



UNITROPEAN COMMISSION
DIRECTORATE-GENERAL FOR HEALTH AND FOOD SAFETY

Crisis preparedness in food, animals and plants
Animal health

**SUBMISSION OF ERADICATION PROGRAMMES FOR CATEGORY B AND C
DISEASES OF TERRESTRIAL ANIMALS BASED ON GRANTING DISEASE-FREE
STATUS AT ESTABLISHMENT LEVEL**

**Template in accordance with Article 10 of Commission Implementing Regulation (EU)
2020/2002**

BOVINE ANIMALS

1. Date of submission

31 May 2021

2. Member State:

Portugal

3. Name of the disease¹:

Infection with *Brucella abortus*, *Brucella melitensis* and *Brucella suis* as regards kept bovine animals.

4. Contact details:

a. E-mail:

b. Responsibility within the competent authority:

Head of Epidemiology and Animal Health Unit.

c. Name:

5. Territorial scope with a description and demarcation of the geographical and administrative areas covered by the eradication programme and the names of the zones and Regions, if more than one Region is included in the territorial scope of the programme.

The eradication programme will be implemented at the following geographical and administrative areas:

Administrative areas of the territorial scope	Geographical areas “Distritos /Islands” with eradication programme
Directorate for Food and Veterinary of the Norte Region (DSAVRN)	Viana de Castelo Braga Bragança Porto Vila Real
Directorate for Food and Veterinary of the Lisboa and Vale do Tejo (DSAVRLVT)	Santarém Lisboa Part of Setúbal
Directorate for Food and Veterinary of the Alentejo Region (DSAVRALT)	Portalegre Évora Beja Part of Setúbal
Directorate of Veterinary Services of Autonomous Region of Açores (RAA)	Island of S. Miguel
Directorate for Food and Veterinary Services of the Autonomous Region of Madeira (RAM)	Islands of Madeira and Porto Santo (Not cofinanced by the EU)

¹ This template is to be used for the submission of the following eradication programmes for the following listed diseases:

Category B-diseases

a) Infection with *Brucella abortus*, *B. melitensis* and *B. suis*

b) Infection with *Mycobacterium tuberculosis* complex

Category C-diseases

a) Enzootic bovine leishmaniasis

c) Infectious bovine rhinotracheitis/infectious pustular vulvovaginitis

d) Infection with Aujeszky’s disease virus

e) Bovine viral diarrhoea

6. A description of the epidemiological situation for each zone or Region, if more than one Region is included in the territorial scope of the programme:

Portugal has been implementing the co-financed bovine brucellosis eradication programme (BBEP) since 1991, and herds covered by the programme have a health status attributed in accordance with Council Directive 64/432/EEC of 26 June and its amendments and National Decree-Law 244/2000 and 79/2011.

The BBEP 2020 was carried out in the non-officially free Regions of Portugal (3 Regions of the mainland area – Norte, Lisbon and Tagus Valley (LVT) and Alentejo) and in 3 islands of the Autonomous Region of Açores – S. Miguel, Terceira and S. Jorge) resulted in a decrease on herd apparent prevalence.

Due to the improvement of brucellosis status, the Autonomous Region of Açores (RAAçores) obtained the officially free status of more two Islands in 2021 - Terceira and S. Jorge. At present RAAçores has 8 of its 9 islands in free status (the other six islands obtained this status in 2002 - Graciosa, Pico, Flores and Corvo and 2009 - Santa Maria and Faial).

Regarding mainland, by 2012 Algarve Region was recognized as officially free and later on, in 2020, the Centro Region. This Region includes the districts of Aveiro, Viseu, Guarda, Coimbra, Leiria and Castelo Branco and some councils of four of these districts that are part of two other Regions, namely the Norte (Aveiro, Viseu and Guarda) and the Lisboa e Vale do Tejo (Santarém).

The programme is advancing in a pre-eradication phase.

Mainland:

Evolution of herd prevalence of bovine brucellosis, from 2019 to 2020:

- herd apparent prevalence increased from 0.12% to 0.145%.
- herd incidence increased 0.10 % to 0.12%.
- % positive animals decreased (from 0.06% to 0.03%).

The comparison of national indicators from 2019 to 2020 is not direct because data on sampled population from Centro Region was excluded from the programme when this region acquired the free status, therefore the observed increase in herd prevalence do not correspond to a real increase in the disease. In fact, in 2019 there were 38 new positive holdings and in 2020 there were only 26.

The All Regions are below 0.3% in herd prevalence.

The variation of herd apparent prevalence by Region, from 2019 to 2020, was the following:

- Norte – from 0.10% to 0.11%.
- LVT – from 0.08% to 0%.
- Alentejo – from 0.35% to 0.28%.

By the end of 2020, 7 herds were classified with an infected status. There were 4 infected herds in the Norte (in 3 counties in Vila Real and Braga), 2 infected herds in the Alentejo (Portalegre) and 1 infected herd in the Lisboa e Vale do Tejo (Setúbal).

There were 26 herds with at least one positive animal and 22 were new positive (84,6%).

Positive animals were subjected to sanitary slaughter and those coming from newly infected herds were subjected to organ collection for bacteriology. The percentage of slaughtered animals sampled with isolation of *B. abortus* was 61,90 % (39/63). From these total, 38 isolates were from Norte and one from Alentejo.

The percentage of free (B3) and officially free (B4) herds was 93,3% in 2020.

Evolution over time of the epidemiological indicators and vaccinated herds and animals are in the file below.

The BBEP foresees the investigation of positive and infected herds in order to access the origin of infection. For this evaluation a specific data collection questionnaire is used (epidemiological enquiry – “EI”). These EI is implemented with the objective to establish possible links to other farms (trace-back and trace-forward) and characterize possible risk factors and sources of infection. There were 13 epidemiological inquiries with probable reasons of introduction of infection, resulting in animal movement and contacts with animals of other herds in common pastures, as the main sources. There were no notified abortions regarding bovine.

Compulsory pre-movement testing has been applied to avoid the entrance of infected bovine in brucellosis free and officially-free herds, with the following data registered at 2020 for the mainland: 15.707 herds and 72.759 animals.

Autonomous Region of Açores (3 Islands)

Herd apparent prevalence and herd incidence decreased from 0,251% in 2019 to 0,037% in 2020. The percentage of positive animals also decreased from 0,007% to 0,001%. There were no seropositive animals at Terceira and S. Jorge islands and there was one seropositive animal at S. Miguel island. This positive animal was subjected to sanitary slaughter and organ collection for bacteriology, with no *B. abortus* isolation (infection not confirmed). By the end of 2020 no herds had an infected status.

The percentage of free (B3) and officially free (B4) herds was 99.95%. The main reasons for attributing a suspended status was due to non-negative results to serology.

Due to the excellent results, Terceira and S. Jorge islands stopped vaccination in 2015 and S. Miguel island during 2019.

The variation of herd apparent prevalence by island from 2019 to 2020 was the following:

- S. Miguel – from 0.57% to 0.085%
- Terceira – remained 0.0%
- S. Jorge – remained 0.0%

No infected animals have been detected since September 2006 at Terceira and since February 2009 at S. Jorge. The last isolation of *B. abortus* in S. Miguel island was in October 2014.

There were no cases of Brucellosis infection but 22 epidemiological inquiries were carried out in S. Miguel, all due to false positive reactors. There were 18 notified abortions tested for Brucellosis, all with negative results.

For the 3 islands, pre-movement testing has been applied with the following data in 2020: 398 herds and 2.001 animals.

During 2021, Terceira and S. Jorge islands obtained the disease-free status from infection with *Brucella abortus*, *B. melitensis* and *B. suis* in bovine animal populations according to Chapter 1 of Part I of Annex I of the Commission Implementing Regulation (UE) 2021/620 and will undergo to a surveillance programme, joining the other 6 disease-free islands.

(a) the number of establishment keeping animals of the targeted animal population by health status (Disease-free, infected or unknown) excluding establishment falling under the derogation referred to in point (6)(f) at 31 December;

Health status - establishments - bovine - by 31 December 2020			
Regions	Unknown status	Infected status (B2.1)	Disease free status
Norte	0	4	15 033
Lisboa e Vale do Tejo	0	1	1 652
Alentejo	0	2	4 673
Açores (3 islands)*	0	0	4 265
Portugal - Total	0	7	25 623

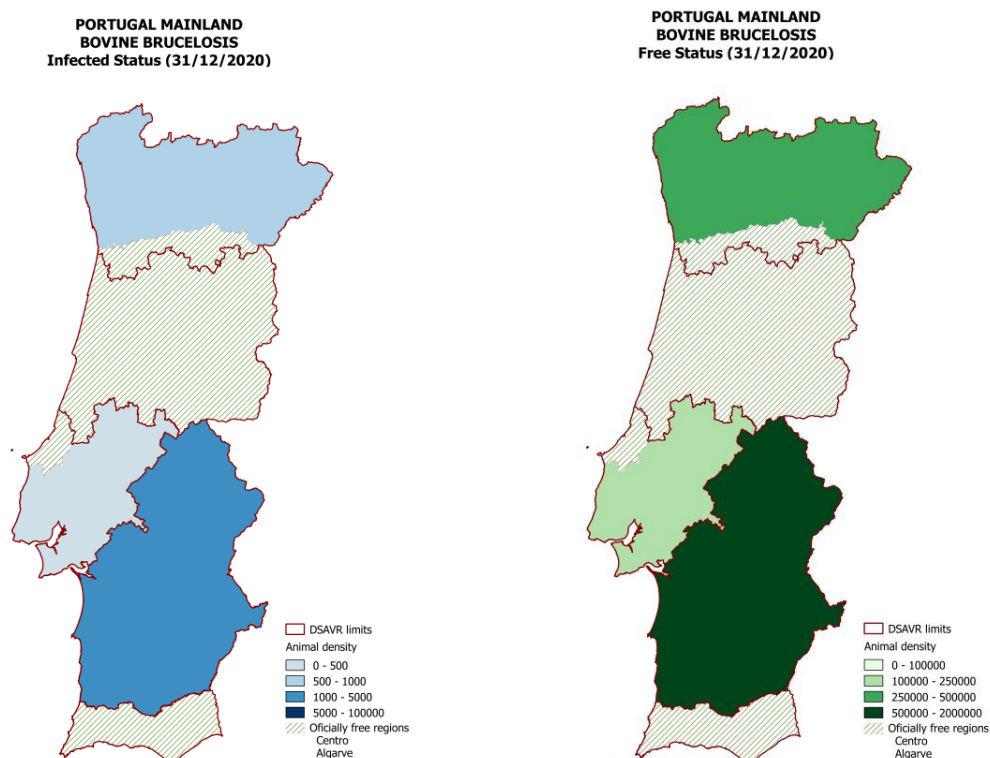
* S. Miguel: 2.141 establishments with disease free status

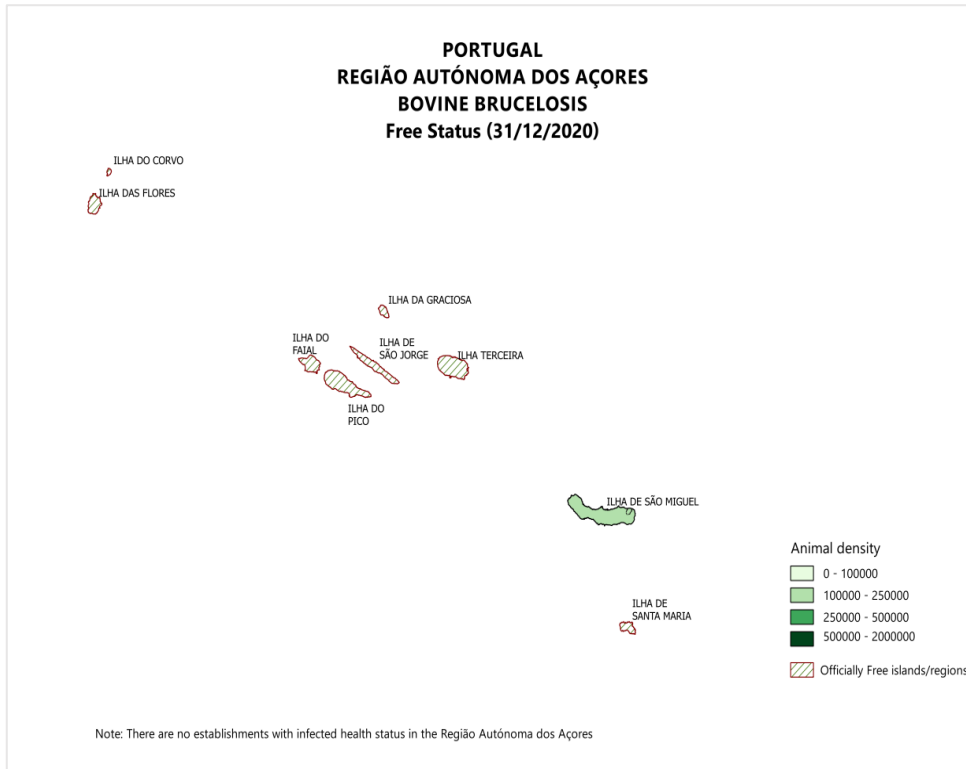
(b) the number of animals of the targeted animal population kept in the establishment referred to in point (a) by health status;

Health status - data on bovine animals - by 31 December 2020			
Regions	Unknown status	Infected status (B2.1)	Disease free status
Norte	0	132	304 396
Lisboa e Vale do tejo	0	11	175 637
Alentejo	0	476	683 548
Açores (3 islands) *	0	0	212 125
Portugal - Total	0	619	1 375 706

* S. Miguel: 129.151 animals kept in the establishments with disease free status

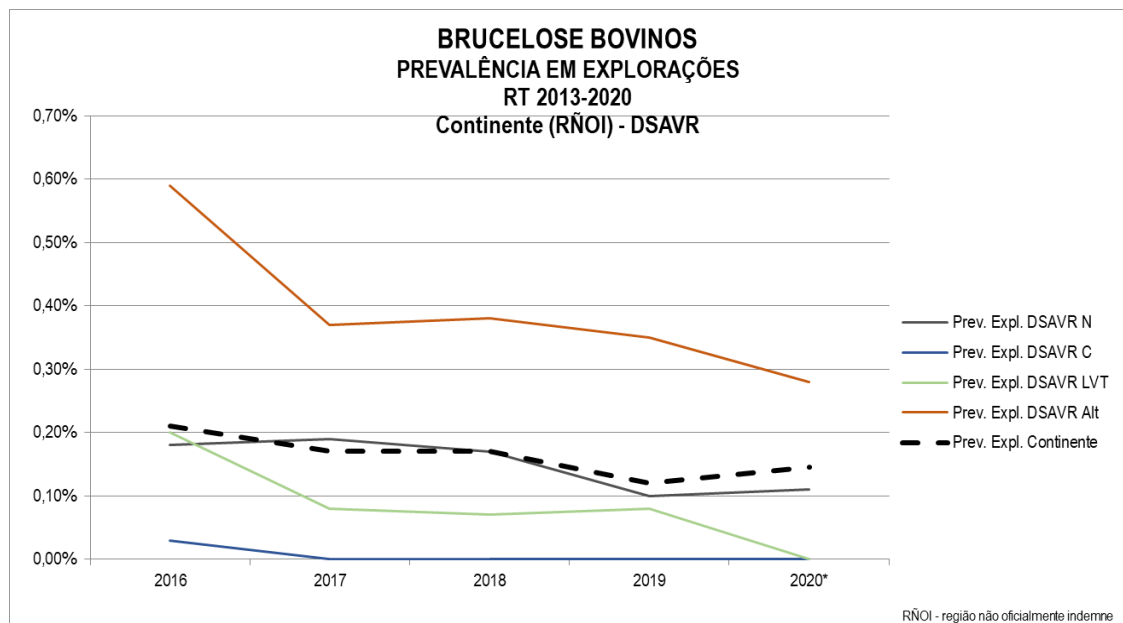
(c) maps indicating the density of the targeted animal population referred to in point (b) by health status;



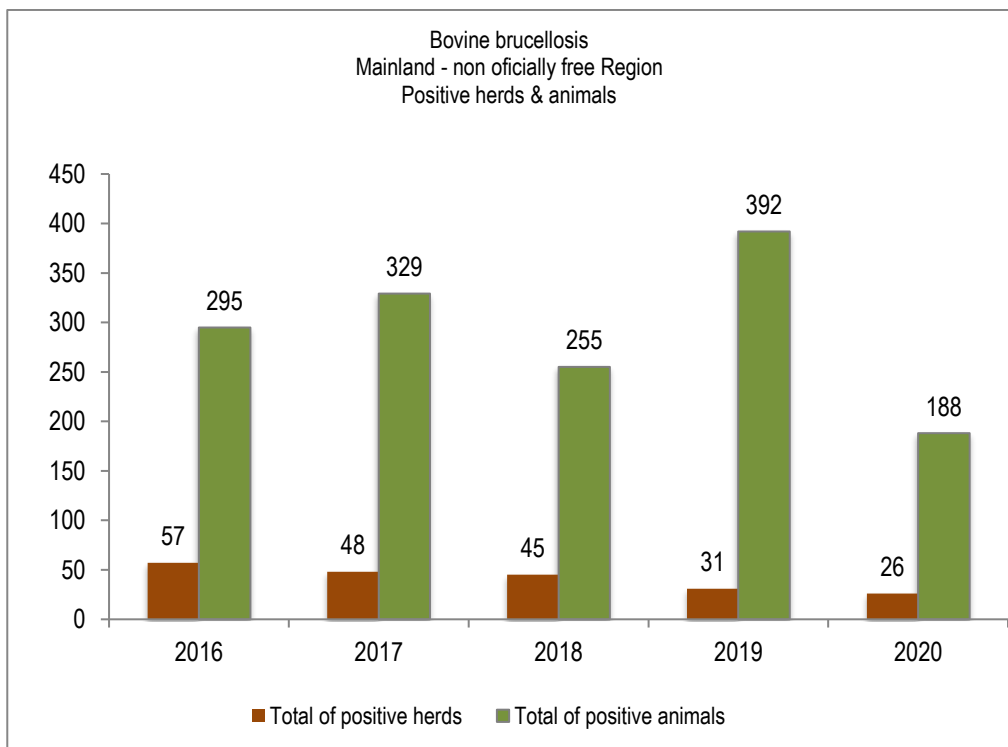


(d) **timeline with prevalence, incidence data and, where relevant, vaccination history covering at least the past 5 years; and**

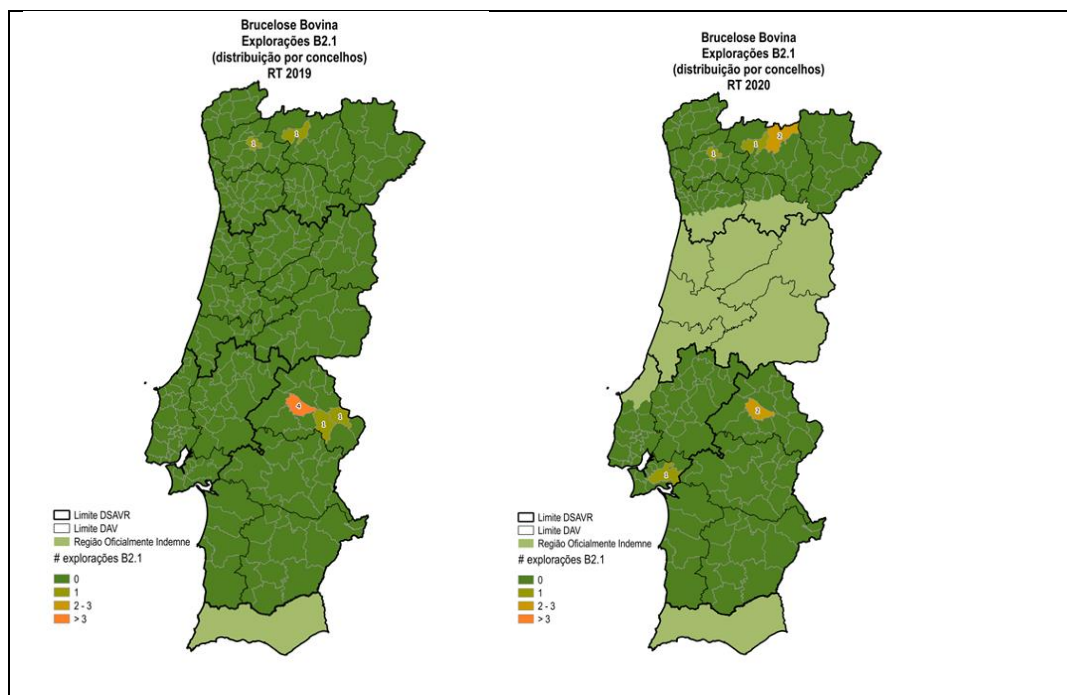
Mainland
Evolution of prevalence by DSAVR (region)



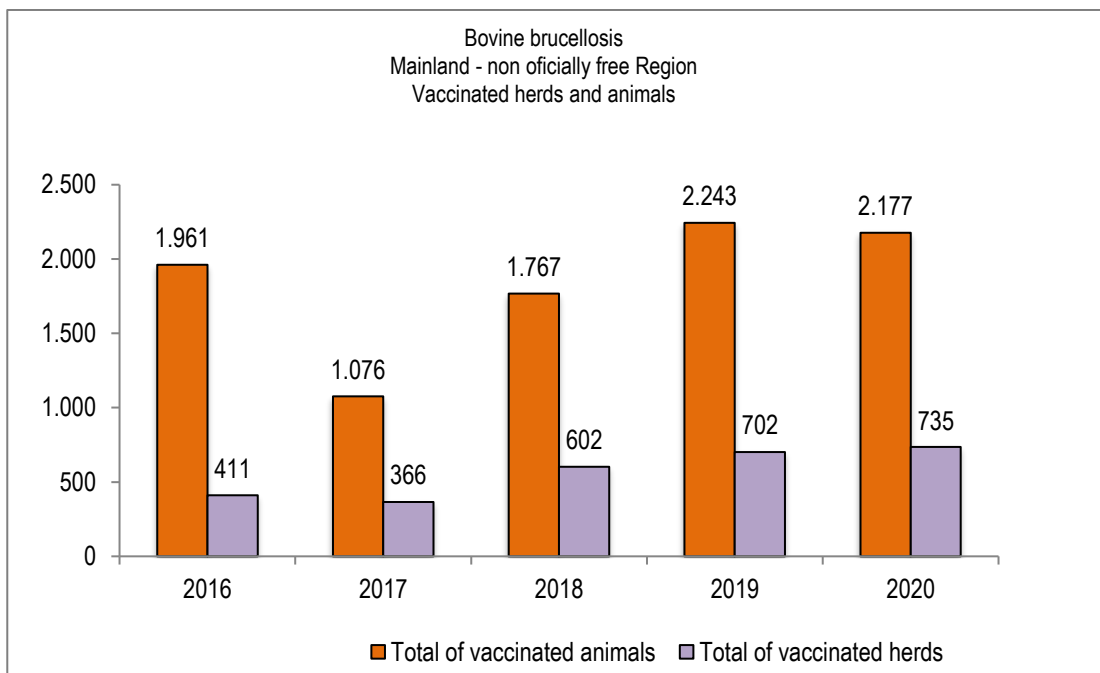
Evolution of positive herds and bovines - Mainland



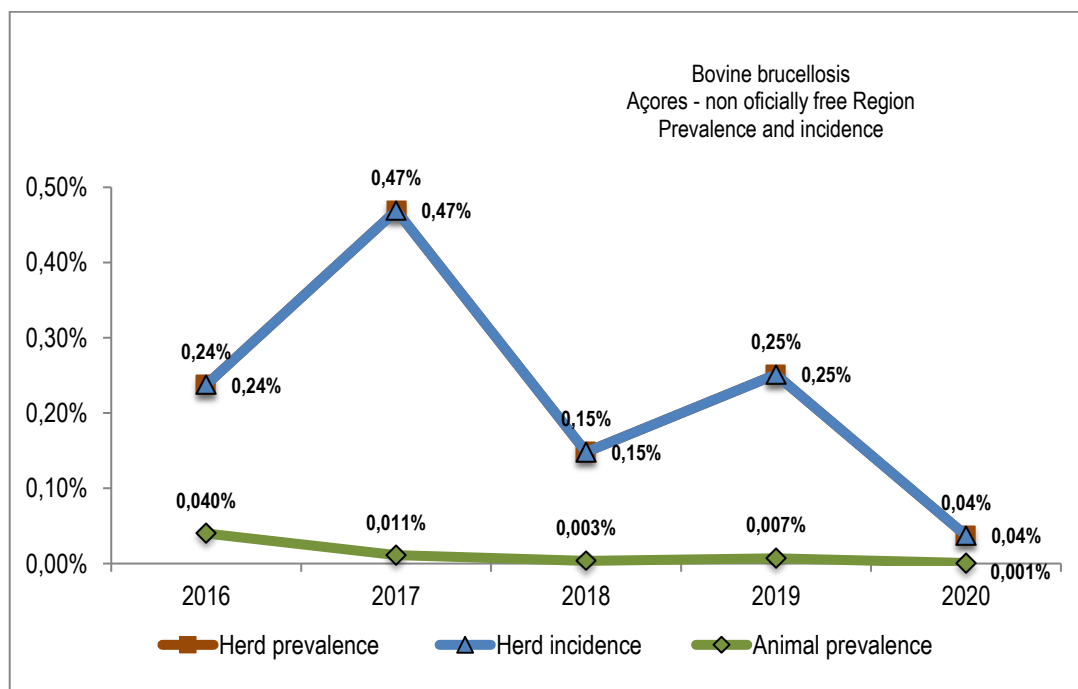
Infected herds (B2.1) in 2019 and 2020 - Mainland



Evolution of vaccinated herds and bovine animals - Mainland

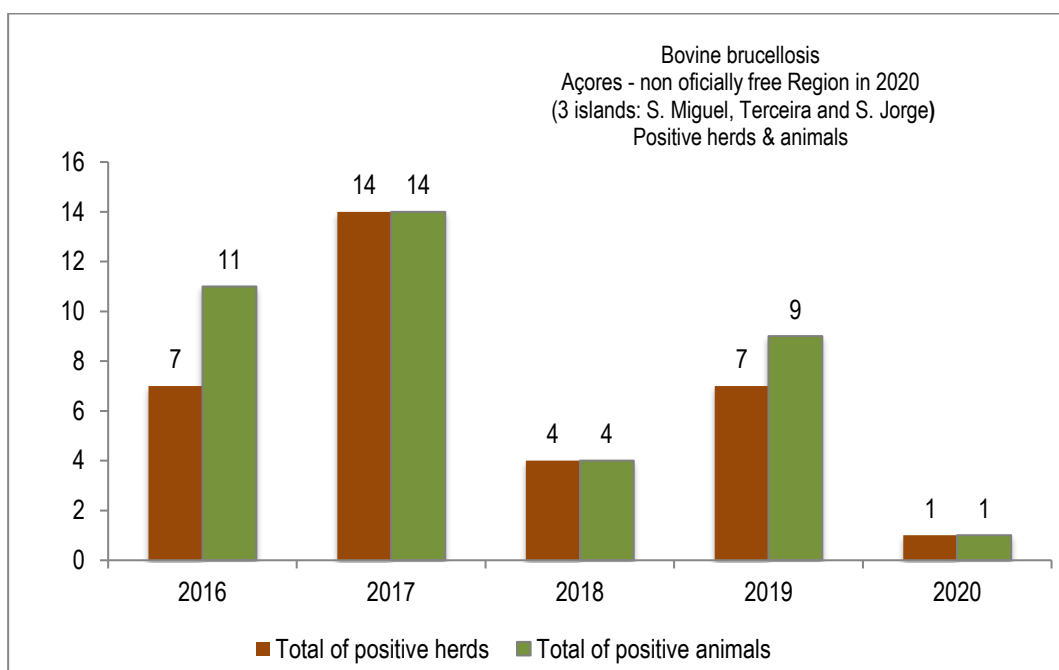


RA Açores (3 islands: S. Miguel, Terceira and S. Jorge) Evolution of bovine brucellosis epidemiological indicators

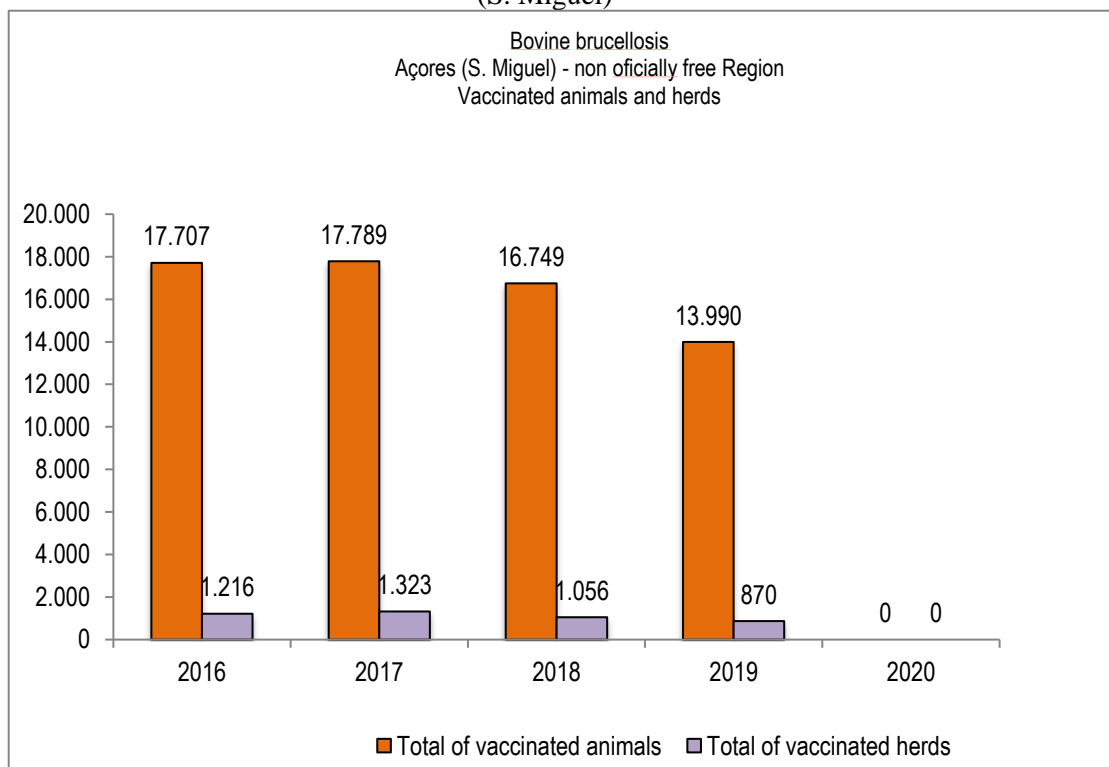


No infected animals have been detected since September 2006 at Terceira and since February 2009 at S. Jorge. The last isolation of B. abortus in S. Miguel island was in October 2014.

Evolution of positive herds and bovines - Azores



Evolution of vaccinated herds and bovine animals RAAçores (S. Miguel)



(e) information as regards the epidemiological situation in additional animal populations, where relevant.

Not relevant

7. A description of the disease control strategy of the eradication programme in accordance with Article 16 of Commission Delegated Regulation (UNIT) 2020/689 including at least:

Disease-specific requirements for the granting, maintenance, suspension and withdrawal of the disease-free status at the level of herds/establishments (or epidemiological units were in accordance with Council Directive 64/432/EEC of 26 June 1964 and national legislation and will be adapted in accordance with the Animal Health Law (AHL) and the requirements of Annex IV to the Commission Delegated Regulation (EU) 2020/689 of 17th December 2019.

Health status is assigned or amended by the Regional official veterinary services (DSAVR). The DSAVR shall grant, suspend or withdraw disease-free status at establishment level when the conditions for these measures have been met and shall specify the maximum period of time during which disease-free status may be suspended (365 days).

The existing health status for establishment, adapted to AHL are as follows:

- B4 - establishment with the disease-free status from infection with *Brucella abortus*, *B. melitensis* and *B. suis* without vaccination.
- B3 – establishment with the disease-free status from infection with *Brucella abortus*, *B. melitensis* and *B. suis* with vaccination.
- B2 - establishment with the disease-status not free from infection with *Brucella abortus*, *B. melitensis* and *B. suis*.

In complement, and for the purpose of management of the eradication programme:

- B2.1 – establishments infected with *Brucella abortus*, *B. melitensis* and *B. suis* (withdraw the disease-free status, when isolation of *Brucella abortus*, *B. melitensis* and *B. suis* is officially confirmed, post-mortem or other).
- B4S - establishment with the suspended disease-free status from infection with *Brucella abortus*, *B. melitensis* and *B. suis* without vaccination.
- B3S – establishment with the suspended disease-free status from infection with *Brucella abortus*, *B. melitensis* and *B. suis* with vaccination.

The operators of establishment where bovine animals are kept, shall comply with the general and specific requirements ordered by DGAV/DSAVR, in order to obtain and maintain the disease-free status of their establishments.

The eradication programme related to bovine animals is based on the health status of the establishment through a test and slaughter policy, with compensation of slaughtered animals to operators. Before compensation is paid out, the respective DSAVR must confirm that the operator has complied with the legal provisions related to the eradication programme, with the animal movement and the specific animal health measures imposed in the notification issued by the DSAVR. If the aforementioned check reveals evidence of non-compliance by the operator, the DSAVR must initiate the relevant penalty process, and payment of compensation will depend on the final decision in the case.

In some areas of the North Region vaccination with RB51 is included in the programme.

Brucellosis is a notifiable disease and treatment is strictly prohibited.

Notification of abortions is compulsory and must give rise to an epidemiological investigation and the collection of material for bacteriological diagnosis. There are procedural rules for collecting and sending material from abortions to the laboratory, drawn up jointly by DGAV and the INIAV I.P. (the national reference laboratory for animal brucellosis), published on the website of both Institutions.

(a) the sampling schemes and diagnostic methods to be used in accordance with Annex IV to Delegated Regulation (EU) 2020/689:

In accordance with section 1 of annex III of Reg. (EU) 2020/689, the following serological diagnostic tests will be used for the granting and maintenance of disease-free status:

a) tests for blood samples:

- buffered Brucella antigen tests, i.e, Rose Bengal Test (RBT)
- complement fixation test (CFT)

b) tests for milk samples:

- I-ELISA

c) Bacteriology.

a) Serological Rose Bengal test (RBT) is used as screening test and the Complement Fixation Test (CFT), as confirmatory test, when serial diagnostic is applied.

In a B3 and B4 establishment, a bovine animal is considered serologically positive if it is simultaneously positive to RBT and has $FC > = 20$ IU / ml (reading in series).

In B3 and B4 establishment, blood samples from animals with 12 or more months of age, are collected and send to approved private laboratories or to INIAV and RBT are applied to all samples. RBT positive samples undergo CFT. If at least 1 of the RBT positive samples is also positive to CFT, all other RBT negative samples are also tested for FCT and, accordingly to the results, the DSAVR can consider animals positive with only one positive test.

In non-disease free establishment, a positive animal is one that has a positive result to RBT or CFT (reading in parallel).

In line with the objective, serological diagnosis is part of:

- The surveillance activities for maintenance of the health status;
- The eradication measures and validation of absence of infection when applied following detection of a positive animal;
- To clarify risk situations, when applied in contact establishment following an epidemiological investigation;
- In establishment with animals to be vaccinated, blood samples are taken at the time of vaccination;
- In pre-movement tests (PreMT) and post-movement tests (PosMT):
 - o For the pre- testing (“PreMT”), during the 30 days prior to their introduction into the establishment, blood samples with negative results to RBT and CFT are taken from entire bovine animals over 12 months of age.
 - o For the pos-movement testing (“PosMT”), during the 30 days following their introduction into the establishment, blood samples with negative results to RBT and CFT are taken from entire bovine animals over 12 months of age provided that they have been kept isolated during this period.

b) In addition, Milk ELISA test is also used as a diagnostic test in dairy establishment, for the purposes of maintaining B3 and B4 health status.

Serological testing can be performed by executing Milk Elisa tests on samples from the lactating cows and simultaneously testing blood samples from the remaining animals over 12 months of age.

Milk ELISA test is not applied in the following situations:

- Where the DSAVR has previously determined that the conditions for the collection of milk samples are not met;
- In establishment of the DAV of Porto (area from the Norte Region), since this area is not yet free of enzootic bovine leukosis (EBL), therefore blood sampling is compulsory.

Regarding the Autonomous Region of Açores, that is closer to the pre-eradicated phase of the disease, in S. Miguel the whole bovine population under the programme is covered serologically (individually) tested, in accordance with Reg 2020/689. Sampling of all animals over 12 months, will be carried out during 2022. ELISA test is used as a complementary screening test, every quarter, at all establishment of S. Miguel and positive results will be evaluated case by case.

c) Bacteriology is an important tool to confirm infection. Bacteriological diagnosis is applied in serological positive animals detected in establishment where brucellosis was not yet confirmed (all non B.2.1 establishment). Samples are collected from animals subjected to sanitary slaughter by official veterinarians and tests are performed only in the National Reference Laboratory (NRL). Isolation of *Brucella* is also followed by typing.

Regarding the Autonomous Regions of Açores (S. Miguel), bacteriological investigation is carried out in all cases of sanitary slaughter and the tests are performed in Regional Veterinary Laboratory.

A brucellosis “infected animal” is the one with bacteriological isolation.

An infected establishment is the one where *Brucella* was isolated, either through bacteriological confirmation, detection of brucellosis after confirmed abortion, through epidemiological evidence or when the repetition of tests with positive results does not allow discarding the presence of brucellosis.

(i) for the granting of the disease-free status to establishment and the maintenance of that status.

Establishment / Unit with B4 status

The status free from infection with *Brucella abortus*, *B. melitensis* and *B. suis* without vaccination (B4) may only be granted to an establishment keeping bovine animals if:

a) during the past 12 months there has been no confirmed case of infection with *Brucella abortus*, *B. melitensis* and *B. suis* in bovine kept in the establishment.

b) during the past 3 years none of the bovine animals in the establishment has been vaccinated against infection with *Brucella abortus*, *B. melitensis* and *B. suis*.

c) all bovine animals over 12 months of age present in the establishment at the time of sampling have tested negative to serological tests on the following occasions:

- First negative test must be carried out on blood samples taken 30 days after slaughter of all positive /confirmed bovine animals, all undergo to RBT and FCT.
- Second negative test must be carried out on blood samples taken not earlier than 3 months after the removal of the last positive/confirmed case;
- Third negative test must be carried out on blood samples taken not earlier than 6 months and not later than 12 months, following the date of sampling referred above.

d) Animals showing clinical signs consistent with infection with *Brucella abortus*, *B. melitensis* and *B. suis*, such as abortions, have been subjected to investigations with negative results;

e) Since the beginning of the first sampling referred above, all bovine animals (and all germinal products of bovine origin) introduced into the establishment must have been originated from B3 or B4 establishment, animals must have not been vaccinated during the past three years and originate from a free Member state (MS) or free Region and, if not, are entire bovine animals over 12 months of age must have tested negative in a PreMT or a PosMT.

The B4 status may be granted to an B3 establishment if requirements for granting the status are fulfilled and if all entire bovine animals over 12 months of age are serologically tested with negative results at appropriate intervals of not more than 12 months.

The status (B4) may only be maintained if:

- the requirements set for granting continue to be fulfilled and serological testing is carried out with negative results on samples taken from all entire bovine animals over 12 months of age at appropriate intervals of not more than 12 months, taking into account the type of production, the situation of the disease and the identified risk factor or
- are entire bovine animals over 12 months of age kept in establishment located in a MS or a Region free from infection, in accordance with a testing regime set up by DGAV, taking into account the type of production and the identified risk factors.

When serological testing is performed by executing Milk Elisa tests on samples from the lactating cows, it is required simultaneously testing of blood samples from the remaining animals over 12 months of age.

Establishment with B3 status

The status free from infection with *Brucella abortus*, *B. melitensis* and *B. suis* with vaccination (B3) may only be granted to an establishment keeping bovine animals if:

- a) during the past 12 months there has been no confirmed case of infection with *Brucella abortus*, *B. melitensis* and *B. suis* in bovine animals kept in the establishment;
- b) all bovine animals over 12 months of age present in the establishment at the time of sampling have tested negative to serological tests on the following occasions:
 - First negative test must be carried out on blood samples taken 30 days after slaughter of all positive /confirmed bovine animals, all undergo to RBT and FCT.
 - Second negative test must be carried out on blood samples taken not earlier than 3 months after the removal of the last positive/confirmed case;
 - Third negative test must be carried out on blood samples taken not earlier than 6 months and not later than 12 months, following the date of sampling referred above.
- c) animals showing clinical signs consistent with infection with *Brucella abortus*, *B. melitensis* and *B. suis*, such as abortions, have been subjected to investigations with negative results;
- d) Since the beginning of the first sampling referred above, all bovine animals (and all germinal products of bovine origin) introduced into the establishment must have been originated from B3 or B4 establishment or come from a free MS or free Region and, if not, the entire bovine animals over 12 months of age must have been tested negative in a PreMT or PosMT.

The B3 status may be granted to an establishment (as derogation to the above requirements) if all bovine animals come from B3 or B4 establishment and are originate from a MS or a Region free from infection, are entire bovine animals over 12 months of age and must have been tested negative in a PreMT or PosMT.

The status (B3) may only be maintained if the requirements set for granting the status continue to be fulfilled and serological testing is carried out with negative results on samples taken from all entire bovine animals over 12 months of age at appropriate intervals of not more than 12 months, taking into account the type of production, the situation of the disease and the identified risk factor.

When serological testing is performed by executing Milk Elisa tests on samples from the lactating cows, it is required simultaneously testing of blood samples from the remaining animals over 12 months of age.

(ii) to confirm or rule out the disease in the event of a suspected case.

Section 3 and 4 of Chapter 1 for the establishment free from infection with *Brucella abortus*, *B. melitensis* and *B. suis* without vaccination and Section 3 and 4 of Chapter 2 for the establishment free from infection with *Brucella abortus*, *B. melitensis* and *B. suis* with vaccination to confirm or rule out the disease in the event of a suspected case will be followed.

- Whenever there is a suspected case of the disease and until it's confirmation or rule out, the DSAVR shall conduct investigations, shall initiate an epidemiological enquiry (EI), shall suspend the disease-free status of the establishment (B3S/B4S) where it occurred until the investigations and the EI are concluded and shall prohibit movement of germinal products of bovine and of bovine animals from and out of the establishment, unless the animals are for immediate slaughter in a designated slaughterhouse. Besides the animal movement restrictions, operators are also notified to isolate the suspected cases, where technically possible.

The suspension of status is carried out on the following occasions:

- If one bovine animal from a B3 or B4 establishment is positive to a serological test (positive to RBT and has CFT \geq 20 IU/ml).
- As a result of the detection of brucellosis following an abortion investigation;
- If the EI reveals the possibility of infection;
- When there are no conditions for the establishment to be classified as B3 or B4 (whenever the plan is not being fulfilled);
- For any other reason considered relevant to the strategy against brucellosis by the veterinary services.

The above measures shall be maintained until the presence of the disease has been ruled out or confirmed and, if necessary, can be extended to additional susceptible species kept in the establishment and to any establishment with epidemiological link with the establishment where the suspected case occurred.

The B3 or B4 status may only be restored if the requirements set out for the granting and maintenance for this status are fulfilled and the results of further investigations substantiate absence of infection with *Brucella abortus*, *B. melitensis* and *B. suis* and the status of all suspected cases has been determined.

Regarding the serological positive animals, DSAVR sets the following standard procedures:

- serological positive animals are marked (and in certain occasions, also other animals in the same unit) and are destined for compulsory slaughter; the remaining animals are submitted to serological test following the removal of the last positive animal for slaughter;
- collection and transport of the animals destined for compulsory slaughter shall be under official supervision within 30 days following the date of official notification of the operator;
- collection of material for laboratory diagnosis (bacteriology), except from animals of infected unit;
- preparation of a dossier for the payment of compensation.

A procedure is established to investigate isolated positive serological reactions, which might not be related to brucellosis, which after an epidemiological investigation determining very low risk of disease introduction in the establishment, allows the re-testing animals from B3 or B4 establishment

after 30 days, whenever animals identified as positive are isolated. This consists to an alternative, to the slaughter of the sero-positive animal and post-mortem sampling for bacteriology. In the retested animal is negative the B3 or B4 status may be regained.

b) disease control measures to be applied in the event of a confirmed case.

An establishment is considered to be infected, if the presence of *Brucella abortus*, *Brucella melitensis* and *Brucella suis* is confirmed, by its isolation in a bacteriological examination of samples taken from sanitary slaughter. In these cases, the B3 and B4 health status of the establishment must be withdrawn and it shall be classified as infected (B2.1). The withdrawal may be limited to the epidemiological units where the case was confirmed.

The B3/B4 status may only be regained if all confirmed cases and all animals that have tested non-negative have been removed and the remaining animals fulfil the requirements set out for the granting status.

DSAVR shall applied measures to the infected establishment, namely conduct investigations and the epidemiological enquiry (EI), as mentioned to the suspicious case; shall prohibit movement of germinal products of bovine and of bovine animals from and out of the establishment, unless the animals are for immediate slaughter in a designated slaughterhouse.

The EI requires the characterization of the establishment, the investigation of possible sources of infection including introduction of animals, contacts at pasture and possible contacts with wildlife. It also investigates all unit that have contacts with the one in question. Non-compliances identified are subjected to penalties and contact establishment must be controlled.

Besides the animal movement restrictions, operators are also notified to isolate the suspected and confirmed cases, where technically possible, up to its removal. Restriction remains in place until the establishment has regained its status. Consequently, all B4S, B3S, B2 and B2.1 establishment are under sanitary restrictions.

Following measures shall also be in taken by the DSAVR, such as:

- notifying the operator on the results obtained and the imposition of animal movement restrictions, prohibiting on moving susceptible animals to and from the establishments;
- compulsory slaughter of all confirmed cases and all animals that have tested non-negative, under the official supervision in a designated slaughterhouse and with appropriate compensation for their operators (owners). Animals shall be slaughtered no later than 30 days following the official notification of the operator;
- data on slaughtered animals shall enter in the animal health data base (PISA.NET) and the veterinarians of the Livestock Producers Organisations (OPP- most field activities of the programme are implemented by private veterinarians) shall be informed so that they may assist with the subsequent checks;
- the use of depopulation (total slaughter) of outbreaks, when necessary;
- the remaining entire bovine animals over 6 months of age present in the establishment at the time of sampling have to be tested negative to the following serological tests of infected establishment (B2.1), until they achieve the B3 or B4 status and will be carried out as following:
 - a) First test carried out on all animals 30 days after slaughter of the positive animal(s). All sera undergo to RBT and CFT to entire bovine animals over 6 months of age.
 - b) Second test of all the animals not earlier then 60 days later (=3 months after the removal of last confirmed case).

If all the results of the serological test referred above are negative, the establishment will cease to be regarded as infected (B2.1) and will from then on be regarded as non-disease free status (B2) undergoing rehabilitation;

c) A third test will be carried out on all the animals over 12 months of age not earlier than 6 months and not later than 12 months, following the date of above sampling (=180 days after the previous negative result).

If infection with *Brucella suis biovar 2* is confirmed in a single bovine animal, the B3/B4 status may be regained after negative testing obtained on the sampling carried out not earlier than 60 days later after the removal of the animal.

Operators are notified of the following requirements:

- milk from confirmed cases shall either be fed only to animals in the same establishment after it has been processed to ensure the inactivation of the disease agent or it shall be disposed of.
- manure, straw, feed or any other matter and substance that have been into contact with a confirmed case or with contaminated material shall be either collected and disposed of as soon as possible, after treatment with officially approved disinfectant solution;
- foetuses, still-born animals, animals which have died from the disease after birth and placenta shall be collected and disposed of.
- the grazing areas where infected animals were kept may not be used within 120 days in winter or 60 days in summer, although it is recommended that the depopulation period should not be less than 120 days;
- cleaning and disinfection with officially approved disinfectants and other measures to prevent the spread of infection, of all parts of the establishments that may have been contaminated after the removal of the confirmed and suspected cases and before repopulation as well as cleaning and disinfection of all means of transport, containers and equipment after the transport of animals or products from infected establishments.

A system is established for the verification of cleaning and disinfection by the OPP or the DSAVR, after the slaughter of positive animals or after total slaughter (depopulation), prior to reintroduction of animals. The cleaning and disinfection shall be supervised and the disease-free status shall not be restored or granted again until it considers that this measure has been completed. There is an approved protocol.

(c) biosecurity and risk mitigating measures to be implemented.

During epidemiological inquiries, operators are faced with a range of questions related to biosecurity measures and management which have also informative and educational purposes. Subjects as management of pregnant animals, use of pastures, risk on sharing equipment, and the scope for direct or indirect contact with other epidemiological units are referred.

The notification to the operator related to sanitary restrictions "sequestro" contains instructions related to cleaning and disinfection of the stables and outbuildings areas and loading points, of the materials or substances from animals or been in contact with them, as well as containers, utensils and other objects used by animals.

Once the laboratory results are known, the official services schedule with the transporters (hired for the purpose), the compulsory slaughter of the animals and the respective slaughterhouse.

The animal is collected on the agreed date, without prior knowledge of the operator, and transported for slaughter on the same day.

After unloading the animal at the abattoir, the vehicle is cleaned, washed and disinfected in the cleaning and disinfection facility approved by the DGAV existing at the abattoir, with hot water and appropriate product for the effect (also approved by the DGAV). These procedures are under the sanitary inspector's supervision.

After this operation, the slaughterhouse issues a statement attesting to the cleaning and disinfection, which must accompany the vehicle's movement documentation.

(d) type of vaccine(s) to be used and the vaccination scheme, if relevant;

Vaccination is considered a useful tool for brucellosis control as it increases immunity and decreases environmental contamination. Vaccination with lyophilised vaccine contains the RB51 strain of *Brucella abortus* can only be introduced in an epidemiological unit (unit) once a written compromised is established (protocol) between the DSAVR and the Operator with the participation of the private veterinarians of the OPP, where measures to be undertaken are laid down in order to control the *Brucella* infection in the unit. The protocol includes the vaccination strategy of young and/or adult female cattle among other measures (such as the schedule of testing). Bovine's males are not vaccinated.

Vaccine application against bovine brucellosis at national territory is under DGAV authorisation and it consists on the administration of 2 ml subcutaneously.

The necessary requirements to stop vaccination in particular areas depend on the favourable evolution of the epidemiological situation at the epidemiological units, and it includes no infected epidemiological units for the last 3 years (efforts will be carried out in order to perform depopulation) and no clinical or other sign of *Brucella* infection.

For the following epidemiological units vaccination programmes with RB51 vaccine will continue to be implemented:

- DSAVR Norte: counties of Montalegre and of Ribeira de Pena and contiguous parishes;
- DSAVR Alentejo (certain units), only in 2021.

Vaccination at the DSAVR Norte

On B3 or B4 establishments which through an epidemiological investigation a relationship with an infected unit is identified, vaccination will be carried out depending on the risk assessment as follows.

- If high risk, vaccination of all replacement females over 4 months of age and non-pregnant adult female;
- If low risk - vaccination of young replacement females between 4 and 12 months of age.

Depending on the evolution of the epidemiological situation at the different unit, revaccination of adult females and young, after 6-12 months of age, can be determined.

Vaccination in DSAVR Alentejo (till the end of 2021 programme,)

Vaccination will be applied in certain unit according to the following schedule:

- Primo-vaccination to all heifers with more than 4 months of age and revaccination to young females 6 to 12 months after the first inoculation;
- After this first vaccination, annual vaccination with one inoculation to all replacing young females between 4 and 12 months age;
- Adult and young females introduced into the unit will be vaccinated at the entrance.

(e) measures to be implemented as regards additional animal populations, if relevant;

Not relevant

(f) derogations to be applied in accordance with Article 19 of Delegated Regulation (EU) 2020/689, if relevant;

Not relevant

(g) coordinated measures with other Member States or third countries, if relevant.

Not relevant

(h) targeted animal populations and when applicable, additional animal populations

The eradication programme covers all bovine animals. Another eradication programme is applied to ovine and caprine animals.

8. A description of the organisation, supervision and roles of the parties involved in the eradication programme including at least:

a) the authorities in charge of coordinating and supervising the implementation of the programme.

Continental territory (mainland):

The Directorate-General for Food and Veterinary (DGAV) is the authority responsible for the control and eradication of infection with *Brucella abortus*, *B. melitensis* and *B. suis* as regards kept bovine animals and its central service, the Directorate for Animal Protection (DSPA) is responsible for coordinating and monitoring the programme.

Three Regional Directorates for Food and Veterinary (DSAVR) of the mainland, decentralized services of DGAV (Norte, Lisboa e Vale do Tejo and Alentejo), are responsible for overseeing the implementation of the various activities under the eradication programme in their area, for the attribution of the health status for the establishment and the implementation of restrictions in positive establishment. DGAV/DSAVR is also responsible for monitoring compliance with the legal requirements arising from the agreements signed with the OPPs.

Regarding the Autonomous Region of Açores, S. Miguel island, the authority responsible for control and eradication of infection with *Brucella abortus*, *B. melitensis* and *B. suis* is the Regional Directorate of Agriculture, via the Directorate for Veterinary Services. The implementation of measures is co-ordinated on the island via a veterinarian who is a Head of Division or Head of the Veterinary Service Sector of the Agricultural Development Service of the Island, who will request cooperation from veterinarians from other bodies. The measures under the plan are implemented by technicians from the agricultural development services of the Island.

(b) responsibilities of all stakeholders involved.

Continental territory (mainland):

Most field activities of this programme are implemented by private veterinarians from Livestock Producers Organisations (OPPs) which annually submit sanitary programmes to be approved by the official services. There is one veterinary co-ordinator per OPP. Each OPP is assisted by several veterinarians. OPP is responsible for: animal identification, vaccination, blood sampling, computerization of the data in PISA.Net and communication to the Regional veterinary services of all irregularities.

The entity that collects the samples is also responsible for submitting them to the laboratory. Sampling during sanitary slaughter is carried out by the official veterinary inspector of the DSAVR.

All laboratories involved in Brucellosis Eradication Programmes are accredited by Portuguese Accreditation Body, named IPAC, and formally recognised by DGAV.

The National Institute for Agrarian and Veterinary Research (Instituto Nacional de Investigação Agrária e Veterinária, I.P., hereinafter - INIAV, I.P.) is the national reference laboratory (NRL) for food safety, animal and plant health.

INIAV, I.P., is the NRL for brucellosis and is responsible for the coordination and technical supervision of the official laboratories and the harmonization of the testing methods used, following guidelines supplied by EURL and OIE Manual. Since 2014, there are two delegations of INIAV performing official samples for brucellosis diagnosis. One is located in the Norte (Vila do Conde - Vairão) and the other in Alentejo (Évora). These delegations perform Rose Bengal Test (RBT), Complement Fixation Test (CFT) and ELISA. Brucella bacteriological examination and typing of Brucella are only performed at NRL at central level and the results are electronically communicated to DGAV.

In the continental territory, there are 6 private official laboratories located at the following districts:

- SEGALAB (Laboratório de Sanidade Animal e Segurança Alimentar, S.A) – Póvoa do Varzim - Porto;
- PROLEITE (Cooperativa Agrícola de Produtores de leite, C.R.L) – Oliveira de Azeméis - Aveiro;
- LMV (Laboratório de Medicina Veterinária, Lda.) – Santarém;
- SOCLAB (Polo Litoral Alentejano) – Santiago do Cacém - Setúbal;
- COPRAPEC (Cooperativa Agrícola de Compra e Venda de Montemor-o-Novo, C.R.L) – Montemor-o-Novo - Évora;
- ACOS (Associação de Agricultores do Sul) – Beja.

These laboratories carry out RBT, CFT and milk ELISA and record the results in PISA.Net.

NRL provides technical training for staff either for initial qualification or for requalification on RBT, CFT and ELISA techniques. NRL provides official Labs with Positive Control Serum for RBT and CFT techniques.

Operators and traders have the responsibility to provide access and the necessary means to carry out the interventions on the animals, to comply with the rules on identification and animal movement, to allow loading and transport for slaughter of positive animals and to comply with the movement restrictions and depopulation periods imposed, following total slaughter. Operators have the right to be compensated for the value of their animals compulsory slaughtered or culled provided that they fulfill their responsibilities under the applicable legislation.

Regarding the Autonomous Region of Açores, in S. Miguel Island, the Official Services have protocols firm with Farmers Associations and those private veterinarians perform the blood sampling, unless in positive herds, that is performed by Official Veterinarians.

Serological diagnostic testing, milk ELISA testing and bacteriological examinations for the isolation, identification and typing of *Brucella* are carried out in the LRVA - Regional Laboratory of the Autonomous Region of Açores.

9. The estimated duration of the eradication programme.

Targets in WD SANTE 2021/10502 foresee a reduction of 50% of bovine brucellosis, for the first year and then zero. The targets prevalence (0.14) and incidence (0.11) for Portugal expressed in this WD included in the denominator regions that meanwhile obtained disease-free status. For the remaining zones in the eradication programme, the national indicators in 2019 and the expected evolution, reducing 50% are the following:

- Prevalence (2019 in 3 regions – 0.18): 2021 – 0.017 / 2022 – 0.008 / 2023 – 0
- Incidence (2019 in 3 regions – 0.15): 2021 – 0.017 / 2022 – 0.008 / 2023 – 0

10. The intermediate targets of the eradication programme including at least:

(a) the expected annual decrease of the number of infected establishment

Expected number of newly infected herds

DSAVR	Districts	2021	2022	2023
N	Viana do Castelo	0	0	0
	Braga	0	0	0
	Porto	0	0	0
	Vila Real	5	3	0
	Bragança	0	0	0
LVT	Santarém	0	0	0
	Lisboa	0	0	0
	Setúbal	1	0	0
ALT	Portalegre	3	1	0
	Évora	0	0	0
	Beja	0	0	0
RA Açores	S. Miguel	0	0	0

(b) the expected annual increase of the number of disease-free establishment;

The expected number of disease-free establishments are the entire bovine population except four holdings in 2022 and all entire population in 2023.

(c) the expected vaccination coverage, where relevant.

The expected coverage of vaccination is 100% of the planned.

Regarding Norte Region:

District of Braga: will stop vaccination in 2022;

District of Vila Real: will continue vaccination in 750 holdings (750/1406=53% to be vaccinated).

Regarding Alentejo Region:

District of Portalegre – will stop vaccination by the end of 2021.