



MANUAL OF INVESTIGATION OF VESICULAR DISEASE

1st Edition

MINISTRY OF AGRICULTURE, LIVESTOCK AND FOOD SUPPLY

Ministry of Agriculture, Livestock and Supply
Secretariat of Animal and Plant Health

MANUAL OF INVESTIGATION OF VESICULAR DISEASE

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PREFACE

The technical procedures that must be performed by vesicular physicians trained in the care and investigation of a notification of suspected vesicular disease were initially described in the "Action plan for foot-and-mouth disease, volume I, care for the notification of suspected vesicular disease" , published in 2009.

This document is an update of that document and contains the procedures for acting on suspected vesicular disease in the investigation and alert phases. Its content must be known and mastered by all veterinarians working in the official veterinary service (SVO) and used in the routine of the SVO, in addition to serving as a basis for training in this area.

Considering the constant advances in knowledge regarding the dynamics of infectious diseases, the availability of laboratory analysis methods and control and eradication strategies, the manuals need to be revised and updated. This version was prepared based on current national and international standards and guidelines on foot-and-mouth disease, and was preceded by technical discussions with the participation of different sectors of the Ministry of Agriculture, Livestock and Supply - Map, Pan American Foot-and-Mouth Disease Center - Panaftosa and of the State Veterinary Services - SVE.

DEFINITIONS FOR THIS DOCUMENT

Vesicular disease: set of communicable diseases characterized by the presence of vesicles or vesicular lesions in the mouth, muzzle, paws or udder regions, associated with clinical and epidemiological conditions that show evidence of previous contact with a causal infectious agent, which must be confirmed or discarded by laboratory diagnosis;

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

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

Epidemiological unit: group of animals with a defined epidemiological relationship and with similar probabilities of exposure to a particular pathogen, according to the characterization performed by the SVO. It can 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 management practices and biosafety conditions;

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

Inspection: observation of the animals in the herd, being able to make them walk or run, in order to observe clinical signs compatible with vesicular damage (salivation, lameness, vesicles on the teats, feet or mouth).

LIST OF ABBREVIATIONS

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

GTA - Animal Transit 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 Surveillance Program of Foot-and-Mouth Disease SFA - Federal Superintendence of Agriculture

SVE – State Veterinary Service

SVO – Official Veterinary Service

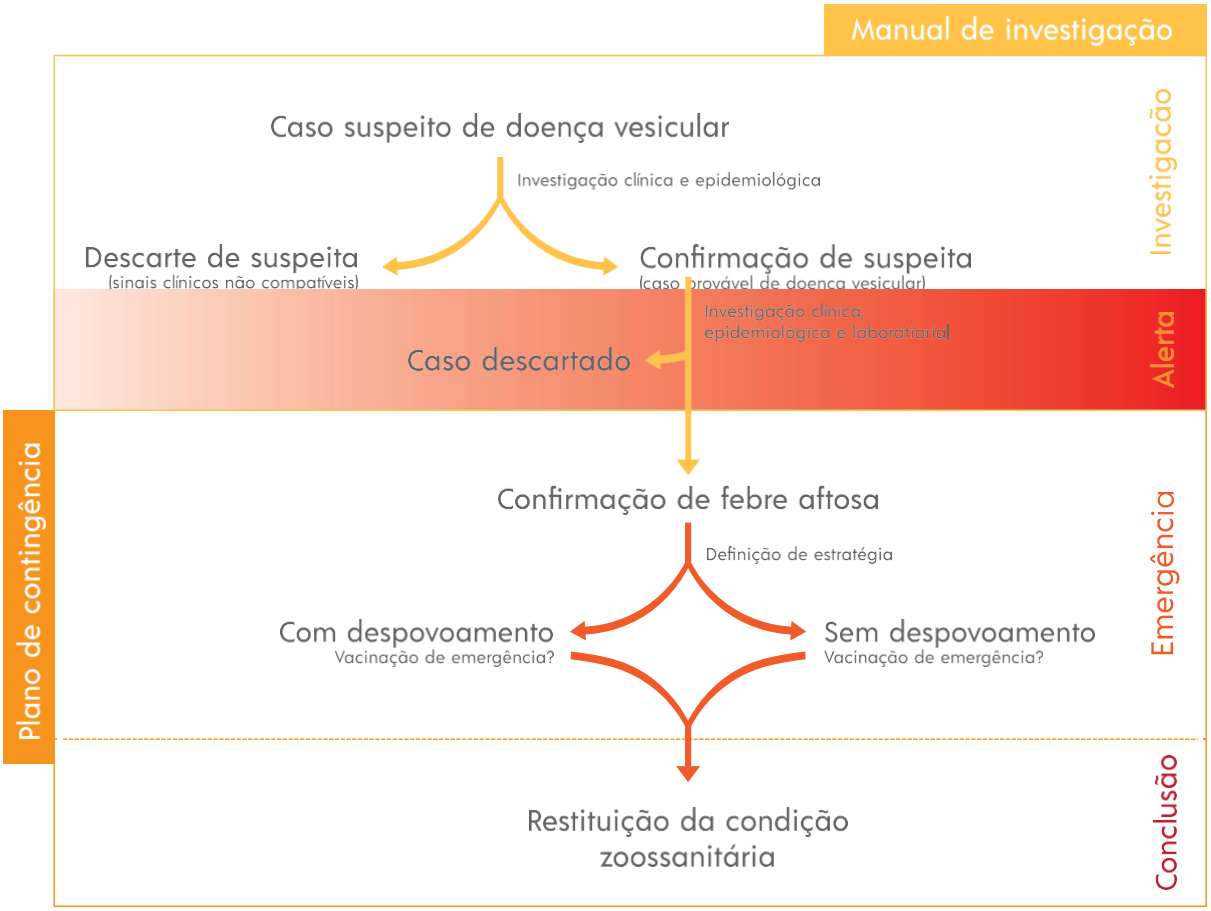
UVL – Local Veterinary Unit

1. INTRODUCTION

This document describes the procedures for investigating suspected cases of vesicular disease. In a didactic way, this process is grouped into two phases: Investigation 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 an outbreak of foot-and-mouth disease is confirmed, there are two other stages, 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 phases of the surveillance system for foot-and-mouth disease in the country are described.



Contingency plan	Investigations Manual	Investigation
	Suspected case of vesicular disease Clinical and epidemiological investigation Dismissal of suspicion (non-compatible clinical signs) Suspicion confirmation (probable case of vesicular disease)	

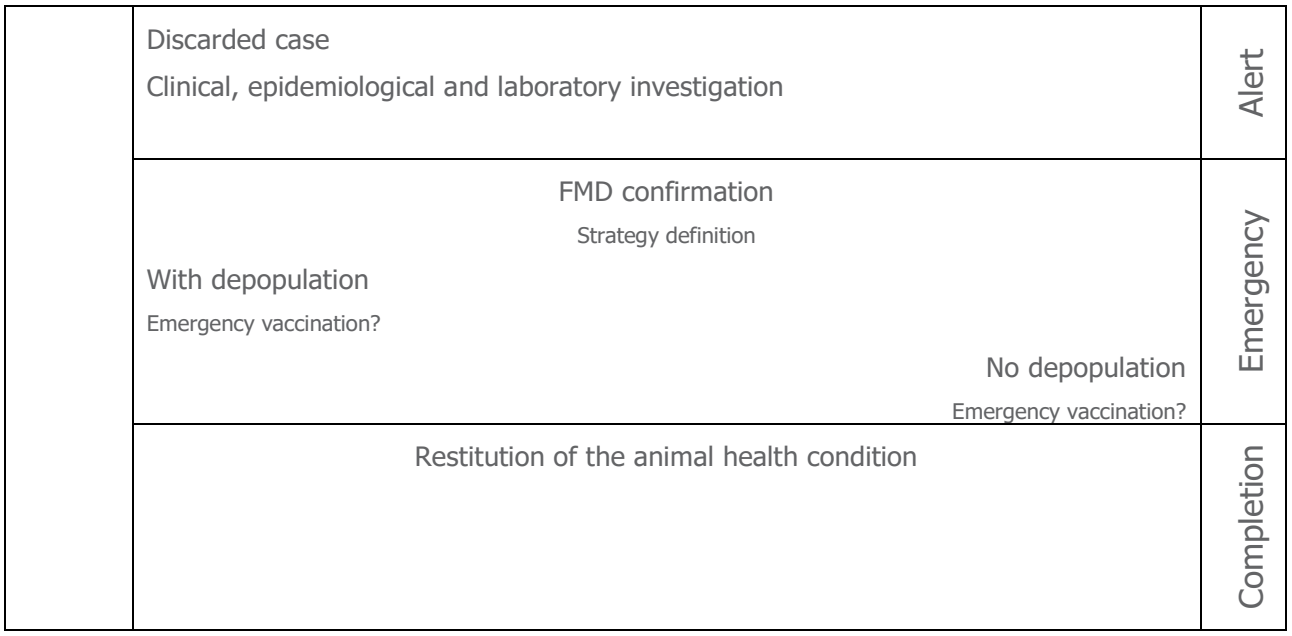


Figure 01. Representation of the main phases of the surveillance system for vesicular disease.

2. INITIAL RECOMMENDATIONS

To improve the effectiveness of vesicular disease surveillance actions and the capacity for prompt reaction in emergencies to contain and eliminate FMD outbreaks, in addition to prepared human resources, basic equipment and financial resources, it is necessary to have some information in advance and specific structures, detailed below:

2.1 Information and database needed for investigation

1. Database referring to properties, rural producers and herds: The SVE, both local and central, must have, electronically, the updated list of rural properties and herds existing in the geographic area of its operation, of according to the guidelines defined by the Map. Special emphasis must be given to the coding and georeferencing system of rural properties, in accordance with the standards established by the Mapa. This information helps during investigation and alert actions, in addition to being essential in animal health emergencies;
2. Animal movement database: The SVE, both local and central, must have a computerized system for the control and issuance of GTAs, with timely access to animal movement data from any rural property.
3. Other information: The SVE, both local and central, must have knowledge and record, electronically and in a standardized way, a series of data and information that will be of great importance in the alert phase and also for a timely response in the case of an emergency in foot-and-mouth disease. This data must be updated at least once a year. A breakdown of the required information is described in Annex 1.

2.2 Equipment, resources and procedures for surveillance activities

1. **Means of transport and communication**: every UVL must have an adequate way to travel and communicate in its area of operation.

2. **Material for care and investigation of suspected cases of vesicular disease:** The material must be available and in use condition. For this, there is a need for discipline and organization on the part of the MVO responsible for the UVL, who must systematically check the available equipment. If the material is incomplete, the professional must officially notify his superior. It is also up to the SVE to create control and monitoring mechanisms, at the central level of the SVE, of the completeness of the service kits in all the UF's UVL. The PNEFA focal point at the SVE must implement a validation methodology by sampling or census of the UVL, in order to assess every six months the situation of this material in the UF, taking the necessary measures to maintain 100% of the UVL with suitable material for care of suspected cases of vesicular disease. **Annex 2** presents the list of materials and

instruments needed to provide adequate care for a suspected case of vesicular disease. Checking the availability of the material includes checking the expiration date of detergents, disinfectants, antiseptics and means of preserving samples. In relation to the latter, the color and appearance must also be observed (cloudy and color-changing solutions must be replaced even before the expiration date), in addition to periodically checking the pH (even for solutions containing indicator of pH in its constitution).

3. **Established and described procedures for the rapid registration and transport of samples for laboratory examination**: The SVE must maintain contracts with transport companies to send samples to the laboratory following the existing biosafety rules for packaging and shipping biological samples, as well as having described the alternative measures to ensure this logistical support in case you have a problem with the contract in force. The sending of the material by the SVE must be preceded by contacting the destination laboratory, to agree details of time and delivery method, which must be confirmed by telephone or email. The records of the investigation in e-SISBRAVET must be carried out prior to sending the samples to the laboratory, so that the PNEFA focal point at the SFA is already aware of the situation and can follow the progress of the investigation. It is the responsibility of the PNEFA focal point at SVE and SFA to monitor the shipment until its arrival at the laboratory.

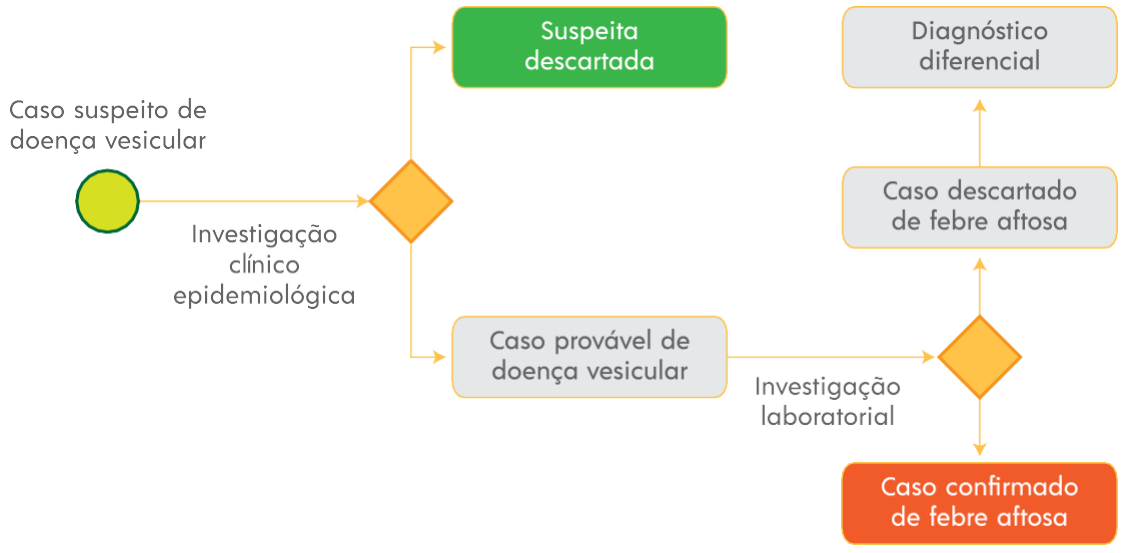
4. **Financial resources:** It is important that the SVE, both at the UVL and at the central level, have established and described administrative procedures for the prompt availability and use of financial resources, in case of need during the investigation and alert phases.

3. INVESTIGATION STAGE

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

- **target disease:** foot-and-mouth disease;
- **classic vesicular diseases (clinically indistinguishable):** vesicular stomatitis, Senecavirus A (SVA) infection, vesicular exanthema and swine vesicular disease (the last two exotic in Brazil);
- **other infectious diseases that, during their course, may present with vesicular or ulcerative lesions:** bovine vaccinia, bovine pseudopox, papular stomatitis, contagious ecthyma, bovine herpetic mamilitis, malignant bluetongue, infectious bovine rhinotracheitis and bovine viral diarrhea;
- **non-infectious diseases that can cause confounding clinical signs** (e.g., lameness, drooling) with infectious vesicular diseases: poisoning by plants, fungi, chemicals, trauma and others.

The definitions of suspected case of vesicular disease, probable case of vesicular disease, ruled out suspicion of vesicular disease, ruled out case of foot-and-mouth disease and confirmed case of foot-and-mouth disease are contained in the disease **datasheet** on the specific site of the Mapa and are in accordance with the criteria of the **OIE** Terrestrial Animal Health Code. Figure 2 demonstrates the flow of investigation of a suspected case of vesicular disease.



Suspected case of vesicular disease		**Discarded suspicion**		**Differential diagnosis**
	Clinical epidemiological investigation			**Discarded case of foot-and-mouth disease**
		Probable case of vesicular disease	**Laboratory investigation**	

				Confirmed case of foot-and-mouth disease

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

3.1. General considerations on the investigation of vesicular disease

The investigation phase begins when the SVO is aware of a suspected vesicular disease.

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

Every suspected case of vesicular disease, regardless of its origin, must be investigated by the SVO within 12 hours. The result of the initial investigation can be discarded suspicion or probable case of vesicular disease. Among reasons for discarded suspicion 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 vesicular disease require further investigation, including collection of material for laboratory diagnosis, and mark the beginning of the alert phase.

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 technically capable of making the decision on the progress of the investigation, requiring knowledge about the pathogenesis and epidemiology of vesicular diseases, training for investigation of vesicular disease, including material collection, and mastery of semiology techniques.

Table 1 shows the main stages of the pathogenesis of foot-and-mouth disease, while **Figure 3** shows the theoretical evolution of the biological reactions expected in a post-infection animal without a history of vaccination, highlighting the ideal times to collect material for viral isolation. This information was adapted from materials prepared by the Pan American Foot-and-Mouth Disease Center – Panaftosa - and is important for the veterinarian, after the clinical evaluation, to be able to inform the date of the probable onset of clinical symptoms in the evaluated animals.

Between the introduction of the virus (intracellular penetration) and the appearance of the first lesions, there is the so-called incubation period, which lasts up to 14 days, and is characterized by two distinct stages: **the eclipse stage and the prodromal stage**. In the eclipse phase, the virus is not isolated even if sophisticated means of investigation are employed. This phase can last for a few hours and corresponds to the intracellular penetration of the agent and the formation of the first complete viral particles. From the moment these particles are disseminated throughout the body, through the blood (viremia) and lymphatic

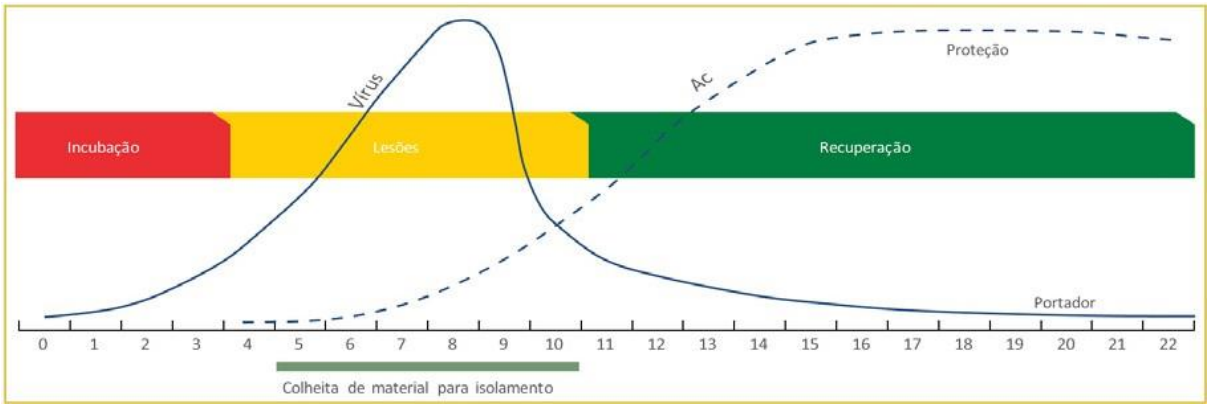
pathways, the prodromal phase begins, which lasts until the appearance of typical foot-and-mouth disease lesions. In the prodromal phase, animals show nonspecific signs (febrile reaction, depression and anorexia), common to several infectious diseases.

The vesicular diseases considered clinically indistinguishable from foot-and-mouth disease are vesicular stomatitis, Senecavirus A infection, swine vesicular disease and swine vesicular exanthema, being possible to differentiate between them only through laboratory tests. For additional knowledge about the main vesicular diseases, **Annex 3** provides electronic addresses that direct you to technical files, with information on foot-and-mouth disease and confusing vesicular diseases. In **Annex 4**, it is possible to find information about the characteristics of the lesions and the susceptibility of domestic species, and of the human species, regarding the group of vesicular diseases that can be confused with foot-and-mouth disease.

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

Pathogenesis of Foot-and-Mouth Disease	Estimated time
a - Virus inhalation b - Infection of cells in the nasal cavity, pharynx and esophagus c - Virus replication and spread to adjacent cells d - Passage of the virus to blood and lymphatic vessels and - Infection of lymph nodes and other glands f - Infection of cells in the oral cavity, paws, udder and rumen	24 – 72 hours (1 to 3 days)
g - Onset of fever h - Appearance of vesicles in the oral cavity, paws, udder and rumen i - Salivation, nasal discharge and lameness	72 – 96 hours (3 to 4 days)
j - Rupture of vesicles and intensification of symptoms k - End of fever l - End of viremia and start of antibody production	120 hours (5 days)
m - Decrease in virus titer in various tissues and fluids	From the 8th day
n - Injury heals and the animal begins to eat	From the 10th day
o - Gradual disappearance of the virus from tissues and liquids p - Increased antibody production	From the 15th day
a - Complete cure (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



	Virus	Ac Protection
Incubation	Injuries	Recovery
		Carrier
	Collection of material for isolation	

Figure 03. Theoretical evolution of foot-and-mouth disease in an infected cattle in days. Source: PANAFTOSA, 1978.

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

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

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

3) cattle are more susceptible to the foot-and-mouth disease virus, however, in animals with a certain degree of immunity to foot-and-mouth disease, lesions may only occur in the mouth, without generalization in the paws, or only in one or two paws, without the appearance of oral lesions. An example of this situation was the focus registered in Monte Alegre (PA), in 2004, when in the investigation of the suspicion, the SVO identified only one bovine with a mild clinical sign in only one paw. In unvaccinated herds, the susceptibility does not depend on the age of the cattle;

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

5) in sheep and goats, considering mainly the strains present in the South American continent, foot-and-mouth disease occurs in a milder form (with mild symptoms), even in animals not being vaccinated. These animals have 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) depending on the FMD virus strain, not all susceptible species are always affected, even living in the affected epidemiological unit. For example, in outbreaks recorded in 2000 and 2001 in Rio Grande

do Sul, despite the existence of swine and sheep living with cattle, only the latter showed clinical signs;

7) vesicular stomatitis, in turn, is endemic in some regions of Brazil. The important difference is the equine susceptibility. However, there are cases in which the disease has been identified in cattle and pigs, but it does not manifest itself in horses. In cattle, 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 may occur simultaneously. Thus, even in the concomitant presence of clinical signs in cattle and horses, one cannot rule out the possibility of foot-and-mouth disease without laboratory testing in bovine samples;

9) Senecavirus A infection affects swine and is endemic in some regions of Brazil, occurring mainly in technified farms. The first records in the country took place from 2015 onwards. It is commonly detected in slaughterhouses with the detection of healing or healed lesions. For this reason, it is important to raise the awareness of the producer and the technical responsible veterinarians for timely notification even on the farms, in order to allow the collection of suitable material for diagnosis;

10) 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;

11) swine vesicular rash was diagnosed only in the United States and Iceland. The disease was considered eradicated in 1959 and, since then, no more cases have been registered in any other part of the world.

3.2. Clinical inspection of animals and epidemiological assessment

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

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

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

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

4) salivation and lameness (in pigs there is greater difficulty in locomotion);

5) bright red, moist and non-bleeding secondary erosions, with or without fibrin deposits, in the muzzle, nostrils, mouth, crown band (crown) of the hooves, interdigital space, teats and udder;

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

7) distribution of animals with clinical signs:

a) in unvaccinated 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 successive stages of vaccination;

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

c) relation of the probable beginning of clinical cases with the entry of susceptible animals into the herd or cattle trucks for loading or unloading animals. In pig farms, special attention must be paid to the

origin of the food.

8) In cases where animals present salivation and lameness simultaneously, with detection or suspicion of vesicular injury, the measures provided for in probable cases of vesicular disease must be taken. In order not to reduce the sensitivity of the diagnosis, the mouth of any limping animal and the paws of animals with lesions in the mouth or nostrils should be examined.

9) The identification of a probable case indicates the possibility of occurrence of foot-and-mouth disease, pointing to another important objective of the investigation phase: the determination of the probable onset of infection. For this, in addition to the information obtained during the interview and anamnesis, it is important to carefully describe the secondary lesions (which develop after the eruption of the vesicles and the beginning of the healing process) to estimate the onset of clinical signs and the likely onset of infection. It is not uncommon for the respondent to make a mistake 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, either by a good anamnesis, or by a good clinical inspection and correct estimation of the age of the lesions. For example, an old lesion, in the healing phase, could not have started two days ago, or the incompatibility between the report of the presence of signs 20 days ago and all animals presenting newly ruptured vesicles. In these cases, the investigation should be expanded as much as possible to clarify all inconsistencies detected in the information provided. Therefore, defining the age of the lesions, particularly the oldest ones, is essential to establish the historical evolution of the focus, with emphasis on defining the origin of the infection and the period of greatest risk of spreading the viral agent. As a source of consultation and estimation of the age of the lesions, the collection of images of foot-and-mouth disease can be consulted.

10) In general, once the vesicles are ruptured, the speed of healing will be influenced by different factors, which allows, in practice, an approximate estimate of the age of the lesion. Until the fifth day, good accuracy is still possible, however, as time passes, the difficulty in estimating the age of the lesion increases. Below are some examples in estimating the age of lesions on the tongue of bovines and on the paws of swine:

a) closed vesicles: up to two days;

b) newly ruptured vesicles 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 sharp edges of fibrous tissue: between three and seven days;

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

3.3. Clinical and epidemiological aspects of other diseases confounded with foot-and-mouth disease

It is important to emphasize that the discard of the suspicion must be technically well-founded and, when in doubt, the professional must 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, what is expected is the presence of clinical signs

in a reduced number of animals, with less severe lesions, which can be verified indiscriminately on the tongue, mouth, interdigital spaces or udder.

In late care of suspects, it is more common to find the presence of secondary lesions, such as erosions, ulcers and crusts. In these cases, the veterinarian must be aware of some illnesses that can confuse the diagnosis of vesicular disease: bovine vaccinia, bovine pseudopox, papular stomatitis, contagious ecthyma, bovine herpetic mamilitis, blue tongue, malignant bluetongue, bovine viral diarrhea / disease mucous membranes, infectious bovine rhinotracheitis/ infectious pustular vulvovaginitis, among others. It is important that the MVO access technical materials and recent publications, in order to gain knowledge of the clinical aspects and the course of these other diseases that may have clinical signs considered to be confounded with vesicular disease.

In addition to confounding infectious diseases, other common cases of discarding suspected vesicular disease involve intoxication and physical or chemical trauma. In the case of poisoning, the substances responsible for photosensitization, caustic or abrasive chemicals, and also fungi of the Clavaria genus 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 Clavaria genus, associated with eucalyptus plantations, in hot times and with high humidity, are important in the southern region of the country, causing intense sialorrhea and necrosis of the lingual epithelium. Congestion of the conjunctiva, corneal opacity in sheep is observed, leading to blindness, difficulty in walking and falling of the animals. In cattle, it is possible to observe detachment of horns, tail hairs or strands of wool in sheep.

As for trauma, different elements can lead to salivation and lameness, especially injuries caused by dry and hard pastures, by recently cut pastures and crops, or by soils with a predominance of gravel. In cattle farms for milk production, foot problems are common, with conditions of: interdigital dermatitis; erosion of the stratum corneum; bead erosion; verrucous dermatitis; vegetative interdigital pododermatitis; digital dermatitis; interdigital phlegmon; diffuse aseptic pododermatitis; circumscribed pododermatitis; necrotizing pododermatitis; nail fissure; white line disease; phalanx fractures; sole and heel abscesses; sole ulcer; pinch ulcer; high arthrosis; dislocations; and sole hemorrhage. Also in relation to foot diseases, in sheep farms the occurrence of contagious ovine pododermatitis (foot root) is common.

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

The procedures to be adopted by SVO veterinarians when reporting a suspected vesicular disease are presented below. It should be stressed that, in addition to the importance of time in the service, another fundamental issue is

the correct and complete recording of the activities carried out.

Upon receipt of a notification at the UVL or by e-SISBRAVET, the UVL shall:

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

When notification is made by telephone, it is recommended, as a precaution, to record the originating telephone number and confirm by callback. However, if the person does not wish to be identified, confidentiality must be guaranteed.

The person who made the notification of the suspicion, if he has been in contact with the animals, must be advised on the necessary biosecurity procedures to prevent the spread of the possible infectious agent, especially regarding the non-movement of the suspected animals and their direct contacts, and do not enter any other property with animals susceptible to foot-and-mouth disease until the end of the investigation that will be carried out by the 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 their classification.

2nd initial survey of information

Initially, the information available in the property register must be evaluated (SVE registration system or e-SISBRAVET "Prepare service" tab), highlighting: identification of the property and its producers, survey of the number of livestock farms and the existing herd; animal movement intensity (mainly ingress and egress occurrence in the last 30 days); date of last vaccination; geographic location and access routes. Also identify the neighboring properties and those with some link (which maintained in the last 30 days some type of relationship – entry/exit – with the property that has animals under investigation). It is still important to obtain information from other properties owned by the same owner and other producers who may be involved in the property. This first survey of information must be carried out in an objective and quick way so as not to compromise the reaction time. Depending on the result of the initial care, new information must be obtained for further analysis.

As a precaution, between the period of notification of a suspected case and its attendance by the SVO, the MVO is entitled to prevent the issuance of an animal transit document that has as its origin or destination the epidemiological unit where the suspected cases of vesicular disease are located.

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

3rd Displacement to comply with the notification

Parallel to the survey of initial information, the preparation of the vehicle for displacement and the kit with the material for the care of suspected vesicular disease must be provided. The UVL team and the immediate superior must be informed of the time and reason for leaving: attending to a 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 surveillance, seek to identify and make prior contact with the owner or person responsible for the animals to agree on the best and fastest 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 the road and lighting conditions at the location, the most recommended service is the service in the early hours of the morning of the following day. In the event that the veterinarian responsible for the UVL is not present at the time of notification, the server receiving it must carry out the initial registration in the system and contact the central unit or 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 person responsible for the animals, the notification may be attended to with the help of the police forces, and all resources must be exhausted before using this action. SVO professionals must carry a functional card or other professional identification document. It is recommended to have a copy or access to the legislation that empowers them to take the necessary measures in the field of animal health protection, with emphasis on entering a rural property or any other place to examine animals with suspected vesicular disease, and for the interdiction of the site, if the risk of the presence and spread of an infectious agent is confirmed.

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

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

4th Actions on the property

Arriving at the property with suspicious animals, the professional must take all precautions with biosafety and pay attention to investigation, 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 in the specific form for investigation of vesicular disease) 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 mangroves 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 the spread of the disease are reduced, the animals can be moved within the property to a location that facilitates the 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 discard the suspicion of infectious vesicular disease;

c) in the batch under investigation, inspect as many animals as possible. For communicable diseases such as foot-and-mouth disease, the order of inspection of animals that are in coexistence has no epidemiological importance, and inspection may start with healthy animals or animals with clinical signs. However, given the need to quickly assess the suspicion presented, and especially in situations where the clinical inspection of animals proves to be complex, it is recommended that the inspection start with the animals with apparent clinical signs, in order to carry out the collection of material for laboratory diagnosis (if necessary). The important thing is that the greatest number of animals be examined, both those with clinical signs and those apparently healthy, in order to assess the spread of the disease and the age of the lesions, in addition to establishing, with the support of the interview carried out, the probable onset of the health episode;

d) the clinical inspection must be extended to other species susceptible to foot-and-mouth disease and equidae existing on the property. The veterinarian responsible for the care must keep in mind that, depending on the clinical and epidemiological situation found, there will be a need for other visits for additional inspections of the herd. The first visit has as priority the disposal or confirmation of the suspicion and, when necessary, the collection of samples to be sent to the laboratory recommended by the Ministry. In **Annex 5**, a basic guide for the examination of animals suspected of vesicular disease can be found;

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

f) The clinical and epidemiological investigations carried out at 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: rule out the suspicion or confirm the occurrence of a probable case of vesicular disease (**Figure 4**).

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

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

a. Suspicion discarded

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

• cases of false reporting or absence of animals susceptible to foot-and-mouth disease;

• occurrence of non-infectious disease (intoxications, foreign bodies, trauma); or

• occurrence of another infectious disease, presenting a clinical picture incompatible with vesicular disease.

The MVO must list all the information that substantiated its diagnosis, recording them in the investigation form, and may make photographic records for insertion into 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 must be closed, recording the final diagnosis and the information that substantiates it, 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 registration information and existing herds. If the property is not registered with the state veterinary service, obtain the necessary information for its inclusion in the database and pass on to the owner or person responsible for the animals the guidelines and information on the legal and sanitary aspects involved.

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, gathering information and biosafety. Next, some procedures and information about each of these activities will be highlighted, to be considered in the place where the probable cases of vesicular disease are located. It is worth emphasizing that, in this alert phase, there is a very important time interval, in which actions must be taken, taking into account the possibility of confirming a case of foot-and-mouth disease, and that are described in the Alert Stage.

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 investigations, requires laboratory support to support the confirmation of the diagnosis. The quality of sample collection and shipment directly affects the time for laboratory processing. At this point, the following guidelines are highlighted:

1) collect at most, at each visit, samples from 10 animals;

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 to perform the tests and, consequently, greater flexibility in processing the samples;

3) samples sent to the laboratory must only be accompanied by the specific

form (FORM LAB) in physical format; it is recommended whenever possible, the photographic record of the injuries, taking all the necessary biosafety precautions;

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

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

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

b.1.1 **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 test of antibodies against the FMD virus can have four causes: natural infection; vaccination; presence of maternal antibodies; and cross-reactions (heterophilic). With respect to 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 FMD virus is easier information to be analyzed, and it should always be associated, however, with the clinical and epidemiological picture found. Therefore, although the identification of antibodies against the FMD virus in animals with clinical signs of vesicular disease represents an important finding in unvaccinated herds, in areas where there is an implemented surveillance system, 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-reference information.

b.1.2 **In the case of service with epithelium collection**

The identification of probable cases that allow the collection of material for viral isolation is the most desired situation, giving more security to the final diagnosis and indicating that notification and care by the SVO occurred in a timely manner. It is important that the following recommendations are followed:

1) The material of choice is composed of vesicular fluid and fragments of

epithelium from newly ruptured vesicles, including the edges of the lesions;

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

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

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

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

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

7) The material collected from each region (oral, nasal, foot and udder) should be placed in separate bottles. Never mix materials from different animals in the same bottle. The bottles must be properly sealed, identified, packed in sealed bags and kept under refrigeration or, preferably, frozen (-20 °C). Once sealed, the bags must be cleaned and disinfected before being stored in the isothermal box (the use of small sprayers or manual sprayers, with a 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, in which the veterinarian must assess the number of animals for inspection. If new cases are found, with easy sampling, the professional must increase the number of animals inspected (without compromising the service time), in order to detect the oldest lesions to support the definition of the probable onset of the disease. On the contrary, if only old lesions are found, with difficulty in collecting material, the professional should inspect as many animals as possible, with the objective of finding newer lesions, with greater possibility of viral isolation;

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

10) In pigs, material collections carried out in slaughterhouses should preferably be carried out on the animals before the scalding process. If suggestive lesions are detected in the inspection lines, the MVO must check the existence of

animals from that batch that have not yet entered the slaughter room for a clinical evaluation, with the objective of collecting samples in the arrival and selection pen.

b.1.3 In the case of care where it is not possible to collect the epithelium

In peculiar situations, such as in ruminants tested for transit purposes and which have reactive results in the serology for foot-and-mouth disease, without presenting clinical symptoms, the investigation can be carried out using techniques aimed at collecting paired samples of esophageal-pharyngeal fluid (LEF), with an interval of 15 days, using an appropriate collection cup. It is worth noting that LEF collection for foot-and-mouth disease is carried out only in ruminants. These situations must be recorded on the standard investigation forms. The collection of LEF requires specific training and the animals must fast for a minimum of 12 hours (collection procedures described in **Annex 7**). The esophageal-pharyngeal fluid must be stored in an equal amount of MEM and frozen as quickly as possible, proceeding with the measurement of the pH of the preservation medium before collecting the material. In the case of negative samples, it is recommended to perform another collection, with an interval of at least 15 days, in order to obtain a more consistent diagnosis.

In pigs, one must choose to collect fragments from the tonsils and keep them frozen until arrival at the Laboratory.

The Vallée and MEM media, used for the conservation of the epithelial and LEF samples, have a distinct composition, and are described in **Annex 6**. In addition to the conservation function, these media aim to prepare the samples for the different procedures to which they will be submitted in 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 presents the recommendations for using the medium by type of material collected.

Table 2. Recommended conservation methods for the different materials:

	Vallée liquid	MEM
Epithelium	X	X
Vesicular fluid	-	X*
Vesicles swab	-	X
LEF	-	X

* only in samples with a volume of less than 200 microliters

In **exceptional** cases, in which it is not possible to use these means, contact the laboratory responsible for diagnosis or the screening sector of the SVE for specific guidance.

b.1.4. Specimen collection for differential diagnosis

Considering that the target disease of PNEFA is foot-and-mouth disease, it is essential to emphasize that tests for differential diagnosis will only be performed in the event of negative results for foot-and-mouth disease. With a view to a conclusive diagnosis, depending on the quality and quantity of 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 SVA) are part of the differential diseases researched in routine laboratory analysis, in investigations of probable cases of vesicular disease.

b.2. Survey of information (epidemiological investigation)

After finding a probable case of vesicular disease, the gathering of information should be deepened through an interview with the owner or those responsible for the animals. The questions should seek to determine the likely starting day of the health event and its possible origin, and assess the degree of risk of dissemination. For this, remember that in the case of foot-and-mouth disease the incubation period is up to 14 days, at most, being between 2 and 7 days more common. Determining the links is very important, and information on animal movements covered by GTA and even those carried out informally should be sought.

The MVO responsible for the service must meet with the people directly involved to carry out the interview, at which time they must provide guidance 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 likely onset of the disease, and making a relationship between the information found and the chronology and age assessment of the lesions examined on the date of the visit. A detailed flow of assistance can be seen in **Annex 8**.

b.3. Biosafety activities

Biosecurity measures are the set of activities used to avoid or minimize the risk of spreading the disease. **Annex 9** contains the biosecurity procedures at the entrance and exit of the place where there are probable cases of vesicular disease, for the professionals involved in the service. The main procedures that must be adopted while still on the property are described below:

1) draw up an interdiction term and pass on, in a clear and objective manner, the guidelines on the precautions to be taken to avoid the spread or aggravation of the health problem. The UVL must have interdiction and release forms for ready use.

The interdiction term must contain the reason for its application, its legal basis, space for knowledge of the owner or person responsible for the herd, and the main established prohibitions;

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

a) prohibit the removal of animals and products at risk for the spread of foot-and-mouth disease. Non-susceptible animals are also included, given the risk of mechanical transmission of the foot-and-mouth disease virus;

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 the material used to store these products;

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

d) leave the lot with probable cases of vesicular disease under the responsibility of only a small group of workers, who cannot have access and contact with other susceptible animals on the property;

e) guide 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 had direct contact with sick animals);

f) prohibit visits by anyone without authorization, including veterinarians, technicians who work with artificial insemination and other professionals and producers, especially those who have contact with animals susceptible to foot-and-mouth disease;

g) 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 swine). Milk represents a direct risk and also a 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 while the risk of spreading the disease persists. Even knowing that this is a measure that involves several economic and social issues, one must consider that milk has a low unit value and it is often safer to recommend its destruction, with compensation to the producer. Alternatives to be used and recommended in relation to milk include:

I) destination for the manufacture of products submitted to thermal processing (mozzarella, cream cheese, among others) within the property;

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

III) destruction, with the use of chemical products that change the pH (for example, vinegar or caustic soda), discarding the product in an open trench for this purpose. Do not spill the product into rivers or other water collections.

In the matter of biosafety, special attention must be given to the disinfectants used in different situations. **Annex 10**, adapted from the **Manual of Procedures for the Care of the Occurrences of Foot-and-Mouth Disease and other**

Vesicular Diseases, of Panaftosa, presents information and a list of chemical products that can be used in cleaning and disinfection work.

5th Return to UVL

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

Once a probable case of vesicular disease is confirmed, the **Alert Phase** begins.

4. ALERT STAGE

4.1. Surveillance activities

The alert phase involves the period between the confirmation of a probable case of vesicular disease and the definitive diagnosis supported by laboratory testing. This phase must be conducted considering the probability of occurrence 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 risk of dissemination of the possible viral agent; and continue to gather information to, if necessary, implement emergency animal health actions. Activities must be conducted with great caution, so as not to produce turmoil or panic in the local community. Only the professionals necessary to carry out preventive and complementary epidemiological investigation operations should be involved.

On return to 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, properly packaged and identified, to the screening laboratory of the central unit of the SVE;

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

material by the UVL.

3) deepen the analysis of links involving the herd with probable cases of vesicular disease. Confirm all properties located around the establishment with affected animals (defined as properties with an epidemiological link due to geographic proximity) and those that, in the last 30 days in relation to 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 GTA of the investigated property suspended and, together with the central unit of the SVE, suspend the issuance of GTA of the related properties.

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

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 Ministry, which will be responsible for informing the SVO of the neighboring country;

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

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

8) Consider that, in the case of foot-and-mouth disease, animals can eliminate virus from three days before the onset of clinical signs, which makes it necessary to provide a team for each linked property, in order to reduce the risk of dissemination of the disease. Even not observing clinical signs compatible with vesicular disease, 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 banned establishment, until confirming or discarding the case of foot-and-mouth disease, in order to monitor the evolution of clinical cases; assess compliance with established restrictions and gather additional information that can support the epidemiological investigation, especially the onset date and origin of the disease (use the FORM COM to record the information obtained during the complementary investigations at the facility, recording the data and loading the forms and photos in e-SISBRAVET).

10) Review all the information in **Annex 1.**

At the SVE central unit:

After knowing the probable case of vesicular disease, the focal points of the PNEFA in the SVE together with the epidemiology sector should:

1) Analyze the data registered in e-Sisbravet, investigation forms and available photos;

2) Inform the PNEFA focal point at the SFA;

3) Contact the person responsible for the diagnostic laboratory, informing them about the samples and the date of probable shipment. It is recommended to send an e-mail with FORM LAB, well in advance, so that the laboratory staff can schedule the receipt and analysis of samples in the shortest time possible;

4) Prepare and send the collected material to the laboratory indicated by the Division of Foot and Mouth Disease of Mapa, in the shortest possible time. It is the responsibility of the PNEFA focal point at the SVE, together with the PNEFA focal point at the SFA, to monitor the shipment until its arrival at the laboratory. **Annex 11** contains recommendations on packaging, storage and shipping of infectious material to the laboratory, in accordance with international regulations. Samples for identification of the agent taken from probable cases of 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);

5) Also carry out the analysis of the property register and the movement of animals, identifying properties with an epidemiological link, especially in the 30 days prior to the probable onset of the disease and neighboring properties;

6) Delimit in advance a probable emergency area, contemplating the initial geographic space for possible interdiction and intervention, if the result is confirmed. The initial delimitation must be carried out by the epidemiology sector of the SVE, adopting as a criterion the total area of the municipalities covered by a radius of 25 km, measured from the property with a probable case. This preliminary work aims to obtain information necessary to optimize the response time in the hypothesis of confirmation of the FMD case;

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

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, dumps, veterinary hospital, rendering plants, etc.);

f) All properties and points in the delimited region must have their geographic location data extracted from the system and made available in spreadsheets so that, in case of confirmation, the file can be accessed in a situation where there is no internet; -,

g) Measure the need for people, equipment and materials for investigations within the delimited areas.

h) Review all the information contained in **Annex 1** related to the related municipalities (investigated property and links).

i) The central unit of the SVE, together with the SFA, is responsible for coordinating and monitoring the entire investigation process.

In linked properties:

The investigation and surveillance work on properties with an epidemiological link must be careful and precise, including the following activities:

1) Employ all biosecurity 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 vesicular disease, consider it as a probable case of vesicular disease and follow all the steps of the investigation and alert stage; or

4) If there are no signs of vesicular disease, register the activities on the FORM VIN and e-SISBRAVET. The properties must be under surveillance, awaiting the laboratory results. Until the laboratory results of the property under investigation, return every three days to properties with an epidemiological link, for a new evaluation;

5) Considering the possibility of animals being in the incubation period, even if no signs of vesicular disease are observed, the professionals responsible for the investigation must 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 surveillance system on property (active surveillance), from notifications (passive surveillance) and serological surveillance (seroepidemiological studies, carried out only in free zones with vaccination), the surveillance system for foot-and-mouth disease has two more components that can detect probable cases of 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 breeding establishments, thus Avoiding any movement or shipment of animals with injuries compatible with infectious diseases to slaughterhouses, crowding events or any issue of GTA that leads to the movement of suspicious animals, aiming to provide care even in the establishment of origin in order to prevent the spread of diseases, in addition disturbances in slaughterhouses, agglomeration or traffic inspections.

The actions that must be taken in each situation will be reported below:

Identification of probable cases of vesicular disease in animal slaughter establishments susceptible to foot-and-mouth disease

In ante-mortem and post-mortem examinations, in case of detection of vesicular lesions, the animals must be segregated and the SVE urged to support the investigation in the establishment of slaughter and in the property of origin of the animals. Flocks with animals identified as probable cases of vesicular disease should be slaughtered last, avoiding direct contact with others. After separation, the slaughter of healthy batches can proceed. Carcasses, viscera and other products from the slaughter of the day, both from the batch with clinical signs and from batches without clinical signs, must be segregated and kept under the control of the Inspection Service until the investigation is completed. Other measures must be applied until

the final results of the investigation ruling out the disease are:

1) Survey of information on the origin of animals and activation of the SVE to investigate the rural properties involved. The SVE will be responsible for the precautionary suspension of the issuance of GTA from the property of origin until the completion of the clinical-epidemiological investigation at the origin;

2) Suspension of the output from the slaughterhouse of the products obtained from the slaughter on the day on which the probable case was found,

3) The output of products submitted to thermal treatment sufficient to inactivate the virus is allowed, provided that the biosafety measures that guarantee the inactivation of the infectious agent in transport vehicles are adopted;

4) The movement of people, as well as other materials, objects and means of transport that can carry the infectious agent from the slaughterhouse, is subject to biosafety measures defined by the SVO.

5) After the end of activities on the day on which a probable case was detected and the establishment's complete cleaning and disinfection, provided that the biosafety measures ensure the inactivation of the viral agent, the slaughter can be released the following day and its products can be marketed.

6) The exit of stored products from slaughter prior to service may be allowed by the competent authority, after assessment and adoption of risk mitigation measures by the Inspection Service (tracing of batches, cleaning and disinfection of transport vehicles, etc.).

Actions in swine slaughter establishments:

In the case of swine slaughter establishments, when the Inspection Service finds vesicular lesions and the batch is accompanied by SVE documentation declaring prior investigation on the property (up to 30 days prior to slaughter) and disposal of suspected foot-and-mouth disease, either by clinical-epidemiological evaluation or by the negative laboratory test report, the slaughter may proceed normally (Joint Circular Letter DSA/Dipoa 01/2020).

The referred SVE documentation must include: dates of initiation and conclusion of the investigation; identification of the breeding establishment; criteria for the conclusion of the investigation (use the following options: 1 –

suspicion of vesicular disease ruled out by clinical and epidemiological criteria, or 2 – case of foot-and-mouth disease ruled out by laboratory criteria); telephone number, identification and signature of the MVO responsible for the service. Only a copy of this document must accompany the GTAs, excluding investigation forms and laboratory results reports.

In case of detection of recent vesicular lesions that are not compatible with the date of the clinical and epidemiological evaluation on the farm, or in batches unaccompanied by the SVE documentation evidencing previous care and discarding suspected foot-and-mouth disease, the SVO should consider it as a case probable of vesicular disease and adopt the measures foreseen in this manual.

Identification of probable cases of vesicular disease in livestock events

Upon 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 the 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:

1) restrictions on the movement of animals, means of transport, objects, materials and people, in order to prevent the spread of the virus;

2) ban on leaving all animals, which must remain in their respective stalls, with access to food and water;

3) collection of samples for laboratory tests;

4) permission for people and means of transport to leave the establishment only after biosecurity measures and authorization from the SVO;

5) suspension of GTA issuance from properties of origin of animals considered probable cases of vesicular disease, and properties with an epidemiological link; and

6) epidemiological investigation to identify the source of infection.

Identification of probable cases of vesicular disease during animal transit

Due to the difficulty of carrying out an adequate clinical inspection, it is quite uncommon to be able to detect a probable case of vesicular disease during the inspection of animals in transit, both in mobile inspections and in fixed posts. Listed below are the recommended procedures if during this activity, considered a sanitary management action to mitigate the irregular transit of animals and their products, if probable cases of vesicular disease are detected:

1) If the identification of animals with clinical signs compatible with vesicular disease has occurred at inspection posts located on interstate borders, prevent entry into the state, retain the vehicle with the animals and immediately notify the central unit of the SVE. This 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;

2) The property of origin of the animals must be inspected and interdicted, and properties with the possibility of an epidemiological link must have the movement of animals suspended and be inspected. It is recommended that properties located in the path of the animals are classified as links and, therefore, the surveillance recommended in this type of property is carried out, especially in the case of transport of cattle on foot or in those where there was a stop to rest the animals;

3) Samples must be collected from suspected animals for laboratory examination, their records carried out and all biosafety procedures recommended for an investigation of a probable case of vesicular disease;

4) Issues related to the place to carry out the collection and to keep the animals until the processing of the samples and the final result of the investigation must be analyzed, considering the following points:

5) Assess the possibility of identifying a nearby location for temporary abduction of the animals. This location must not contain other susceptible animals. The choice must consider the risks involved and seek a solution that compromises the smallest possible area and facilitates the actions to eliminate the animals, if the occurrence of foot-and-mouth disease is confirmed;

6) If the presence of sick animals has also been found on the property of origin of the animals and if the distance between the property and the place of interruption of traffic is not too great and does not put other properties at risk, the possibility can be evaluated. of return of the animals to the origin;

7) In the case of transporting the cattle on foot, the animals must be boarded in appropriate vehicles to be sent to the identified location for kidnapping, considering the biosecurity measures;

8) In any event, the transport of animals must be carried out in vehicles escorted by the animal health defense service, with police support. Transport vehicles must be subjected to cleaning and disinfection soon after disembarking the animals;

4.2 Completion of 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 conclude the investigation with a discarded case of foot-and-mouth disease, 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 in **Annex 12**.

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

Inappropriate material for diagnosis: due to insufficient quantity or conservation problems. This situation should be avoided, but if it does occur,

immediate steps must be taken to visit the property again and collect material (with registration and completion of complementary and laboratory investigation forms). Take the opportunity to update information regarding new cases. The property and those related to the investigation must remain closed.

Negative diagnosis of foot-and-mouth disease: the discontinuation of the property with investigation of a probable case of vesicular disease will occur when the SVO is notified of negative laboratory results, in addition to the clinical and 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-epidemiological evaluation of the pigs on the affected farm. In all cases, the end of the investigation and the final diagnosis must precede the completion of the closing complementary investigation form, with data registration and upload of the form in e-SISBRAVET. It should be noted that it will be up to the MVO, through all the epidemiological, clinical and laboratory information, to define whether or not the investigation should be closed, and depending on the characteristics analyzed in the investigation, it may maintain the property interdiction, even with negative laboratory results, and may carry out new samples and submit new samples to the laboratory.

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

ANNEXES

ANNEX 1. Other important information and database for the alert and emergency stage

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

1. database referring to all slaughterhouses, slaughterhouses, meat processing companies (packaged), dairy factories, processing plants, refrigeration stations and dairy products existing in the geographic area of its operation, containing information on capacity, species, contact of the technical manager, owner and geolocation data of the establishment;

2. have registration and knowledge of the main agricultural characteristics of the area under its jurisdiction, with emphasis on the inflows and outflows of animals, their products and by-products. Analysis of the movement of animals, products and by-products, including main flows, origin and destination, must be carried out annually and with the knowledge of technicians at the central and local level of the SVE;

3. have digital maps of the geographic area in which it operates, including information on geopolitical boundaries, road network, hydrographic network, location of rural properties, milk lines and the path of each line, villages, villages, indigenous reserves, rural settlements, conservation units or environmental protection areas, forest reserves, among other elements of relevance to health intervention activities.

4. updated database, with name, position, address and contact form for municipal authorities (including police forces), representatives of civil defense and representatives of the agricultural sector. When located on the international border or on the state border, include the name, address and contact form of the person responsible for the neighboring UVL, belonging to the neighboring country or state;

5. database with the names of the animal health emergency team at the 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 responsible for 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, education, address and form of contact for self-

employed professionals, from the private sector and from other institutions that work in the field, mainly veterinarians, zootechnicians and agricultural technicians;

8. Database with contact list of heavy machinery owners, such as backhoes, crawler tractors, tractors with wheel loaders, trucks with buckets, among others, that can be used in animal health emergency activities (including identification and contact form with those responsible for releasing these machines);

9. database with name and capacity of hotels, farm hotels and other establishments that can host a large number of professionals, with geolocation data of establishments;

10. database of airports and runways, 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, gyms, community centers, etc.), with the geolocation data of the establishment, for possible implementation of Coezoo, with the geolocation data of the establishments . The site must be large and available for use for at least three uninterrupted months, and have the following characteristics: capacity for installing a warehouse, patio that can be used as a garage for many vehicles, good lighting and plenty of water, possibility control of 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 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 geolocation data for these locations;

14. database with the registration of owners and drivers of vehicles transporting animals or products at risk and those responsible for animal transporters in the region, including type, quantity and capacity of vehicles per owner or carrier;

15. identification in the registry 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 data for contacting 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

access for susceptible animals within these establishments;

- resellers of veterinary products, including the name of technically responsible veterinarians;

- auction sites and other agglomerations of animals, with identification of the event organizers and respective technical managers;
- grease, tanneries and salting houses, with identification of the technical responsible;

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

List of material for care for suspected vesicular disease			
1	Mouth opener	22	Vallee liquid and MEM
2	Antiseptic	23	Rubber gloves and procedure gloves
3	Needles: . Disposable, sterilized hypodermics (40 x16 mm) . Disposable for vacutainer® tube (0.80 x 25mm) Adapter for multiple harvesting 25 x 8 mm	24	Overalls
4	Hydrophilic cotton and gauze	25	Materials for animal identification: earrings and applicator, tattooing forceps, etc.
5	Plastic bucket	26	Eppendorf microtubes, 2 ml capacity (for serum)
6	Notepad	27	Microtubes with screw cap and sealing ring (for epithelium and swab)
7	Back pump and hand sprayers	28	Absorbent paper (paper towels)
8	Rubber boots	29	Ph indicator paper
9	Box with instruments for necropsy	30	Forceps (mouse tooth type)
10	Styrofoam (isothermal) boxes in various sizes	31	Pasteur pipette (transfer), disposable, sterile, 3 ml capacity
11	Box for storing and transporting materials	32	Plate or banner for identification of prohibited properties
12	Disinfectants, detergents and soap	33	Clipboard
13	Brushes for cleaning boots and hands	34	Disposable 3mm biopsy punch
14	Adhesive	35	Sharp material disposal container
15	Polyethylene adhesive tapes	36	Plastic bags or bags for disposable waste
16	Inquiry forms	37	1 ml (insulin) and 10 ml syringes
17	Ant and pipe for containment	38	Synthetic flocked sterile swab
18	15 and 50 ml Falcon sterile bottles	39	Clinical thermometers for veterinary use
19	Recyclable ice	40	Interdiction and Release Terms
20	Loops or ropes for containment	41	Scissors and Scalpels with Blade
21	Flashlights and Batteries	42	10 ml Vacutainer® test tubes, without anticoagulant

ANNEX 3. Internet addresses with health 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 STOMATITIS:

• 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. 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. SWINE VESICULAR DISEASE:

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

5. VESICULAR RASH:

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

ANNEX 4. Comparative table of the main vesicular diseases

Characteristics	Foot-and-mouth disease	Vesicular stomatitis	Senecavirus A	Swine vesicular disease	Vesicular rash
Morbidity	High (60% to 100%)	Low to medium (5%-10%);	High in piglets (5 to 60%)	High (25-65%) - subclinical infections occur	High
Mortality	Low (in young animals it can be high)	In dairy cattle herds up to 85%	Low	Low	Low (<5%)
Transmission	Contact, aerosols, meat products. Doubts about the role of carriers. By wind, only under special conditions (temperature, humidity, pressure, wind).	Doubts about the role of contacts, carriers and vectors. milking machines	Oronasal	contact - meat products (persists in meats chilled/frozen) -through foot wounds - nasal and oral secretions	contact - meat products (persists in refrigerated/frozen meat) through foot wounds - nasal and oral secretions
Susceptible Species	Cattle, swine, sheep and goats	Cattle, pigs, horses, goats and humans	Swine	Swine	Swine
Observations	Persistence in cattle. Viruses in feces, urine, milk, esophageal-pharyngeal fluid, respiratory aerosols and lesions. 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 the infection/ streaming. Wild fauna?	Vesicular lesions, mainly in sows and finishing animals and neonatal mortality. In addition to the lesions, viruses are also present in oral, nasal and feces secretions. Detection of virus in tonsils	Zoonosis - related to human Coxsackie B5 virus. Virus very resistant to inactivators/environment. Elimination/ feces – 3 weeks. Contamination of fomites. No vertical transmission has been demonstrated.	Persistence in chilled/frozen meat. Post-infection immunity – 20 months – but no cross-immunity with other serotypes. Mortality may be higher in young animals. Abortions and females that do not allow fomites to breastfeed are not a problem. No vertical transmission has been demonstrated

Source: adapted from the Manual of Procedures for the Care of the Occurrences of Foot-and-Mouth Disease and other Vesicular Diseases, Panaftosa

ANNEX 5. Basic guide for examination of animals suspected of vesicular disease

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

a) Before immobilizing the animals, note:

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

2. Properly contain animals and record all details about observed signs and injuries.

a) Record body temperature and estimated age Normal values (variation of + or – 0.5°C may occur)

- cattle = 38.5ºC
- sheep = 39.5°C
- goats, pigs and horses = 39.0°C

b) Describe the vesicles in detail:

- intact or broken (closed or open);
- size;
- color (e.g. whitish, bright red, yellowish etc.);
- depth;
- defined or worn edges (boundaries);
- degree of scarring (presence of fibrin deposits).

3. Cattle

Location of injuries:

- inspect the nostrils;
- in the oral cavity, inspect the tongue, lips, gums and side walls and superiors;
- edges (remove dirt with running water): interdigital space, band coronary artery and beads;
- udders and teats;
- vulva and foreskin.

4. Pigs

important signs

- acute and sudden lameness;
- watch the animal on concrete or another hard surface and encourage it to walk.

Injuries

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

5. Small ruminants

important signs

• acute and sudden lameness (usually affects all extremities), diagnosis differential: foot-rot. Injuries

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

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

ANNEX 6. Composition of solutions used to conserve materials for sending to the laboratory

50% Vallée liquid

(for epithelial collection)

1. KH2 PO4 (1,35 g) Potassium phosphate monobasic
2. K2 HPO4 (7,80 g) dibasic potassium phosphate
3. 1% Phenol Red (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)
7. Sterilize the phosphate and glycerin solutions in autoclave in different vials 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 bottle and mix. Fraction as needed and availability of sterile vials.
8. Add 1000 IU penicline 100 IU neomycin sulfate, 50 IU polymyxin B sulfate and 100 IU mycostatin.

MEM

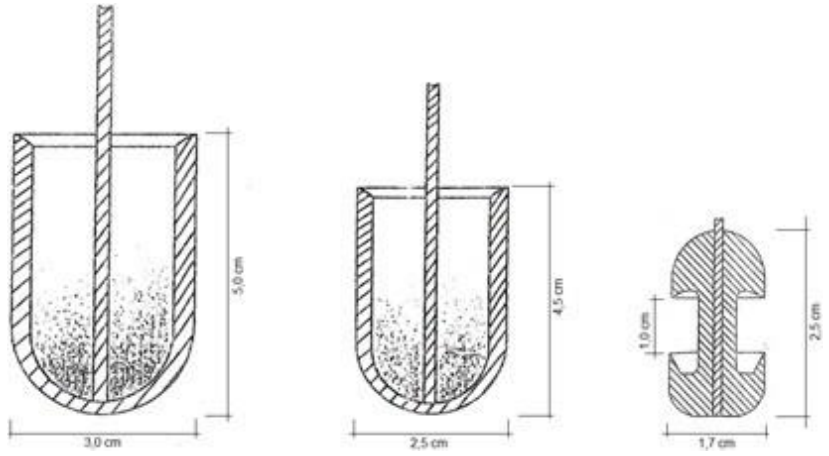
With lactalbumin hydrolyzate and yeast extract (for esophageal-pharyngeal fluid collection - LEF and Suabe)

Eagle's minimal essential medium
With Earle's salts, non-essential amino acids and sodium bicarbonate.
It can be purchased in powder form or ready to use.
If purchased in powder form, hydrate as directed by the manufacturer and sterilize by filtration.
Add 1000 IU of penicillin, 100 IU of neomycin sulfate, 50 IU of polymyxin B sulfate and 100 IU of mycostatin, every 1 liter of the sterilized liquid medium.

Note: The control and recording of the storage temperature and pH of the media must be constant.

ANNEX 7. Technique and procedures for collecting esophageal-pharyngeal fluid (LEF)

Facilities: It is essential that the facilities are suitable for a perfect containment of the animals and allow the head to be immobilized and turned upwards, maintaining an adequate and comfortable position for carrying out the harvest. The correct containment of animals is an important factor to facilitate the work and prevent accidents, both for the 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) attached through the center of the inside to a curved rod approximately 50 cm long.



LEF Collector Models

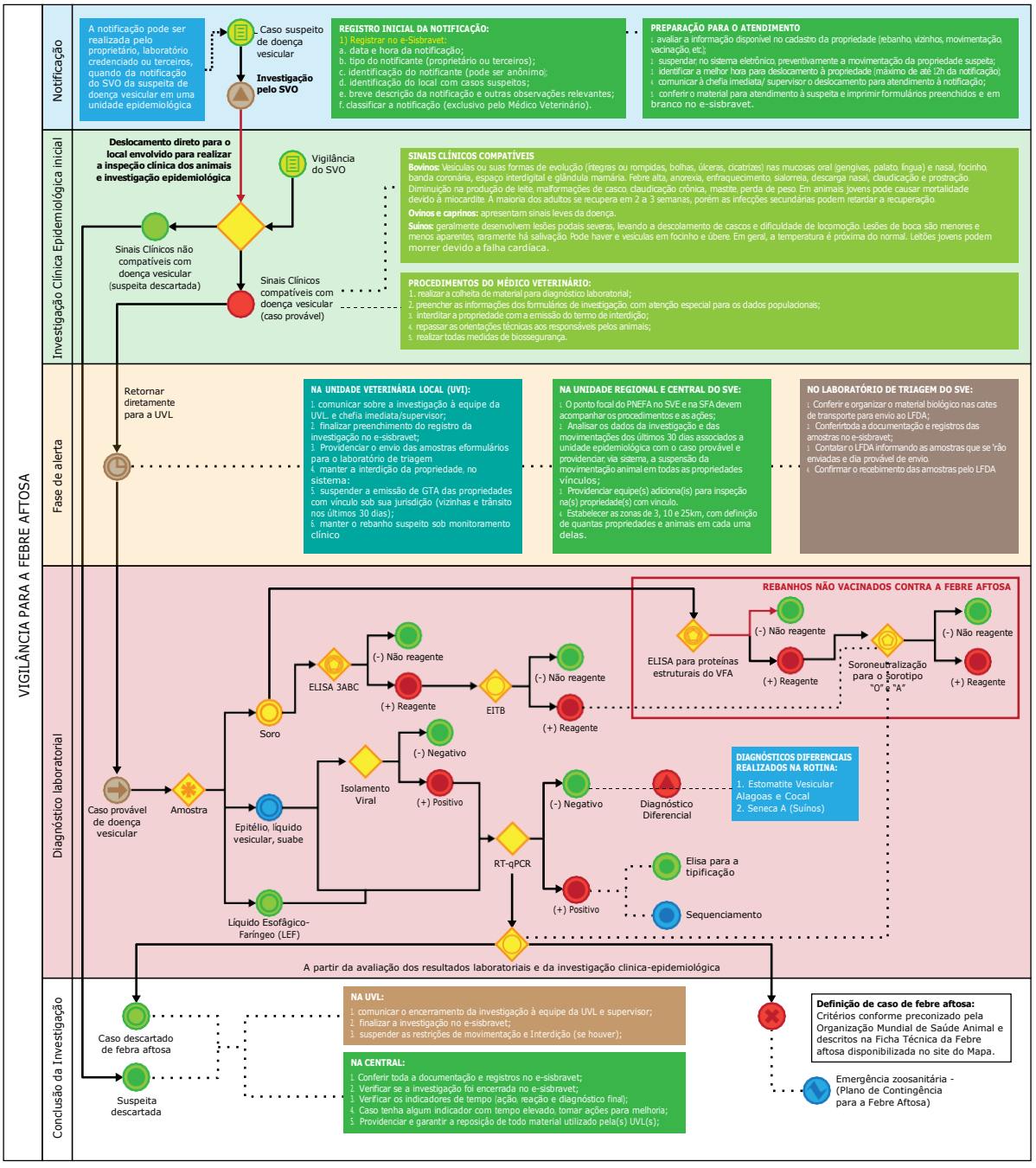
Ruminants: Animals, properly identified, must remain on a water diet for at least 12 hours. One hour before harvesting, water should be administered in order to eliminate any food residues and moisten the esophageal-pharyngeal region. This procedure facilitates the penetration of the collector, as well as the scraping of the mucosa. The use of tranquilizers that cause myorelaxant 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 collection of the sample. In this case, the operator must reject the material and try another harvest, after leaving the animal to rest for a few hours. If you persist, it is advisable to transfer the harvest to another day.

Sample collection: During the work, the operator must take all precautions to avoid possible transmission of virus 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 down, and through the labial commissure, make the collector penetrate carefully, until reaching the pharynx and anterior part of the esophagus. This is

characterized by the animal's voluntary swallowing movement. After swallowing, the esophagus region can be palpated to verify the correct positioning of the collection cup. The cough reflex indicates that the cup is misplaced and should be removed. Once the collector is introduced, it is necessary to scrape the esophageal-pharyngeal mucosa with gentle movements (five to ten times) before removing it. This procedure is essential for sample collection, since the main sites of FMD virus replication are found on the anterior floor of the pharynx and on 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 detach from the esophageal-pharyngeal region at the time of scraping, with the presence of saliva, mucus and food debris. After collection, the operator should wash hands and arms with a disinfectant solution and then with running water.

Conservation and shipment of samples: After removing the collector, the contents of the cup are transferred to a sterile tube of the "Falcon" type. Immediately, an equal amount of MEM, containing antimicrobials, is added. The bottle is identified and properly sealed, 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. Samples must be sent to the laboratory as soon as possible, accompanied by the forms standardized by SIZ.

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



Surveillance for foot-and-mouth disease	Notification	Notification can be made by the owner, an accredited laboratory or third parties, when the SVO is notified of a suspected vesicular disease in an epidemiological unit	Suspected case of vesicular disease SVO investigation	Initial notification record: 1) Register on e-Sisbravet a. date and time of notification; b. type of notifier (owner or third party); c. identification of the notifier (may be anonymous); d. identification of the location with suspected cases; e. brief description of the notification and other relevant remarks; f. classification the notification (only by the veterinarian)	Preparation for the service 1. Evaluate the information available in the property register (herd, neighbors, movement, vaccination, etc.); 2. suspend, in the electronic system, preventively the movement of suspicious property; 3. identify the best time to travel to the property (maximum within 12 hours of notification); 4. inform the immediate/superior manager of the displacement to meet the notification; 5. check the material to deal with the suspicion and print completed and blank forms on e-sisbravet.

Initial clinical epidemiological investigation	Direct travel to the involved location to carry out the clinical inspection of the animals and epidemiological investigation	SVO surveillance	Compatible clinical signs Bovine: Vesicles or their evolutionary forms (whole or ruptured, blisters, ulcers, scars) in the oral (gums, palate, tongue) and nasal mucosa, muzzle, coronary band, interdigital space and mammary gland. High fever, anorexia, weakness, drooling, nasal discharge, lameness and prostration. Decreased milk production, hoof malformations, chronic lameness, mastitis, weight loss. In young animals it can cause mortality due to myocarditis. Most adults recover within 2 to 3 weeks, but secondary infections can delay recovery. Sheep and goats: show mild signs of the disease. Swine: usually develop severe foot injuries, leading to detachment of the hooves and difficulty in walking. Mouth lesions are smaller and less apparent, there is rarely salivation. There may be vesicles in muzzle and udder. In general, the temperature is close to normal. Young piglets can die from heart failure.
	Clinical signs not compatible with vesicular disease (if detected)	Clinical signs compatible with vesicular disease (probable case)	Veterinary Procedure: 1. carry out the collection of material for laboratory diagnosis; 2. fill in the information on the investigation forms, with special attention to population data; 3. interdict the property with the issuance of the interdiction term; 4. pass on the technical guidelines to those responsible for the animals; 5. perform all biosecurity measures.

Alert phase	Return directly to UVL	At the Local Veterinary Unit (UVL): 1. communicate the investigation to the UVL team and immediate/superior leadership; 2. finish filling out the investigation record in e-sisbravet; 3. arrange shipment of samples and forms to the screening laboratory; 4. maintain the property interdiction in the system; 5. suspend the issuance of GTAs for properties linked under its jurisdiction (neighbors and transit in the last 30 days); 6. keep the suspect herd under clinical monitoring.	At the SVE regional and central unit: 1. The PNEFA focal point in the SVE and SFA must monitor the procedures and actions; 2. Analyze the investigation data and movements of the last 30 days associated with the epidemiological unit as a probable case and arrange, via the system, to suspend animal movement in all linked properties; 3. Provide additional inspection teams for linked properties; 4. Establish zones of 3, 10 and 25 km, with definition of how many properties and animals in each one of them.	In the SVE screening laboratory: 1. Check and organize the biological material from the transport boxes to be sent to the LFDA; 2. Check all documentation and records of samples in e-sisbravet; 3. Contract the LFDA, informing the samples that will be sent and the probable day of delivery; 4. Confirm receipt of samples by LFDA.

Laboratory diagnosis	Probable case of vesicular disease	Sample							Herds not vaccinated against foot and mouth disease			For swine species only	
			Serum	Elisa 3ABC	(-) Non-reactive				(-) Non-reactive		(-) Non-reactive	Elisa for vesicular stomatitis and senecavirus	
					(+) Reactive	EITB	(-) Non-reactive	Elisa for VFA structural proteins	(+) Reactive	Seroneutralization for serotype "O" and "A"	(+) Reactive	(+) Reactive	(-) Idiopathic swine vesicular disease
			Epithelium, vesicular fluid, swab	Viral isolation	(-) Negative		(+) Reactive						
					(+) Positive	RT-qPCR	(-) Negative	Differential diagnosis	Differential diagnoses performed in routine: 1. Alagoas and Cocal vesicular stomatitis 2. Seneca A (swine)				
			Esophageal-pharyngeal fluid (LEF)				(+) Positive	Elisa for typing					
					From the evaluation of laboratory results and clinical-epidemiological investigation			Sequencing					

Completion of the investigation	Discarded case of vesicular disease	At UVL: 1. communicate the closure of the investigation to the team at UVL and above; 2. finalize the investigation on e-sisbravet; 3. suspend movement restrictions and interdiction (if any);	Definition of foot-and-mouth disease case: criteria as recommended by the World Organization for Animal Health and described in the Technical Datasheet for foot-and-mouth disease available on the Mapa website.
		At the center: 1. check all documentation and records on e-sisbravet; 2. check if the investigation was closed in e-sisbravet; 3. check the time indicators (action, reaction and final diagnosis); 4. if you have any indicator with high time, take actions for improvement; 5. provide and guarantee the replacement of all material used by the UVLs.	Animal health emergency (Contingency plan for foot-and-mouth disease)

ANNEX 9. Biosafety Procedures

Biosecurity measures must be rigorous 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:

In order to better organize the material and facilitate disinfection, the materials should be placed in resistant plastic boxes or bags, labeled and closed, highlighting:

1. Personal Protective Equipment (PPE): coveralls, disposable latex gloves, heavy duty rubber gloves and tall rubber boots.
2. adhesive tapes;
3. disinfectants;
4. large plastic bags, ideally having at least 2 different colors for transporting waste material or for disinfection;
5. resistant brushes and buckets for disinfection and drums for transporting water;

General measures to prevent contamination:

1. avoid unnecessarily walking through potentially contaminated areas;
2. avoid direct contact with potentially contaminated materials, surfaces and vehicles;
3. before placing the PPE, check that it has no tears or holes;
4. Do not carry items such as: cigarettes, candies, food, beverages etc.

Precautions that must be taken to minimize equipment contamination:

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 packaged 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 place, preferably near the gate, avoiding entering if it is a small property. In the case of large properties, drive in close to the livestock facilities, 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 clinical animal inspection activities.

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 your watch, rings, bracelets, necklaces, etc. and leave it in the vehicle. Cell phones, camera and GPS device must be placed in individual plastic bags and sealed.
2. While working on the property, eating, smoking or drinking should be avoided.

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 must be washed with water, soap and a 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 should be placed in plastic bags of disposable material for destruction. Piercing or sharp materials must be placed in specific devices or "pet" bottles before being placed in garbage bags.

Leaving the property:

If ruled out: no specific biosafety procedures required
In probable case, adopt the following procedures:

1. Clean and disinfect material boxes, bag equipment and transfer to vehicle.
2. 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 phones, cameras and GPS bags;
b. clean and disinfect rubber boots and brush gloves, including soles;
c. clean and spray the overalls with disinfectant, or dip it in a bucket with disinfectant solution, then place it in a clean bag;
d. remove the gloves, being careful not to touch the outside of your hands, and place them in the disposable bag. If they are reusable rubber gloves, they must be washed, disinfected and placed in the bags together with the overalls;
e. close the plastic bags containing the samples, equipment, boots and coveralls using masking tape;
f. put on shoes;

g. clean and disinfect hands, wrists and arms;

h. dump the disinfectant residues on the vehicle wheels;

i. place bags with non-disposable materials and garbage disinfected externally in the car (trunk or body); and

j. disinfect the vehicle's wheels, pedals and floor before leaving the property.

3. When returning from the property, provide:

a. proper destination for biohazard disposable material;

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 Procedures manual for the attention occurrences of foot-and-mouth disease and other vesicular diseases, Panaftosa)

1. 2% citric acid

Preparation: two parts citric acid to 98 parts water. Indications: laboratory objects and vehicle cabins.

Note: It is low corrosive to metals and painted surfaces.

2. 4% sodium carbonate solution

Preparation: dissolve 400 g of sodium carbonate in 10 liters of water. Contact time: 10 minutes.

Application method: spraying, sprinkling, footbath and immersion.

Precaution: when applying the disinfectant indoors, boots, gloves and a mask are recommended.

Indications: facilities, people and animals, vehicles, clothing, utensils, hides, skins, bones, hay and straw.

3. Iodophor compounds.

Preparation: mix 1 liter of 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 manure.

It should be noted that in the zoo sanitary emergency episodes for the elimination of FMD outbreaks carried out 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, conserve and use, and can be used both as disinfectants and as antiseptics, changing only the concentration/dilution, according to the manufacturers' recommendations.

4. 2% Acetic Acid

Preparation: 2 parts glacial acetic acid to 98 parts water. Indications: laboratory objects and vehicle cabin.

Note: It is low corrosive to metal objects, but has little action on organic matter.

5. Potassium monopersulfate triple salt solution

Preparation: dilute the powder in running water, as directed by the manufacturer. Contact time: 30 minutes.

Application method: spraying, spraying droplets and immersion. Precaution: it is not toxic or irritating.

Indications: disinfection of stables, corrals, industrial processing plants, surface of limbs and feet of animals, vehicles and farm equipment.

Limitations of use: do not mix with alkaline substances, as the product works at a pH of 2.5 for a 1% solution.

Note: As the effectiveness of acids and alkalis as viricides depends on their pH, it is important

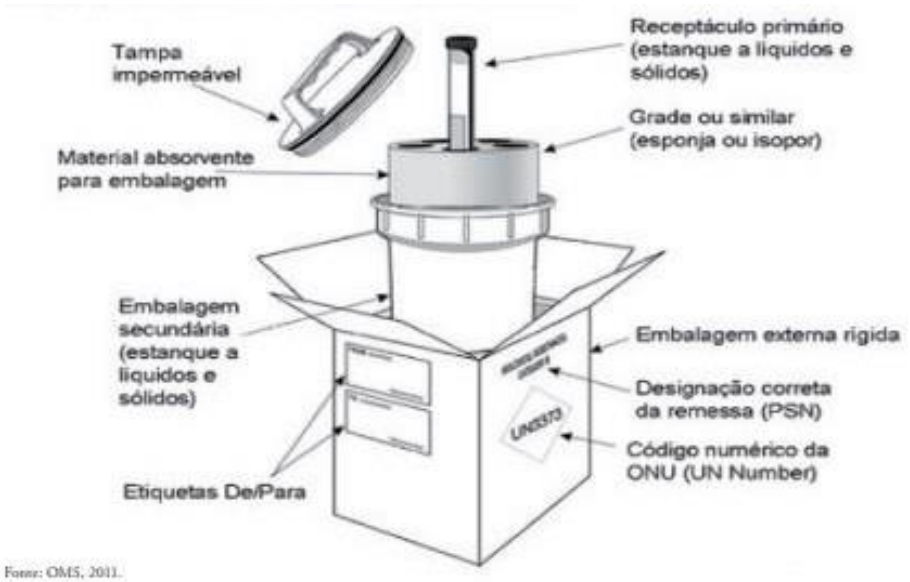
that they do not mix. Surfaces treated with one type must not be subjected to the action of another, unless a water wash is interspersed. Never use washing soda and an acid to disinfect the same item.

- Disinfectants recommended for FMD 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, storage and shipping samples for laboratory testing

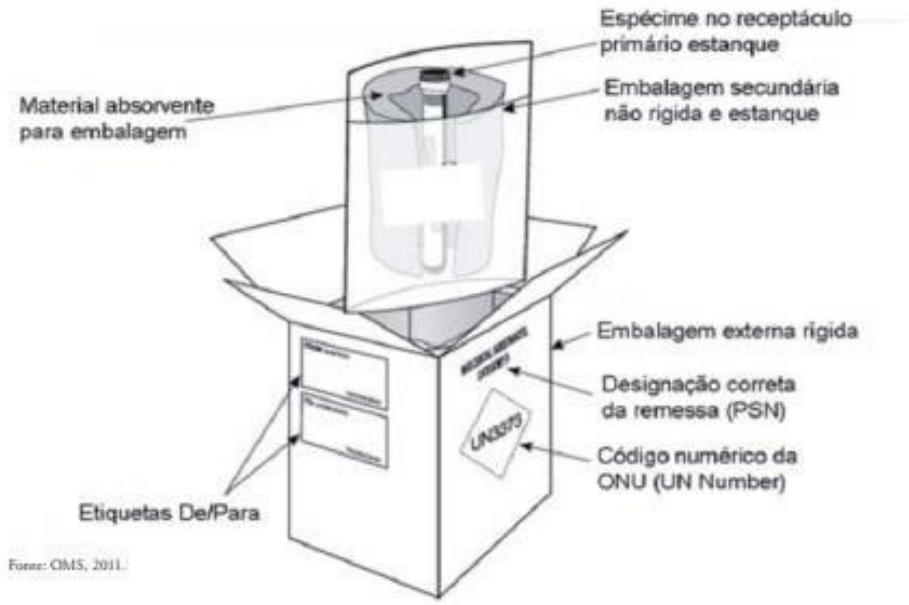
The packaging must be of good quality, strong enough to withstand the loads and impacts that normally occur during transport, including transshipment, stacking, manual or mechanical handling. Packages must be constructed and closed in such a way as to prevent any loss of contents under normal transport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

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



Fonte: OMS, 2011.

Waterproof cover	Primary receptacle (tight to liquids and solids)
Absorbent material for packaging	Grid or similar (sponge or Styrofoam)
Secondary packaging (tight to liquids and solids)	Rigid outer packaging
From/to tags	Correct shipping designation (PSN)
	UN numeric code (UN Number)



Absorbent material for packaging	Specimen in the watertight primary receptacle
	Secondary non-rigid and watertight packaging
From/to tags	Rigid outer packaging
	Correct shipping designation (PSN)
	UN numeric code (UN Number)

The primary container must be wrapped in absorbent material enough to contain all the material without compromising the integrity of the cushioning product or the secondary packaging. The primary container must be protected by a secondary packaging that, under normal transport conditions, does not break or puncture. If several fragile primary containers are placed in the same secondary packaging, they must be wrapped individually or separated, in order to avoid contact between them.

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

The secondary packaging must be able to prevent the loss of contents when the primary container fails to seal and will be accommodated inside the outer packaging with an appropriate cushioning material.

When using ice or dry ice (carbon dioxide), they must be placed outside the secondary packaging, that is, in the outer packaging or in a cooler (polystyrene). Internal wedges must be placed to keep packages immobilized when 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 break 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.

UN1845 dry ice
Quantidade líquida ____kg

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.

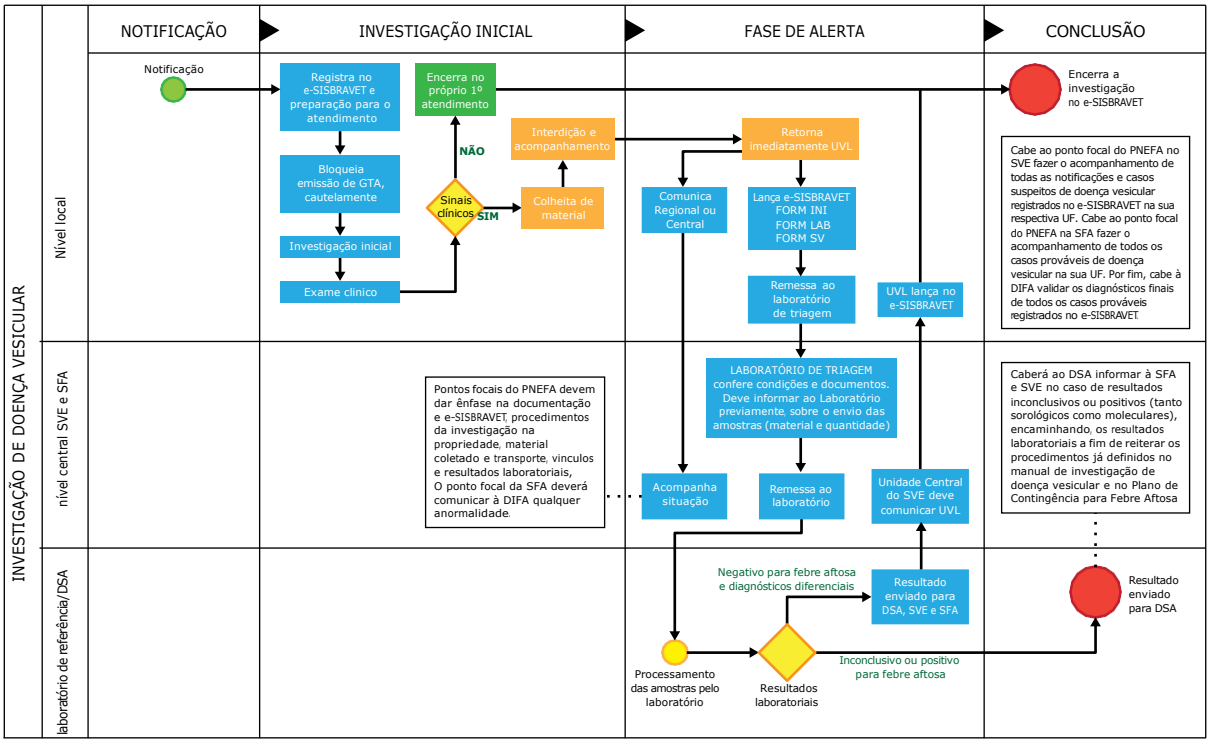
	Dry ice label (carbon dioxide, solid) This label must be affixed to the outside of every package that contains dry ice.
UN1845 dry ice Net quantity ___ kg	Marking for dry ice (carbon dioxide, solid) The net weight of dry ice must be indicated on the outside of every package that contains dry ice.

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

The outer packaging must also be labeled with the name, address and telephone number of the sender and recipient, including an emergency telephone number. The term Biological Substance, Category B / Biological Substance, Category B must also be included. The transport of samples must comply with international standards, as per the IATA standard.

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

ANNEX 12. Information flow for the investigation of vesicular disease in the country



VESICULAR DISEASE INVESTIGATION		NOTIFICATION	INITIAL INVESTIGATION				ALERT STAGE			COMPLETION
	Local level	Notification	Register in e-SISBRAVET and prepare for service	Closed on the 1st service				Returns immediately UVL		Closes the investigation in e-SISBRAVET
			Blocks GTA issuance, in precautionary measure	No		Interdiction and monitoring	Communicates Regional or Central	Launches e-SISBRAVET FORM INI FORM LAB FORM SV		It is up to the PNEFA focal point at the SVE to monitor all notifications and suspected cases of vesicular disease registered in e-SISBRAVET in their respective UF. It is up to the PNEFA focal point at the SFA to monitor all probable cases of vesicular disease in its FU. Finally, DIFA is responsible for validating the final diagnoses of all probable cases registered in e-SISBRAVET.
			Initial investigation	Clinical signs	Yes	Material collection		Shipment to the screening laboratory	UVL launches on e-SISBRAVET	
			Clinical exam							
	central level SVE and SFA		PNEFA focal points should emphasize documentation and e-SISBRAVET, on-farm investigation procedures, material collected and transport, links and laboratory results. The SFA focal point shall report any abnormality to DIFA.				SCREENING LABORATORY confers conditions and documents. The laboratory must be informed in advance about the sending of samples (material and quantity)			It will be up to the DSA to inform the SFA and SVE in case of inconclusive or positive results (both serological and molecular), forwarding the laboratory results in order to reiterate the procedures already defined in the vesicular disease investigation manual and in the Foot-and-Mouth Contingency Plan .
							Follow the situation	Shipping to the laboratory	SVE Central Unit must communicate UVL	
	Reference laboratory/DSA							Negative for foot-and-mouth disease and differential diagnoses	Result sent to DSA, SVE and SFA	Result sent to DSA
							Sample processing by the laboratory	Laboratory results	Inconclusive or positive for foot-and-mouth disease	

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