommended to collect a maximum of serum samples from 10 animals.

According to the World Organization for Animal Health - OIE - a positive reaction to the detection of antibodies against the FMD virus can have four causes: natural infection; vaccination; presence of maternal antibodies; and cross-reactions (heterophilic). Concerning maternal antibodies, the OIE indicates that they are normally found up to six months of age in cattle, but can be detected longer in some individuals.

When it comes to regions where vaccination is not practiced, the identification of antibodies against the foot-and-mouth disease virus is

information that is easier to analyze, and should always be associated, however, with the clinical and epidemiological picture found. Therefore, although the identification of antibodies against the foot-and-mouth disease virus in animals with clinical signs of vesicular disease represents an important finding in unvaccinated herds, in areas where a surveillance system is in place it is more likely that confirmation of the case will be carried out by viral isolation and identification.

In places where vaccination is carried out, the use of laboratory tests to detect antibodies against the FMD virus is of limited value when the analysis is individual. The MVO responsible for investigating suspected vesicular disease must take special care in obtaining a history of vaccination against foot-and-mouth disease, seeking to cross-check information.

b.1.2 In the case of assistance with epithelium collection

The identification of probable cases that allow the collection of material for viral isolation is the most desired situation, providing more security to the final diagnosis and indicating that notification and care by the SVO occurred promptly. It is important to follow these recommendations:

1) The material of choice is composed of vesicular fluid and fragments of recently broken vesicle epithelium, including the edges of the lesions;

2) If the vesicles are intact (not ruptured), collect the liquid and the epithelium separately. Vesicular fluid must be obtained in insulin-type syringes, which must be transferred to microtubes without preservatives. You should not send the liquid to the laboratory inside the syringes;

3) If the volume is less than 200 microliters (0.2 ml), an equal volume of preservation medium should be added to the vesicular liquid sample and freeze the material;

4) In small vesicles, where it is not possible to aspirate the liquid, or recently broken, make a swab. The swab must not be made of cotton, as this material inhibits PCR reactions. Some swab options are dracon, polyester, and others. The tip of the swab used must be cut and added to a microtube, containing 1 ml of preservation medium;

5) In the case of discrete injuries, such as those observed in injuries caused by Poxvirus, the use of punch is suggested. The obtained fragments must be placed in microtubes with phosphate buffer in sufficient volume to cover them;

6) 6) The material collected from the oral and nasal regions is more suitable due to the lesser presence of dirt. The legs and udders must be washed with clean water to remove dirt before harvesting (do not use any type of soap or antiseptic). Pack the material collected in separate bottles, for each animal involved, containing Vallée's Liquid (ANNEX 6) in sufficient volume to cover the tissues. Small fragments of epithelium should preferably be sent in microtubes;

7) 7) The material collected from each region (oral, nasal, foot, and udder)

should be placed in separate flasks. Never mix materials from different animals in the same bottle. The bottles should be properly sealed, identified, packed in sealed bags, and kept refrigerated or, preferably, frozen (-20 ° C). Once sealed, the bags must be cleaned and disinfected before being packed in the isothermal box (the use of small sprayers or manual sprayers, with disinfectant solution, facilitates this operation);

8) It is suggested to evaluate animals at different stages of the disease, seeking to establish the age of the lesions. This is an important point, where the veterinarian must assess the number of animals for inspection. If new cases are found, with an easy collection of samples, the professional must increase the number of animals inspected (without compromising the service time), to detect the oldest lesions to support the definition of the probable onset of the disease. On the contrary, if you find only old lesions, with difficulty in collecting material, the professional should inspect the largest possible number of animals, to find newer lesions, with a greater possibility of viral isolation;

9) The owner or person in charge of the animals must be informed of the prohibition of treating animals that show clinical signs so as not to compromise further sampling, if necessary;

10) In swine, material harvesting from refrigerators should preferably be carried out on animals before the scalding process. If suggestive lesions are detected in the inspection lines, the MVO should check for the existence of animals from that batch that have not yet entered the slaughter room for a clinical evaluation, to collect samples in the arrival and selection pen.

b.1.2 In the case of assistance where the epithelial collection is not possible

In peculiar situations, such for example in ruminants tested for transit and which have resulted in a positive serology for foot-and-mouth disease, without presenting clinical symptoms, the investigation can be carried out using techniques that aim to collect paired samples of esophageal-pharyngeal fluid (LEF), with an interval of 15 days, using an appropriate collecting cup. It is worth noting that LEF collection for the foot-and-mouth disease is carried out only in ruminants. These situations must be recorded on standardized investigation forms. LEF harvesting requires specific training and animals must be fasting for at least 12 hours (collection procedures described in ANNEX 7). The esophageal-pharyngeal liquid must be stored in an equal amount of MEM and frozen as soon as possible, proceeding with the measurement of the pH of the preservation medium before the material is collected. In the case of negative samples, it is recommended to perform another collection, with an interval of at least 15 days, in search of a more consistent diagnosis.

In pigs, one should choose to collect fragments of the tonsils and keep them frozen until arrival at the laboratory.

The Vallée medium and MEM, used for the preservation of the epithelium and LEF samples, have a different composition and are described in ANNEX 6. In addition to the conservation function, these means aim to prepare the samples for the different procedures to be submitted to the laboratory. Thus, the use of these means must respect their specific purposes, and it is not appropriate to substitute one for the other. The table below shows the recommendations for the use of the medium by type of material harvested.

Table 2. Recommended preservation mediums for different materials

	Vallée liquid	MEM
Epithelium	X	X
Vesicular liquid	-	X*
Vesicles Suab	-	X
LEF	-	X

*only in samples inferior to 200 microliters

In **exceptional** cases, when it is not possible to use these means, contact the laboratory responsible for the ~~EVS~~ diagnosis or screening sector for specific guidelines.

b.1.4. Sample collection for differential diagnosis

Considering that the PNEFA's target disease is foot-and-mouth disease, it is essential to highlight that the tests for the differential diagnosis will only be carried out in the face of negative results for foot-and-mouth disease. ~~With a view to~~ a conclusive diagnosis, depending on the quality and quantity of the samples collected during the first clinical inspection, there may be a need to return to the property or the place where the animals are to collect new samples.

Vesicular diseases clinically indistinguishable from foot-and-mouth disease and which are endemic in the country (Vesicular Stomatitis and VES) are part of the differential diseases investigated in routine laboratory analyzes, in investigations of probable cases of the vesicular disease.

b.2. Information survey (epidemiological investigation)

After finding a probable case of vesicular disease, the collection of information should be further investigated through interviews with the owner or those responsible for the animals. The questions ~~should seek to~~ determine the probable start date of the health event and its possible origin and to assess the degree of risk of diffusion. For this, remember that in the case of the foot-and-mouth disease the incubation period is up to 14 days, ~~at most,~~ being more common between 2 and 7 days. The determination of the bonds is very

important, and information on animal movements ~~covered by~~ GTA should be ~~sough~~, and even information carried out informally.

The MVO responsible for the service must meet with the people directly involved to conduct the interview, at which time they must advise on biosafety recommendations. At the end of the interview, it should be verified that all information for filling out the forms has been collected, paying special attention to the probable onset of the disease, and making a relationship between the information found and the chronology and assessment of the age of the lesions examined on the date of the visit. A detailed service flow can be viewed in ANNEX 8**.**

b.3. Biosafety activities

a) Biosafety measures are the set of activities used to avoid or minimize the risks of spreading the disease. ANNEX 9 contains the biosafety procedures at the entrance and exit of the place where there are probable cases of the vesicular disease, ~~for the professionals involved in the service~~. The main procedures that must be adopted on the property are described below:

b) 1) draw up an interdiction term and pass on, clearly and objectively, the guidelines on the care to be taken to avoid the spread or aggravation of the health problem. UVLs must have forms of interdiction and de-interdiction for ready use. The interdiction term must contain the reason for its application, its legal basis, ~~space for~~ the owner or person responsible for the herd, and the main prohibitions established;

c) 2) among the main guidelines and prohibitions that must be applied, adapting them especially to issues such as property size and the predominant livestock production system, the following items stand out:

d) a) prohibit animals and products to leave the property at risk for the spread of foot-and-mouth disease. Also ~~not~~ included ~~are~~ non-susceptible animals, due to the risk of mechanical transmission of the FMD virus;

e) b) products not directly associated with the risk of spreading the disease can spread it mechanically, so all measures must be taken to disinfect the means of transport and packaging material for these products;

f) suspend work with tractors and machinery that may increase the chances of mechanical spread of the virus;

g) leaving the batch with probable cases of vesicular disease under the responsibility of only a small group of workers, who will not be able to have access and contact with the other susceptible animals on the property;

h) advise those present so that they do not visit other properties with animals susceptible to foot-and-mouth disease and do not maintain contact with other people who also deal with animals susceptible to the disease (such conduct should be stricter for those people who maintained direct contact with sick animals);

i) forbid visits by anyone without authorization, including veterinarians,

technicians working with artificial insemination, and other professionals and producers, especially those who have contact with animals susceptible to foot-and-mouth disease;

j) milk production must be retained on the property. Do not use the product and its derivatives in the feeding of susceptible animals (especially calves and pigs). Milk represents a direct risk and also of mechanical diffusion, through the transport truck and the people who handle its collection. Regardless of the quantity produced, the removal of the product from the property cannot be authorized as long as the risks of spreading the disease persist. Even though it is a measure that involves several economic and social issues, it must be considered that milk has a low unit value and it is often safer to recommend its destruction, with compensation to the producer. Alternatives to be employed and recommended concerning milk include:

I) ~~the destination for the manufacture of products submitted to thermal processing (mozzarella, curd, among others) inside the property;~~

II) internal milk consumption of healthy animals, after boiling for at least five minutes, if it is not possible to perform the inactivation process recommended by the OIE;

III) destruction, with the use of chemicals that lead to a change in pH (for example, vinegar or caustic soda), discarding the product in an open ditch for this purpose. Do not spill the product into rivers or other water collections. In the matter of biosafety, special attention should be given to disinfectants used in different situations. In ANNEX 10, adapted from Panaftosa's **Manual of Procedures for Attention to Foot-and-Mouth Disease and other Vesicular Diseases**, information and a list of chemicals that can be used in cleaning and disinfection work.

5º Return to UVL

After returning directly to UVL, the veterinarian must communicate the result of the investigation to the superiors and other members of his work team, and complete the registration of the activities carried out in the forms and in e-SISBRAVET, with the upload of the forms and photos.

Upon confirmation of a probable case of vesicular disease, the Alert Phase begins.

4. Alert phase

4.1. Surveillance activities

The alert phase involves the period between the confirmation of the probable case of vesicular disease and the definitive diagnosis supported by laboratory tests. This phase should be carried out considering the **probability of foot-and-mouth disease**. The main objectives of the actions developed in this phase are: to start activities to assess the

possibility of the disease occurring in other herds; restrict the movement of animals susceptible to foot-and-mouth disease to minimize the risks of spreading the possible viral agent; and continue to gather information to, if necessary, implement animal health emergency actions. Activities should be conducted with great caution, so as not to cause turmoil or panic in the local community. Only the professionals needed to carry out prevention and complementary epidemiological investigation operations should be involved.

Upon return to the UVL:

1) prepare, identify, record, and properly pack the collected material. After adjusting the logistics with the superior, send the material, as soon as possible, packaged and properly identified, to the screening laboratory of the central EVS unit;

2) review and insert all information on the forms in e-SISBRAVET; immediate notification to the higher authorities and the DSA is done through this route, and it is no longer necessary to send forms by email. The dates of probable start, notification, attendance, registration, and result will generate the performance indicators for investigations of vesicular disease. FORM LAB must be generated from sample information inserted in the system and this can also be done by the central unit, avoiding delays in the shipment of the material by UVL.

3) to deepen the analysis of the links involving the herd with probable cases of the vesicular disease. Confirm all properties located around the establishment with affected animals (defined as properties with an epidemiological link due to geographical proximity) and those that, in the last 30 days concerning the possible onset of the disease, maintained a link of entry or egress of susceptible animals with the herd under investigation. Keep the issuance of the investigated property's GTA suspended and, together with the central EVS unit, suspend the issuance of linked properties' GTA.

4) all link information, formal or informal, must be entered in e-SISBRAVET (sub-tab Linked establishments of the Epidemiological Investigation tab). The system generates investigation notifications for the UVLs involved, at the UF itself and at other UFs, if applicable;

5) 5) If the property involved is located in international border regions, the veterinary services of the countries involved must be notified immediately. It will be up to the SFA of the state involved to inform the Animal Health Department of the Map, which will be responsible for informing the SVO of the neighboring country;

6) In the case of regions with dairy production, communicate and guide those responsible for collecting milk or other dairy products. The collection lines must be identified and the sections that involve the properties under investigation must be closed, defining alternative routes;

7) Estimate the number of teams needed to carry out the investigation on the properties with links in your area of jurisdiction and forward the demand to the EVS central unit for immediate measures.

8) Consider that, in the case of foot-and-mouth disease, animals can eliminate

viruses from three days before the onset of clinical signs, which makes it necessary to provide a team for each linked property, to reduce the risks of spreading disease. Even if no clinical signs compatible with the vesicular disease are observed, it is important that, under these conditions, all biosafety procedures are adopted when entering and leaving the properties.

9) Schedule additional daily inspections of the prohibited establishment, until confirming or discarding the case of foot-and-mouth disease, to monitor the evolution of clinical cases; assess compliance with the established restrictions and collect additional information that may support the epidemiological investigation, especially the start date and origin of the disease (use FORM COM to record information obtained during complementary investigations at the establishment, recording data and loading data forms and photos on e-SISBRAVET).

10) Revisar todas as informações constante no **ANNEX 1**.

Na unidade central do SVE:

After becoming aware of the probable case of vesicular disease, the PNEFA focal points in EVS together with the epidemiology sector should:

1) Analyze the data recorded on the e-Sisbravet, investigation forms, and photos provided;

2) Inform the PNEFA focal point in SFA;

3) Contact the person responsible for the diagnostic laboratory, inform about the samples and the date of the probable shipment. It is recommended to send an email with FORM LAB, in due time, so that the laboratory team can program itself for the receipt and analysis of the samples in the shortest time possible;

4) 4) Prepare and send the collected material to the laboratory indicated by the FMD Division of the Map, in the shortest possible time. It is the responsibility of the PNEFA focal point in the EVS, together with the PNEFA focal point in the SFA, to monitor the shipment until it arrives at the laboratory. ANNEX 11 contains recommendations on packaging, packaging, and shipping of infectious material to the laboratory, according to international regulations.

5) The agent identification samples taken from probable cases of the vesicular disease are classified as **UN3373 - BIOLOGICAL SUBSTANCE - category B**, according to the guidelines of the United Nations (Recommendations for the Transport of Dangerous Goods);

6) ~~Also perform the analysis of~~ the property register and animal transit, identifying properties with an epidemiological link, especially in the 30 days before the probable onset of the disease and neighboring properties;

7) Delimit previously a probable emergency area, contemplating the initial geographical space for possible interdiction and intervention, if the result is confirmed. The initial delimitation must be carried out by the EVS epidemiology sector, adopting as a criterion the total area of the municipalities covered by the 25 km radius, measured from the property with a probable case. This preliminary work aims to obtain information necessary to optimize

the response time in the event of confirmation of the FMD case;

8) Once the possible areas of epidemiological risk have been defined (3km periphery; 7km surveillance and 15km protection), the following information by area must be mapped and collected:

a) Total existing properties;
b) Total animals susceptible to foot-and-mouth disease, stratified by animal species;
c) Access routes, identifying possible locations for the implementation of sanitary barriers (with support from the transit sector);
d) Geographical accidents and natural barriers;
e) Important strategic locations (dairy, slaughterhouses, agglomerations of animals, landfill, veterinary hospital, greasers, etc.);
f) All properties and points in the defined region must have their geographic location data extracted in the system and made available in spreadsheets so that, in case of confirmation, the file can be accessed in a situation of lack of internet;
g) Measure the need for people, equipment, and materials for investigations within the defined areas.
h) Review all information contained in ANNEX 1 related to the related municipalities (investigated property and bonds).
i) The EVS central unit, together with SFA, is responsible for coordinating and monitoring the entire investigation process.

~~With~~ properties with a link:

Research and surveillance work on properties with an epidemiological link must be careful and accurate, including the following activities:

1) Employ all biosafety measures when entering and leaving the property (ANNEX 9);

2) Conduct interviews with those responsible for the animals and general examination of the herd;

3) In the face of clinical signs compatible with the vesicular disease, consider as ~~a~~probable case of vesicular disease and follow all steps of the investigation and alert phase; or

1) If there are no signs of vesicular disease, register the activities on FORM VIN and e-SISBRAVET. The properties must be kept under surveillance, awaiting laboratory results. Until the laboratory result of the property under investigation, return every three days to properties with an epidemiological link, for a new evaluation;
2) Because of the possibility of animals being in the incubation period, even if no signs of vesicular disease are observed, the professionals responsible for the investigation should adopt strict biosafety procedures and wait 24 hours for inspection of other susceptible herds;

In other epidemiological units:

In addition to the components of the property surveillance system (active surveillance), from notifications (passive surveillance) and serological surveillance (seroepidemiological studies, carried out only in free areas with vaccination), the surveillance system for the foot-and-mouth disease has two more components that can detect probable cases of the vesicular disease, outside rural properties: surveillance in slaughterhouses and surveillance in livestock events.
It should be noted that the efforts of the private sector (producers, private veterinarians, agricultural technicians, etc.) must be in the sense that the notification of suspected vesicular disease is made immediately to the SVO, with the animals still in the farms, thus avoided any movement or sending of animals with lesions compatible with infectious diseases to slaughterhouses, agglomeration events or any emission of GTA that leads to the movement of suspicious animals, aiming at attending the establishment of origin to avoid the spread of diseases, besides, disorders in slaughterhouses, agglomerations or transit inspections.
Below are the actions to be taken in each situation:

Identification of probable cases of vesicular disease in slaughterhouses for animals susceptible to foot-and-mouth disease

In antemortem and post-mortem examinations, in case of detection of vesicular lesions, the animals must be segregated and the EVS is urged to support the investigation in the slaughter establishment and the animals' origin property. Lots with animals identified as probable cases of vesicular disease should be slaughtered last, avoiding direct contact with the others. After separation, the slaughter of healthy lots can proceed. Carcasses, offal, and other products of the day's slaughter, both from the batch with clinical signs and from the batches without clinical signs, must be segregated and kept under the control of the Inspection Service until the end of the investigation. Other measures must be applied until the final result of the investigation ruling out the disease is:

1) Collection of information on the origin of animals and activation of EVS to investigate the rural properties involved. The EVS will be responsible for the precautionary suspension of the issuance of GTA from the original property until the completion of the clinical-epidemiological investigation at the origin;
2) suspension of the departure from the slaughterhouse of products obtained from the slaughter of the day on which the probable case was found,
3) The exit of products subjected to sufficient heat treatment for the inactivation of the virus is allowed and provided that the biosafety measures that guarantee the inactivation of the infectious agent in the transport

vehicles are adopted;

4) The movement of people, as well as other materials, objects, and means of transport that can transmit the infectious agent of the slaughterhouse are subject to biosafety measures defined by the SVO.
5) After the end of the day's activities in which a probable case was detected and the complete cleaning and disinfection of the establishment, provided that the biosafety measures guarantee the inactivation of the viral agent, the slaughter can be released the following day and the products of this can be marketed.
6) The exit of stored products from slaughtering before service may be permitted by the competent authority, after assessment and adoption of risk mitigation measures by the Inspection Service (traceability of batches, cleaning, and disinfection of transport vehicles, etc.).

Actions in pig slaughterhouses:

In the case of pig slaughter establishments, when the Inspection Service finds vesicular injuries and the batch is accompanied by EVS documentation that declares previous investigation on the property (up to 30 days before slaughter) and ~~disposal of the suspicion of foot-and-mouth disease,~~ either by clinical-epidemiological evaluation or negative laboratory test report, slaughter may proceed normally (Joint Circular Letter DSA / Dipoa 01/2020).

This EVS documentation must include: dates of the beginning and end of the investigation; identification of the breeding establishment; criterion for the conclusion of the investigation (use the following options: 1 - suspicion ruled out of vesicular disease due to clinical-epidemiological criteria, or 2 - case discarded of foot-and-mouth disease by laboratory criteria); telephone, identification, and signature of the MVO responsible for the service. Only a copy of this document must accompany the GTAs, excluding research forms and laboratory results reports.

In case of detection of recent vesicular lesions that are not compatible with the date of the clinical-epidemiological evaluation on the farm, or in unaccompanied batches of EVS documentation proving previous care and discarding the suspicion of foot-and-mouth disease, the SVO should consider this case probable vesicular disease and adopt the measures provided for in this manual.

Identification of probable cases of the vesicular disease in livestock events

1) In the detection of suspected vesicular disease in livestock events (fairs, auctions, etc.), the responsible veterinarian must suspend the reception and departure of animals and immediately notify UVL, which will adopt the investigation procedures provided for in this document. If the official veterinarian identifies a probable case of vesicular disease, the following measures should be applied:

2) 1) restrictions on the movement of animals, means of transport, objects, materials, and people, to prevent the spread of the virus;
3) 2) prohibition on the exit of all animals, which must remain in their respective pens, with access to food and water;
4) 3) collection of samples for laboratory tests;
5) 4) allowing people and means of transport to leave the establishment only after biosafety measures and authorization by the SVO;
6) 5) suspension of the issuance of GTA from the properties of origin of the animals considered as probable cases of the vesicular disease, and properties with an epidemiological link; and
7) 6) epidemiological investigation to identify the source of infection.

Identification of probable cases of the vesicular disease during animal transit

1) Due to the difficulty of carrying out an adequate clinical inspection, it is quite unusual to be able to detect a probable case of vesicular disease during the inspection of animals in transit, both in flying inspections and in fixed stations. Listed below are the recommended procedures if during this activity, considered a health management action aimed at mitigating the irregular transit of animals and their products, probable cases of the vesicular disease are detected:
2) If the identification of animals with clinical signs compatible with the vesicular disease has occurred at inspection posts located on interstate boundaries, prevent entry into the state, retain the vehicle with the animals and immediately notify the central EVS unit. The latter must immediately notify the SFA to activate the states involved, especially the one of origin of the animals, seeking to carry out a joint action;
3) The property of origin of the animals must be inspected and interdicted, and the properties with the possibility of an epidemiological link must have the animal movement suspended and be inspected. It is recommended that the properties located in the animals' path be classified as bonds and, therefore, the surveillance recommended in this type of property be carried out, mainly in the case of transporting cattle on foot or in those that had a rest stop for the animals;
4) Samples of the suspect animals for laboratory examination must be collected, their records made and all biosafety procedures recommended for an investigation of a probable case of vesicular disease;
5) Questions related to the place to perform the collection and to keep the animals until the samples are processed and the final result of the investigation must be analyzed, considering the following points:
6) Assess the possibility of identifying a nearby location for the temporary storage of animals. This location must not contain other susceptible animals. The choice must consider the risks involved and seek a solution that compromises the smallest area possible and facilitates the actions of elimination of the animals if the occurrence of foot-and-mouth disease is

confirmed;

7) If the presence of sick animals has also been found in the property of origin of the animals and if the distance between the property and the place where the traffic is interrupted is not very large and does not put other properties at risk, the possibility can be assessed return of the animals to their origin;
8) In the case of transporting cattle on foot, the animals must be loaded in an appropriate vehicle for dispatch to the identified location for kidnapping, considering biosafety measures;
9) In any event, the animals must be transported in vehicles escorted by the animal health defense service, with police support. The transport vehicles must undergo cleaning and disinfection soon after the animals disembark;

4.2 Completion of the investigation

After completing all stages of the investigation in the epidemiological unit with probable cases of vesicular disease and its links, supported by the laboratory result, the SVO may end the investigation with a discarded case of FMD, request new laboratory tests or, in the case of confirmation of a case of foot-and-mouth disease entering the emergency phase. A summary of the entire flow is available at ANNEX 12.

The laboratory result is an important component of the investigation. From it you can have the following situations:

Inappropriate material for diagnosis: due to insufficient quantity or conservation problems. This situation must be avoided, but if it occurs, immediate arrangements must be made for a new visit to the property and collection of material (with registration and filling out the complementary and laboratory investigation forms). Take the opportunity to update the information concerning new cases. The property and those related to the investigation must remain closed.

Negative diagnosis of foot-and-mouth disease: the removal of the property with the investigation of a probable case of the vesicular disease will occur when the SVO is notified of negative laboratory results, in addition to the clinical-epidemiological evaluation of the animals. Specifically for pigs, when there is only a serological diagnosis, the release will occur after a negative result and clinical and epidemiological evaluation of the pigs in the affected farm. In all cases, the end of the investigation and the final diagnosis should precede the

filling out the complementary closure investigation form, registering the data, and uploading the form to e-SISBRAVET. It is emphasized that it will be up to the MVO, through all epidemiological, clinical, and laboratory information, to decide whether or not to end the investigation, and, depending on the characteristics analyzed in the investigation, may maintain the interdiction of the property, even with negative laboratory results, and may carry out new investigations, harvests and submit new samples to the laboratory.

Positive diagnosis for foot-and-mouth disease: According to the criteria defined for the case of foot-and-mouth disease, it will be up to the Map to decree **ZOOSANITARY EMERGENCY.** In this case, the guidelines and procedures are described in the Contingency Plan for FMD.

ANNEXES

ANNEX 1. ~~Outras informações e base de dados importantes para a fase de alerta e de emergência~~

This information is essential for timely action during animal health emergency actions. The information, in addition to being available in electronic format and, at least once a year, updated and evaluated at the local (UVL), regional, and central EVS level, regarding data consistency and validation of geographical coordinates:

1. database referring to all slaughterhouses, slaughterhouses, meat processing companies (sausages), dairy factories, processing plants, refrigeration stations, and dairy products existing in the geographic area where it operates, including information on capacity, species, contact of the technical responsible, owner and geolocation data of the establishment;
2. have a record and knowledge of the main agricultural characteristics of the area under their jurisdiction, with emphasis on the inflows and outflows of animals, their products, and by-products. The analysis of the movement of animals, products, and by-products, including main flows, origin, and destination, must be carried out annually and of knowledge of technicians at central and local EVS level;
3. have digital maps of the geographic area of its operations, including information on geopolitical limits, road network, hydrographic network, location of rural properties, milk lines and the route of each line, villages, towns, indigenous reserves, rural settlements, conservation units, or areas of environmental protection, forest reserves, among other elements of relevance for health intervention activities.
4. updated database, with name, position, address, and form of contact for municipal authorities (including police forces), representatives of civil defense, and representatives of the agricultural sector. When located at the international border or the state border, including the name, address, and form of contact of the person responsible for the bordering UVL, belonging to the neighboring country or state;
5. database with the names of the animal health emergency team at UF, with address and form of contact between their representatives, especially those responsible for the region where the UVL is located;
6. database with the contacts of the person in charge of municipal social communication and the main means of communication available (TV, newspaper, radio, websites), with the name and address of those responsible or representatives;

7. database with the name, ~~training~~, address, and contact form for self-employed professionals, the private sector, and other institutions working in the field, mainly veterinarians, zootechnicians, and agricultural technicians;
8. database with a contact list of owners of heavy machinery, such as backhoe, crawler tractor, wheel loader, bucket trucks, among others, that can be used in animal health activities (including identification and contact form) those responsible for releasing these machines);
9. database with name and capacity of hotels, farm hotels, and other establishments that can serve as accommodation for a large number of professionals, with the geolocation data of the establishments;
10. database of airports and airstrips, including those for small aircraft, with the establishment's geolocation data; with the geolocation data of the locations;
11. database with a list of spaces (schools, rural schools, technical schools, sports gyms, community centers, etc.), with the geolocation data of the establishment, for possible implementation of COEZOO, with the geolocation data of the establishments. The location must be large and available for use for at least three uninterrupted months and have the following characteristics: the capacity for warehouse installation, a patio that can be used as a garage for many vehicles, good lighting and plenty of water, the possibility to control the entry of vehicles and people, a place for cleaning and disinfecting clothes and vehicles, rooms for work teams, a room reserved for the Coordination and for holding technical meetings, with the possibility of using the telephone and internet;
12. database with identification of possible locations for the implantation of fixed inspection posts, with the geolocation data of these locations;
13. database with main access routes, including traffic conditions, with the geolocation data of these locations;
14. database with the registration of owners and drivers of vehicles transporting animals or risk products and of those responsible for transporting animals in the region, including type, quantity, and capacity of vehicles by owner or carrier;
    - identification in the register of rural settlements, indigenous reserves, and quilombola communities, so that it is possible to filter this information and easily identify these communities;

16. database with location, including geographic coordinates, and contact details for the owner of establishments and points of interest for the animal health defense system:
    - sanitary landfills and ~~dumps~~, including control conditions and whether there is a possibility of access for susceptible animals inside these establishments;
    - resale of products for veterinary use, including the name of the veterinarians in charge of technicians;
    - auction facilities and other agglomerations of animals, with identification of

the event organizers and respective technical officers;

- greasers, tanneries, and salting machines, with identification of the technician responsible;

ANNEX 2. Materials that shall compose a kit to attend to notifications of suspected vesicular disease

List of material for care for suspected vesicular disease			
1	Mouth opener	22	Vallée liquid and MEM
2	Antiseptic	23	Rubber and procedural gloves
3	Needles: . Disposable hypodermic, sterile (40 x16 mm) . Disposable for tube type vacutainer® (0.80 x 25mm) Adapter for multiple ~~ha~~sting 25 x 8 mm	24	Overalls
4	Hydrophilic cotton and gauze	25	Materials to identify animals: earrings and device, tattoo tweezers.
5	Plastic bucket	26	Eppendorf microtubes, 2 ml capacity (for serum)
6	Notepad	27	Threaded cap and ring microtubes sealing (for epithelium and swab)
7	Costal pump and hand sprayers	28	Absorbent paper (paper towel)
8	Rubber boots	29	pH indicator paper
9	Box with instruments for necropsy	30	Tweezers (rat tooth type))
10	Styrofoam boxes of varyi sizes (isothermal)	31	Disposable, sterile, Pasteur pipette (transfer), 3 ml capacity
11	Box for storing and transporting materials	32	Identification plate or band of prohibited properties
12	Disinfectants, detergents, and soap	33	Clipboard
13	Brushes for cleaning boots and hands	34	Disposable punch for 3 mm biopsy
14	Adhesive tape	35	Recipient to discard sharp material
15	Polyethylene adhesive tapes	36	Plastic bags or bags for disposable waste
16	Research forms	37	Serynge 1 ml (insuline) and 10 ml
17	~~Ant and pipe for containment~~	38	A flocked synthetic sterile swab
18	Sterile Falcon type bottles of 15 and 50 ml	39	Veterinary clinical thermometers

19	Recyclable ice	40	Interdiction and Disinterdiction Terms
20	Ties or ropes for containment	41	Scissors and scalpels with blades
21	Flashlights and batteries	42	Test tubes type vacutainer® 10 ml, without anticoagulant

ANNEX 3. Internet addresses with technical records of the main vesicular diseases

1. FOOT AND MOUTH DISEASE:

- http://sistemasweb.agricultura.gov.br/pages/fichas_tecnicas/Ficha_Tecnica_Febre_Aftosa_jan20.pdf
- https://www.oie.int/fileadmin/Home/eng/Animal_Health_in_the_World/docs/pdf/Disease_cards/FOOT_AND_MOUTH_DISEASE.pdf

2. VESICULAR STOMATIS:

- https://www.oie.int/fileadmin/home/eng/animal_health_in_the_world/docs/pdf/disease_cards/vesicular_stomatitis.pdf
- http://www.cfsph.iastate.edu/Factsheets/pt/vesicular-stomatitis-PT.pdf

3. 3. SENECAVIRUS A INFECTION:

- https://ainfo.cnptia.embrapa.br/digital/bitstream/item/141041/1/final8034.pdf
- http://www.cfsph.iastate.edu/Factsheets/pt/senecavirus-a-PT.pdf

4. PIG VESICULAR DISEASE:

- https://www.oie.int/fileadmin/Home/eng/Animal_Health_in_the_World/docs/pdf/Disease_cards/SWINE_VESICULAR_DISEASE.pdf

5. 5. VESICULAR EXANTHEMA:

- http://www.cfsph.iastate.edu/pdf/shic-factsheet-vesicular-exanthema-swine-virus

ANNEX 4. Comparative table of the main vesicular diseases

Features	Fmd	Vesicular stomatitis	Senecavírus A	Vesicular disease of pigs	Vesicular exanthema
Morbidity	High (60% to 100%)	Low to average (5%-10%); In dairy cattle up to 85%	High in piglets (5 to 60%)	High (25-65%) - subclinical infections occur	High
Mortality	Low (in young animals may be high)	Zero or low	Low	Low	Low(<5%)

Transmission	Contact, aerosols, meat products. Doubts about the role of carriers. Wind, only under special conditions (temperature, humidity, pressure, wind).	Doubts regarding the role of contacts, carriers, and vectors. Milking machines	Oronasal route	contact - meat products (persists in meat chilled / frozen) -through foot wounds - nasal and oral secretions	contact - meat products (persists in meat chilled / frozen) -through foot wounds - nasal and oral secretions
Susceptible species	~~Bovino, suínos, ovinos e caprinos~~	Cattle, pigs, sheep and goats humans	Pigs	Pigs	Pigs
Observations	Persistence in cattle. Viruses in feces, urine, milk, esophageal-pharyngeal fluid, respiratory aerosols and injuries. Considered the most contagious disease in veterinary medicine.	Calves are more resistant than adults. New Jersey serotype more virulent than Indiana. Zoonosis. Natural immunity <6 months. The virus does not survive more than a week or two in the environment. Fibrous food exacerbates infection / streaming. Wild fauna?	Vesicular lesions, mainly in sows and animals of termination and neonatal mortality. In addition to the lesions, viruses are also present in oral, nasal, and fecal secretions. Detection of the virus in tonsils	Vesicular lesions, mainly in sows and animals of termination and neonatal mortality. In addition to the lesions, viruses are also present in oral, nasal, and fecal secretions. Detection of the virus in tonsils.	Persistence in chilled/frozen meat. Post-infection immunity - 20 months - but there is no cross-immunity with other serotypes. Mortality may be higher in young animals. Abortions and non-breastfeeding females Fomites piglets are no problem. It has not been demonstrated a vertical transmission

Source: adapted from the Manual of Procedures for the Attention to Foot-and-Mouth Diseases and other Vesicular Diseases, Panaftosa

ANNEX 5. Basic guide for examining animals suspected of having the vesicular disease

1. **For all types of animals susceptible to foot-and-mouth disease.**

a) Before immobilizing the animals, observe:

- apathy;
- signs of claudication;
- excessive salivation;
- noises emitted with the lips ("smack" sound);
- gnashing of teeth.

2. **2. Contain the animals properly and record all details about the observed signs and injuries.**

- a) Record body temperature and estimated age
  Normal values (variation of + or - 0.5º C may occur)
- bovine = 38,5ºC
- ovine = 39,5ºC
- caprine, swine and equine = 39,0ºC

a) Describe the vesicles in detail:

- intact or broken (closed or open);
- size;
- color (eg whitish, bright red, yellowish etc.);
- depth;
- defined or worn edges (boundaries);
- • degree of healing (presence of fibrin deposit).

3. **Cattle**

Localization of lesions:

- Inspect nostrils;
- in the oral cavity inspect the tongue, lips, gums, and side walls and higher;
- edges (remove dirt under running water): interdigital space, band coronary and talons;
- udders and teats;
- vulva and prepuce.

4. **Swine**

Significant signs

- acute and sudden claudication;
- observe the animal on concrete paving or other hard surface and stimulate it to walk.

Lesions

- muzzle, lips, tongue (lesions are generally smaller and less apparent than in cattle), and extremities (separation of the nail from the coronary band can be seen).

5. **Small ruminants**

Significant signs

• acute and sudden claudication (usually affects all extremities), diagnosis

differential: foot-rot.

Lesions

• usually at the extremities, coronary band; lesions in the interdigital space and separation of the nails can also be observed. Small vesicles usually appear on the tooth base and the lips.

6. Record all information legibly and check the quality and correctness text. Fill in all fields of e-Sisbravet forms.

ANNEX 6. Composition of the solutions used to conserve materials to be sent to the laboratory

Vallée Liquid at 50%

(for epithelium harvesting)

1. KH2 PO4 (1.35 g) monobasic potassium phosphate
2. K2 HPO4 (7.80 g) dibasic potassium phosphate
3. Phenol Red 1% (for pH control) 0.1 ml
4. Demineralized H2O - q.s.p. (1,000 ml)
5. Measure the pH. Must be 7.6 ± 0.1
6. Glycerol (1,000 ml)

Sterilize autoclave the phosphate and glycerin solutions in separate bottles for 20 minutes at 121 ° C. Wait for the solutions to reach room temperature. In a biological safety cabinet or clean bench, transfer the two solutions to an appropriate flask and mix. Fraction according to the need and availability of sterile bottles.

6. 7. Add 1000 IU penicillin 100 IU neomycin sulfate, 50 IU polymyxin B sulfate, and 100 IU myostatin.

MEM

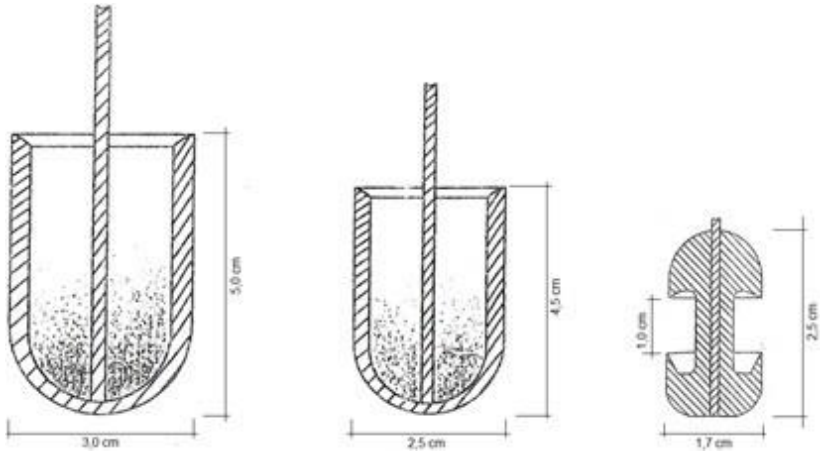
With lactalbumin hydrolyzate and yeast extract (for collection of esophageal-pharyngeal liquid - LEF and Swab)

Eagle minimum essential medium
With Earle salts non-essential amino acids and baking soda.
It can be purchased in powder or ready to use.
If purchased in powder, moisturize according to the manufacturer's guidelines and sterilize by filtration.
Add every 1 liter in the liquid and sterilized medium 1000 IU penicillin 100 IU neomycin sulfate, 50 IU polymyxin B sulfate and 100 IU myostatin

Obs.: The control and recording of the storage temperature and pH of the medium must be constant.

ANNEX 7. Technique and procedures for the collection of esophageal-pharyngeal fluid (LEF)

Facilities: The facilities must be suitable for perfect containment of animals and allow the head to be immobilized and facing upwards, maintaining an adequate and comfortable position for the ~~harvest~~. The correct containment of animals is an important factor to facilitate work and avoid accidents, both for animals and for the operator. LEF samples must be collected with the help of specific collectors, according to the models below. The collectors consist of a stainless steel metal cup, with a rounded bottom and bevel-shaped edges (just enough to scrape the mucosa), fixed from the center of the inner part to a curved rod approximately 50 cm long.



Models of LEF collectors

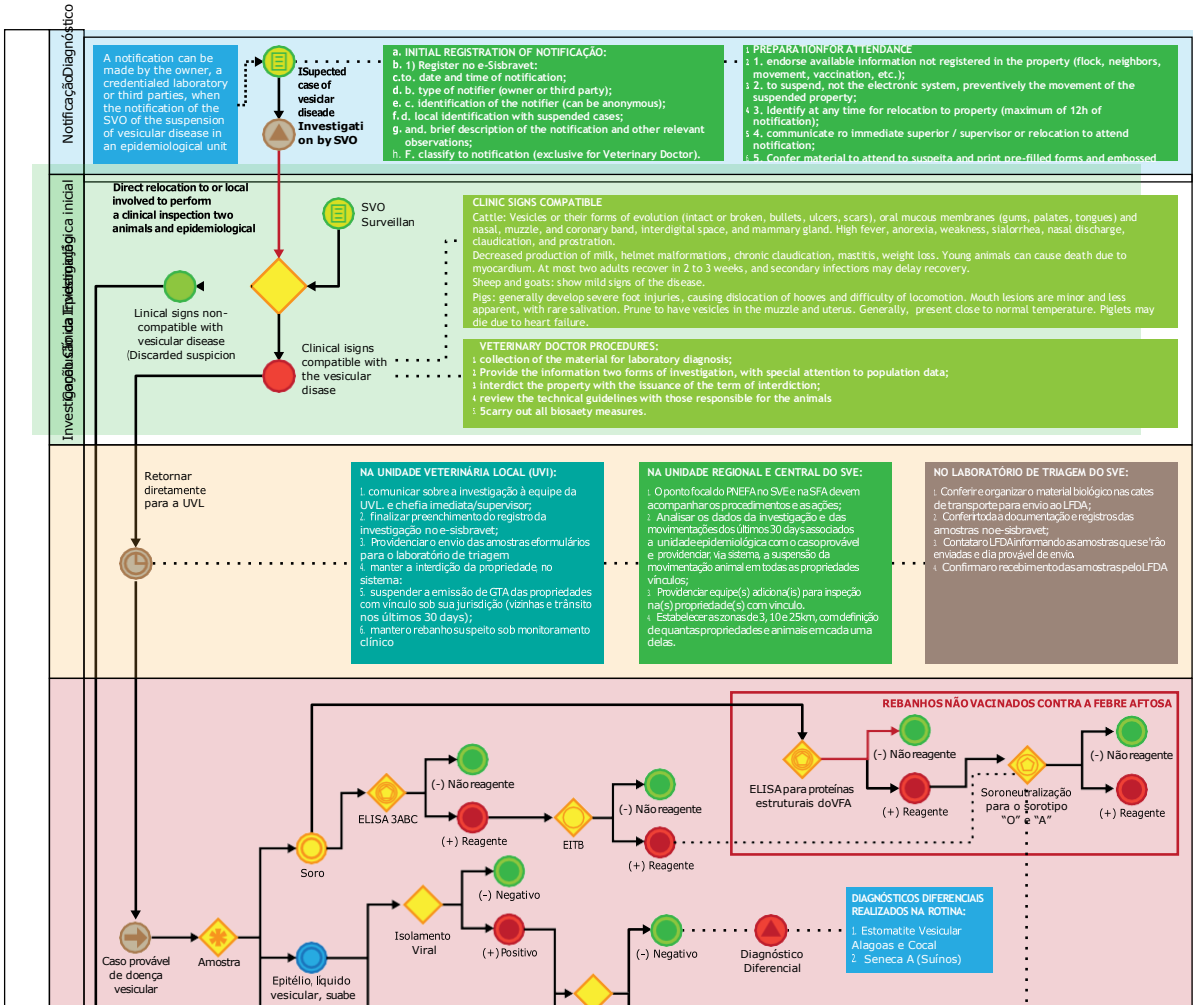
Ruminants: The animals, properly identified, must remain on a water diet for at least 12 hours. One hour before ~~harvest~~, water should be administered to eliminate any food remains and moisten the esophageal-pharyngeal region. This procedure facilitates the penetration of the collector, as well as the scraping of the mucosa. The use of tranquilizers that cause myorelaxing action should be avoided. It is possible that, with the maneuver of introducing the collector through the esophagus, the animal has a vomiting reflex and impairs the sample collection. In this case, the operator must reject the material and try another ~~harvest~~, after letting the animal rest for a few hours. If it persists, it is advisable to transfer the ~~harvest~~ to another day.

Sample collection: During work, the operator must take all precautions to avoid the eventual transmission of viruses from one animal to another, in addition to general biosafety precautions. A sterile collector must be used for each animal. For the introduction of the collector, the operator must open the animal's mouth, pressing the tongue downwards, and through the lip commissure make it penetrate

the collector carefully, until it reaches the pharynx and the anterior part of the esophagus. This is characterized by the voluntary swallowing movement of the animal. After swallowing, the esophageal region can be palpated to verify the correct positioning of the collecting cup. The cough reflex is an indication that the glass is in the wrong position and should be removed. Once the collector is inserted, it is necessary to scrape the esophageal-pharyngeal mucosa through gentle movements (five to ten times) before removing it. This procedure is essential for the collection of the sample since the main sites of replication of the FMD virus are found on the anterior pharyngeal floor and the dorsal surface of the soft palate. In the case of a positive animal, the foot-and-mouth disease virus must be present in the epithelial cells that come off the esophageal-pharyngeal region at the time of ~~shaving~~, with the presence of saliva, mucus, and food debris. After harvesting, the operator must wash his hands and arms with a disinfectant solution and then with running water.

Preservation and sending of samples: After removing the collector, the contents of the cup are transferred to a sterile "Falcon" type tube. Immediately, an equal amount of MEM is added, containing antimicrobials. The flask is identified and properly sealed, and then shaken vigorously to homogenize the sample with the medium. The sample should preferably be frozen and placed in a container that maintains the storage temperature. The samples must go to the laboratory as soon as possible, accompanied by the standardized forms by the SIZ.

ANNEX 8. Representation of the service flow for notification of suspected vesicular disease



Investigation Diagnosis

The notification may be made by the owner, by the accredited lab, or by third parties as o the SVO notice of suspicion of vesicular disease in an epidemiological unit

ANNEX 9. Biosafety procedures

Biosafety measures must be strict during surveillance activities and care for suspected vesicular diseases. Some biosafety procedures for adoption by surveillance teams are highlighted.

Equipment and materials needed for biosafety procedures:

To better organize the material and facilitate disinfection, the materials must be placed in resistant, labeled, and closed plastic boxes or bags, highlighting:

1. personal protective equipment (PPE): overalls, disposable latex gloves, resistant rubber gloves, and high-top rubber boots.
2. adhesive tapes;
3. disinfectants;
4. large plastic bags, the ideal is to have at least 2 different colors for transporting waste material or for disinfection;
5. resistant brushes and buckets for disinfection and drums for water transport;

General measures to prevent contamination:

1. avoid walking unnecessarily through potentially contaminated areas;
2. avoid direct contact with potentially contaminated materials, surfaces, and vehicles;
3. before placing the PPE, check that it is free of tears or holes;
4. Do not carry items such as: cigarettes, candies, food, drinks, etc.

Precautions that must be taken to minimize contamination of the equipment:

1. when taking samples, place the boxes and instruments in a clean bag before placing them in vehicles; and
2. when samples are taken, they must be properly packed and placed in bags that allow external disinfection before being transported.

Suggested procedures for entering properties:

Stop the vehicle in a safe, dry, and clean spot, preferably near the gate, avoiding entry if it is a small property. In the case of large properties, drive the car close to the livestock facilities, but keeping a good distance and choosing a dry and clean place.

Personal protective equipment clothing:

1. put on the overalls. The use of disposable coveralls is recommended;
2. put on the rubber boots;
3. put on disposable gloves. It is also recommended to have more resistant rubber gloves for the activities of clinical inspection of animals.

Suggestions for procedures on the property:

1. Check all the material before entering. Many items are unnecessary (such as bags and keys, among others) and must be kept in the car. Take off the watch, rings, bracelets, necklaces, etc., and leave it in the vehicle. Cell phones, cameras, and GPS devices must be placed in individual, sealed plastic bags.
2. While working on the property, you should avoid eating, smoking, or drinking.

Preparing to leave the property:

1. Take advantage of the farm's washing facilities to remove as much visible dirt from used materials and boots as possible.
2. After the clinical inspection and sample collection procedures, professionals must separate all non-disposable items, which should be washed with water, soap, and brush, and then disinfected and stored in specific non-disposable bags, sealed and disinfected again on the edge of the clean area, before being placed in the vehicle.
3. Used disposable items must be placed in plastic bags of disposable material for destruction. Piercing or cutting materials must be placed in specific devices or "pet" bottles before being placed in garbage bags.

Leaving the property:

In a discarded case: no specific biosafety procedures are required
In a probable case, adopt the following procedures:

1. Clean and disinfect the material boxes, bag the equipment, and transfer it to the vehicle.
2. The removal of personal biosafety equipment must be in order, to protect against exposure to potentially infectious materials. It is recommended to adopt the following points:
    a. clean and disinfect cell phone, camera, and GPS bags;
    b. clean and disinfect rubber boots and gloves with a brush, including soles;
    c. clean and spray the coverall with disinfectant, or dip in a bucket with disinfectant solution, then put in a clean bag;

d. remove the gloves, taking care not to touch the hands on the outside, and put them in the disposable bag. If they are reusable rubber gloves, they must be washed, disinfected, and placed in the bags with the overalls;
e. close plastic bags containing samples, equipment, boots, and overalls using tape;
f. put on shoes;
g. clean and disinfect hands, wrists, and arms;
h. pouring the remains of disinfectant onto the vehicle's wheels;
i. placing the bags with non-disposable materials and externally disinfected garbage in the car (trunk or body);
j. disinfect the vehicle's wheels, pedals, and floor before leaving the property.

3. When returning from the facility, provide:
a. suitable destination for disposable material with biological risk;
b. cleaning and disinfection of reusable materials; and
c. bathing and asepsis of the respiratory airways (nose and throat).

ANNEX 10. List of disinfectants for foot-and-mouth disease (adapted from the Manual of Procedures for Attention to the Occurrence of Foot-and-Mouth Disease and other Vesicular Diseases, Panaftosa)

1. **Citric Acid at 2%**

Preparation: two parts of citric acid to 98 parts of water.
Indications: laboratory objects and vehicle cabins.
Note: it is not very corrosive to metals and painted surfaces.

2. **Solução de carbonato de sódio a 4%**

Preparation: dissolve 400 g of sodium carbonate in 10 liters of water.
Contact time: 10 minutes.
Application method: spraying, sprinkling, footbath, and immersion.
Caution: when applying disinfectant indoors, boots, gloves and a mask are recommended.
Indications: facilities, people and animals, vehicles, clothing, utensils, leather, skins, bones, hay, and straw.

3. **Iodophor compounds.**

Preparation: mix 1 liter of the product in 200 liters of water. Contact time: 10 minutes.
Application method: spraying, sprinkling, footbath, and immersion.
Indications: people, animals, vehicles, clothing, utensils, leather, skin, bone, hay, straw, and dung.
It should be noted that in the zoosanitary emergency episodes, to eliminate outbreaks of foot-and-mouth disease conducted in Brazil between 1997 and 2005, the products chosen for the different applications were based on Iodophor. They are products that are easy to acquire, preserve and use, and can be used both as disinfectants and as antiseptics, changing only the concentration/dilution, according to the manufacturers' recommendations.

4. 4. Acetic acid at 2%

Preparation: 2 parts of glacial acetic acid to 98 parts of water. Indications: laboratory objects and vehicle cabin.
Note: it is slightly corrosive to metal objects but has little action on organic matter.

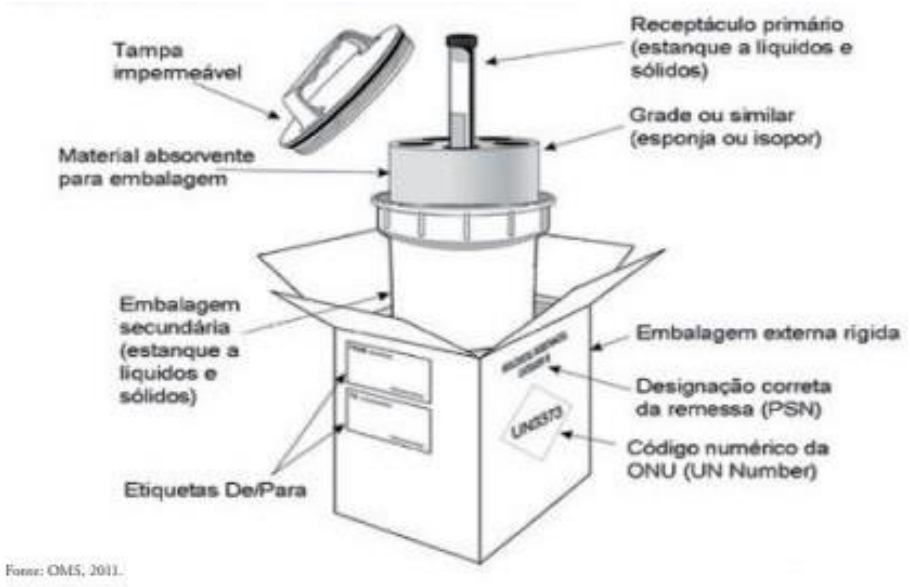
5. Potassium monopersulfate triple salt solution

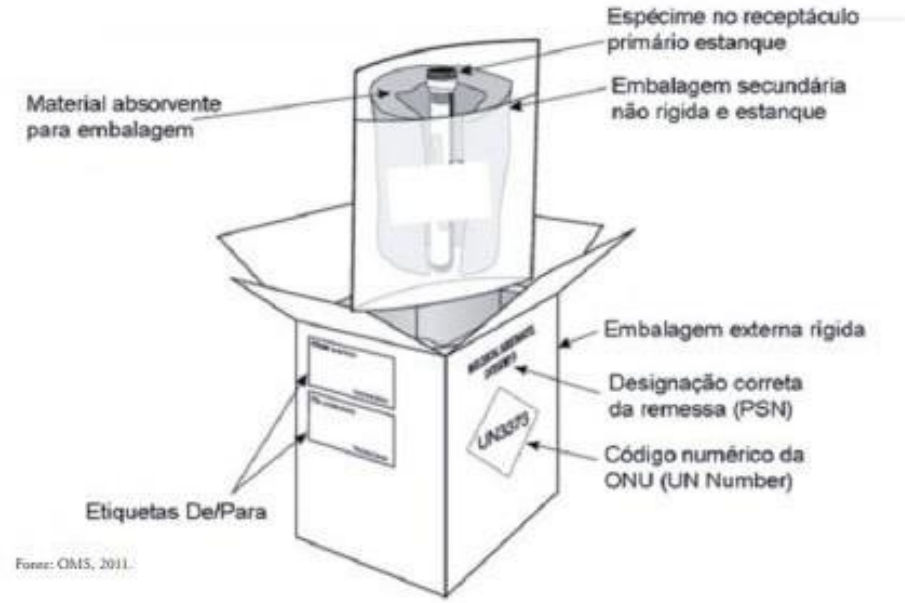
- Preparation: dilute the powder in running water, according to the manufacturer's instructions. Contact time: 30 minutes.
- Application method: spraying, droplet spraying, and immersion. Caution: it is not toxic or irritating.
- Indications: disinfection of stables, corrals, industrial processing plants, animal limbs and legs, vehicles, and farm equipment.
- Usage limitations: do not mix with alkaline substances, as the product works at a pH of 2.5 for a 1% solution.
- Note: Since the effectiveness of acids and alkalis as viricides depends on their pH, it is important that they do not mix. Surfaces treated with one type should not be subjected to the action of another unless a wash with water is inserted. Never use washing soda and acid to disinfect the same item.
- Disinfectants recommended for foot-and-mouth disease are not effective against many pathogenic bacteria and viruses and may lose their specific effectiveness if mixed or applied together with general-purpose disinfectants.

ANNEX 11. Requirements for ~~packaging~~, packaging, and shipping samples for laboratory testing

The packaging must be of good quality, strong enough to withstand the loads and impacts that normally occur during transport, including transshipment, stacking, manual or mechanical handling. The packages must be constructed and closed to prevent any loss of content under normal conditions of transport, due to vibration or changes in temperature, humidity, or pressure.

The UN3373 category B packaging system (figure below) is applied, which has a triple filling, including for local transport by surface, comprising three elements: a primary container, a secondary packaging, and an obligatorily rigid external packaging.





Waterproof cover Primary receptacle (waterproof to liquids and solids) Absorbent material for packaging Grid or similar (sponge or Styrofoam Secondary receptacle (waterproof to liquids and solids) Rigid external package to/from tags correct shipment designation UN numeric code (UN Number)

Absorbent material for packaging Specimen in the primary waterproof receptacle Secondary non-rigid and waterproof packaging to/from tags Rigid external package Correct shipment designation UN numeric code (UN Number
Source: WHO 2011

The primary container must be wrapped in absorbent material sufficient to contain all of the material without compromising the integrity of the damping product or that of the secondary packaging. The primary container must be protected by secondary packaging that, under normal conditions of transport,

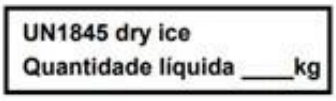
will not break or puncture. If several fragile primary containers are placed in the same secondary packaging, they must be wrapped individually or separately, to avoid contact between them.

Always use good quality plastic bottles with screw caps. Serums should be shipped, preferably, in disposable plastic microtubes, of the 2 ml Eppendorf type. Attention to fill only 2/3 of its capacity, once, when freezing, the liquids expand their volume.

The secondary packaging must be able to prevent loss of content when the primary container fails to seal a[illegible] will be accommodated within the outer packaging, with an appropriate ~~dumping~~ material.

When using ice or dry ice (carbon dioxide), they must be placed outside the secondary packaging, that is, in the outer packaging or a thermal box (Styrofoam). Internal shims should be placed to keep the packages immobilized when the ice melts or evaporates. When dry ice is used, the packaging must allow the gas to escape and prevent the accumulation of pressure that could rupture it, and must be marked with its own label with the indication "Solid carbon dioxide" or "Dry ice".

Etiqueta para gelo seco (dióxido de carbono, sólido)
Esta etiqueta deve ser afixada no exterior de todo pacote que contém gelo seco.



Marcação para gelo seco (dióxido de carbono, sólido)
O peso líquido do gelo seco deve ser indicado no exterior de todo pacote que contém gelo seco.

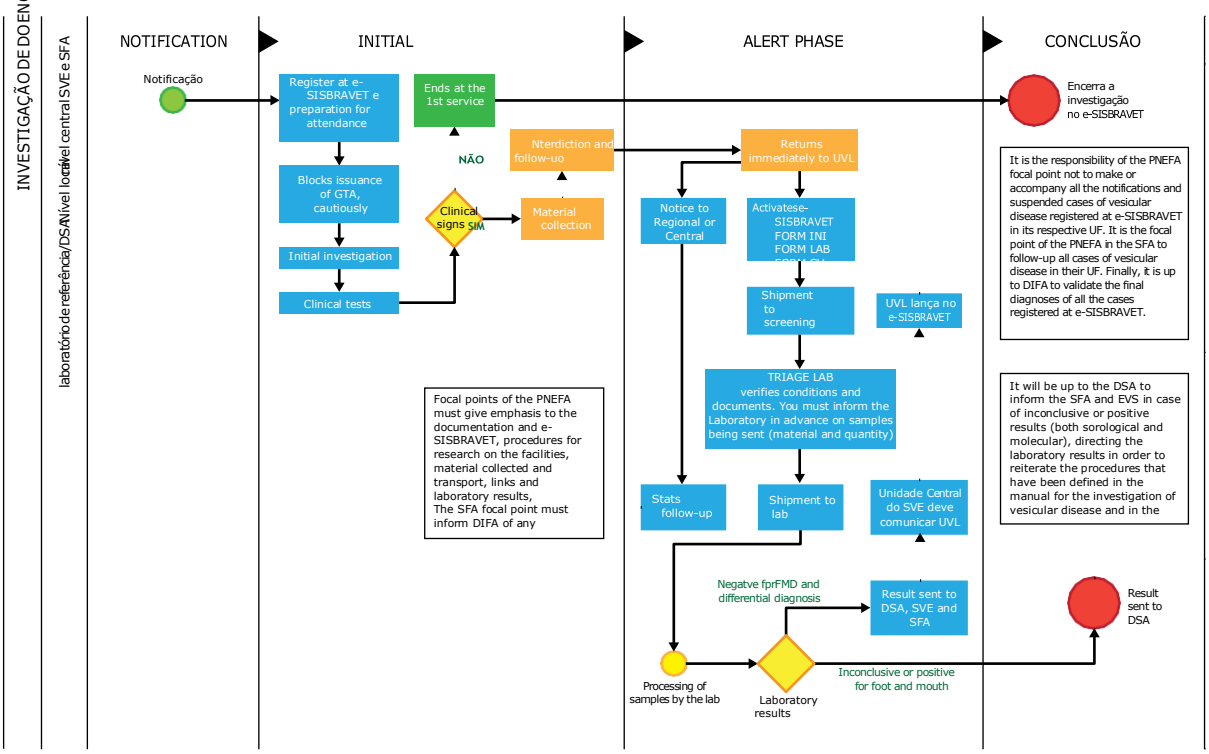
Tag for dry ice (solid carbon dioxide) This tag must be posted on the external part of each package containing dry ice.
Marking for dry ice (solid carbon dioxide) The net weight of the dry ice in the external part of each package containing dry ice.

If liquid nitrogen is used, the outer packaging must bear the corresponding hazard label and, in the case of air transport, the handling label for cryogenic liquids will also be placed.

Labels with the name, address, and telephone number of the sender and recipient must also be placed on the outer packaging, including an emergency telephone number. The term Biological Substance, Category B / Biological Substance, Category B must also be included. The transportation of samples must conform to international standards, according to IATA norm.

Training and awareness are important for all personnel involved in the transport of category B biological substances. Only through appropriate guidance and training can shippers ensure the correct classification of the substance to be sent, as well as the correct selection and preparation of packing. Carriers and other companies whose workers are involved in transportation must train employees in the proper procedures to recognize and handle packages containing biological substances and how to deal with spills, protecting them from exposure.

ANNEX 12. Flow of information on vesicular disease investigation in the country.



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