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DIRECTORATE-GENERAL FOR HEALTH AND FOOD SAFETY

Health and food audits and analysis

DG(SANTE) 2016-8773 - MR

**FINAL REPORT OF AN AUDIT  
CARRIED OUT IN  
PORTUGAL  
FROM 29 FEBRUARY 2016 TO 09 MARCH 2016  
IN ORDER TO  
EVALUATE THE IMPLEMENTATION OF THE BOVINE BRUCELLOSIS AND  
TUBERCULOSIS ERADICATION PROGRAMMES**

## *Executive Summary*

*This report describes the outcome of an audit in Portugal carried out from 29 February to 9 March 2016 as part of the published Directorate General Health and Food Safety audit programme.*

*The objectives of the audit were to evaluate whether:*

- The Programmes for eradication of bovine tuberculosis and bovine brucellosis approved for 2015 have been implemented effectively; and*
- Epidemiological information and knowledge gathered during the monitoring and evaluation of the effectiveness of the bovine tuberculosis and bovine brucellosis Programmes measures were used effectively:*
  - To set the objectives and annual targets of the bovine tuberculosis and bovine brucellosis eradication Programmes to be implemented in 2016.*
  - To select the most efficient and effective measures to achieve the objectives of the Programmes.*

*The competent authorities implemented many of the measures contained in the 2015 eradication Programmes. They availed of comprehensive and functional data management systems which were used to support planning and controls. The systems provide data to analyse the progress of the tuberculosis and bovine brucellosis eradication efforts.*

*The central competent authority considered epidemiological indicators and knowledge gathered during the monitoring and evaluation of the effectiveness of the bovine tuberculosis and bovine brucellosis Programmes when setting the Programmes' objectives and annual targets. The measures in place to detect and eliminate both bovine tuberculosis and bovine brucellosis are effective in keeping the diseases prevalence low.*

*The weaknesses observed include insufficient use of epidemiological data, lack of assessment of fitness for purpose of the current bovine tuberculosis testing scheme, absence of knowledge regarding the impact of not testing animals in fattening herds, absence of analysis of the high rate of new bovine tuberculosis positive herds detected at slaughterhouses. Insufficient analysis and learning from the data prevented objective identification of the most effective and efficient measures to achieve the objectives of the Programmes and to accelerate the eradication of bovine tuberculosis and bovine brucellosis in the country. Also, the exclusion of male animals from testing in some areas in the mainland of Portugal, may have compromised the maintenance of the officially tuberculosis free herd status in these areas during 2015.*

*Acceleration of the eradication of bovine tuberculosis and bovine brucellosis can hardly be expected without critical and objective analysis of the factors that substantiate the persistence and spread of the bovine tuberculosis/bovine brucellosis infection and those that may explain why progress in the elimination of the diseases has slowed down in the recent years. This in particular concerns: (i) the ability of the current field testing scheme to detect the majority of positive animals, (ii) the impact of not testing non-breeding male animals in breeding herds and the exclusion of more than 10000 fattening herds from bovine tuberculosis field testing, (iii) the impact of individual performance and reliability of the work of the field testers, (iv) the impact of non-vaccination of all at risk herds in a bovine brucellosis infected area, (v) the speed and completeness of implementation*

*of risk reduction measures in positive herds. The absence of such analysis will continue to affect understanding of which measures are the most appropriate to achieve the objective of eradication of bovine tuberculosis and bovine brucellosis in Portugal within the established target period.*

*The report makes four recommendations to the competent authorities aimed at addressing areas in which further improvements are required.*

## Table of Contents

1	Introduction .....	1
2	Objectives and scope .....	1
3	Legal Basis .....	2
4	Background .....	2
5	Findings and Conclusions .....	4
5.1	Planning of the implementation of the bTB and bB Programmes measures .....	4
5.2	Implementation of bTB and bB eradication Programmes .....	6
5.2.1	<i>On farm measures to detect bTB and bB</i> .....	6
5.2.1.1	<i>Tuberculin testing</i> .....	6
5.2.1.2	<i>Use of Gamma-interferon assay</i> .....	7
5.2.1.3	<i>Quality control of work of field testers</i> .....	7
5.2.1.4	<i>Vaccination against bovine brucellosis</i> .....	8
5.2.2	<i>Slaughterhouse surveillance</i> .....	9
5.2.3	<i>Measures after slaughterhouse suspicion, detection of bTB reactor or bB positive animals</i> 12	
5.2.3.1	<i>Movement restrictions and official investigations</i> .....	12
5.2.3.2	<i>Slaughter of bB and bTB positive animals</i> .....	14
5.2.3.3	<i>Slaughter of all susceptible animals from an infected herd (depopulation)</i> .....	14
5.2.3.4	<i>Cleaning and disinfection</i> .....	14
5.2.3.5	<i>Epidemiological investigation</i> .....	15
5.2.4	<i>Classification and maintenance of herd status</i> .....	16
5.2.4.1	<i>Herd status as regards bTB</i> .....	17
5.2.4.2	<i>Herd status as regards bB</i> .....	19
5.2.5	<i>Control of movements of animals within the country</i> .....	19
5.2.6	<i>Re-occurrence of bTB and bB</i> .....	20
5.2.7	<i>Measures to detect the bTB infection in wildlife and to prevent its spread</i> .....	21
5.2.8	<i>Training of field testers, slaughterhouse inspectors/workers, hunters</i> .....	21
5.3	Laboratory diagnostic .....	24
5.4	Verification of implementation, evaluation of effectiveness and adjustment of measures ...	26
5.5	Follow-up .....	28
6	Overall Conclusions .....	29
7	Closing Meeting .....	29

8 Recommendations .....30

## ABBREVIATIONS AND DEFINITIONS USED IN THIS REPORT

<b>Abbreviation</b>	<b>Explanation</b>
bB	Bovine brucellosis
bB Programme	Programme for the eradication of bovine brucellosis for 2015, approved by Grant Decision approving national programmes and associated funding, of 30 January 2015 (SANTE/VP/2015/ES/SI2.700826).
BF	Brucellosis Free status of a bovine herd
bTB	Bovine tuberculosis
bTB Programme	Programme for the eradication of bovine tuberculosis in Portugal for 2015, approved by Grant Decision approving national programmes and associated funding, of 30 January 2015 (SANTE/VP/2015/ES/SI2.700826).
CCA	Central Competent Authority
DGAV	Directorate-General for Food and Veterinary Matters
DSAVR	The Regional Directorates for Food and Veterinary Services
ELISA	The enzyme-linked immunosorbent assay
EURL	European Union Reference Laboratory
ICTT	Intradermal Comparative Tuberculin Test
<i>Mycobacterium</i>	<i>Mycobacterium tuberculosis complex (including M.bovis, M.tuberculosis and M. caprae)</i>
NRL	National Reference Laboratory
OBF	Officially Brucellosis Free status of a bovine herd
OPP	Livestock producers organisation
OTF	Officially Tuberculosis Free status of a bovine herd
PISA.NET	Animal Health Database, contains information on implementation of animal sanitary health measures. It also contains information on a herd's health status and communicates this information to SNIRA/iDigital
SNIRA/iDigital	Central Herd Register and Animal Movements Database
SIPACE	Central Database managing the information regarding food establishments, including veterinary inspection at slaughterhouses

## 1 INTRODUCTION

The audit took place in Portugal from 29 February to 9 March 2016. The audit team comprised two auditors from DG Health and Food Safety. The audit was undertaken as part of the DG Health and Food Safety audit programme.

The audit team was accompanied throughout the audit by a representative of the Directorate of Animal Protection of the General Directorate for Food and Veterinary Matters (DGAV), which is the central competent authority (CCA) for the scope of this audit.

At the opening meeting on 29 February 2016 the audit team confirmed the objectives and scope of the audit as well as the itinerary.

## 2 OBJECTIVES AND SCOPE

The objective of the audit was to evaluate whether:

- The Programmes for eradication of bovine tuberculosis (bTB) and bovine brucellosis (bB) approved for 2015<sup>1,2</sup> have been implemented effectively; and
- Epidemiological information and knowledge gathered during the monitoring and evaluation of the effectiveness of the bTB and bB Programme measures were used effectively:
  - To set the objectives and annual targets of the bTB and bB eradication Programmes to be implemented in 2016.
  - To select the most efficient and effective measures to achieve the objectives of the Programmes.

The scope of the audit covered implementation of relevant measures of the approved bB and bTB eradication Programmes, in particular: detection of bTB in the field and at slaughterhouses; supervision and verification of bTB testing; vaccination against bB and testing to detect bB; measures applied in bB and bTB positive herds; classification (e.g. upgrading and downgrading herd status) and maintenance of herd status as regards bTB and bB; control of animal movements; measures to detect bTB in wildlife; diagnostic support for the eradication of bTB and bB.

The audit was conducted by document and data review, on-the-spot verification and interviews/meetings with official and private veterinarians, laboratory experts, farmers and farmer's representatives.

In pursuit of the objectives, the following sites were visited:

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<sup>1</sup> [http://ec.europa.eu/dgs/health\\_food-safety/funding/cff/docs/animal\\_vet-progs\\_2015\\_dec-2015-7437-ec\\_bovine-brucellosis\\_portugal.pdf](http://ec.europa.eu/dgs/health_food-safety/funding/cff/docs/animal_vet-progs_2015_dec-2015-7437-ec_bovine-brucellosis_portugal.pdf)

<sup>2</sup> [http://ec.europa.eu/dgs/health\\_food-safety/funding/cff/docs/animal\\_vet-progs\\_2015\\_dec-2015-7437-ec\\_bovine-tuberculosis\\_portugal.pdf](http://ec.europa.eu/dgs/health_food-safety/funding/cff/docs/animal_vet-progs_2015_dec-2015-7437-ec_bovine-tuberculosis_portugal.pdf)

MEETINGS / VISITS		no.	COMMENTS
Competent Authorities	Central	1	
	Regional	3	
Laboratories		1	
Cattle holdings		3	
Slaughterhouses		1	
Assembly centres		2	
Private veterinarians		3	
Livestock producers organisations (OPP) and other farmers' associations representatives		3	One OPP in mainland Portugal and two farmers association in Azores

### 3 LEGAL BASIS

The audit was carried out under the general provisions of EU legislation and, in particular:

- Article 45 of Regulation (EC) No 882/2004.
- Article 37 of Regulation (EC) No 652/2014 of 15 May 2014 laying down provisions for the management of expenditure relating to the food chain, animal health and animal welfare, and relating to plant health and plant reproductive material, amending Council Directives 98/56/EC, 2000/29/EC and 2008/90/EC, Regulations (EC) No 178/2002, (EC) No 882/2004 and (EC) No 396/2005 of the European Parliament and of the Council, Directive 2009/128/EC of the European Parliament and of the Council and Regulation (EC) No 1107/2009 of the European Parliament and of the Council and repealing Council Decisions 66/399/EEC, 76/894/EEC and 2009/470/EC.
- Article 10 of Council Directive 77/391/EEC introducing Community measures for the eradication of brucellosis, tuberculosis and leucosis in cattle.

Annex I comprises a full list of EU legislation providing the criteria for this audit. The annex refers, where relevant, to the last amended version.

### 4 BACKGROUND

Portugal has been implementing measures to eradicate bB and bTB for many years with EU financial support (e.g. eradication of bTB has been co-financed since 1992). Measures, for example, include testing and slaughter of bB and bTB positive animals, controls on cattle movements, identification and reduction of adverse practices that substantiate the diseases introduction and spread. In two regions on mainland Portugal and three islands of the autonomous region of Azores, the CCA applies RB51 vaccination to increase immunity to

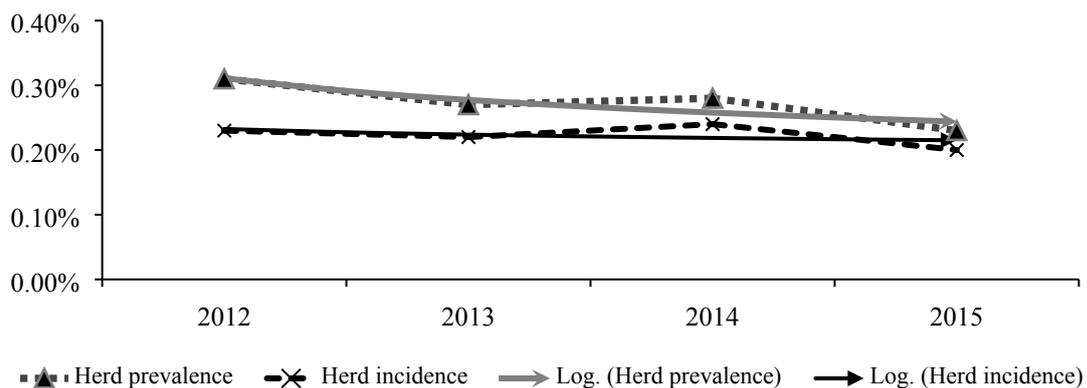
brucellosis infection and to reduce both the risk of abortions and excretion of the bacteria into the environment, thus supporting bB eradication efforts.

According to the data provided by the CCA during this audit, there are 1,572,100 cattle kept in 44,500 herds in Portugal (1,307 540 cattle are kept in 36,848 herds on the mainland and 264,560 in 7,652 herds on the Azores Islands). In 2015, around 75% of cattle herds on the mainland (except the Algarve region which is officially free from both diseases) were under the eradication Programmes for bTB and bB. On the Azores, 99% of cattle herds were under the bTB eradication Programme and the bB eradication Programme is implemented on three islands, with 77% of herds under the Programme.

From 2012 to 2015, annual apparent between herd prevalence and incidence of bB (*Graph 1*) is decreasing very slowly, and trends in 2014 and 2015 remain stable. During the same period, there is no significant decrease in the apparent prevalence and incidence of bTB (as shown on *Graph 2*).

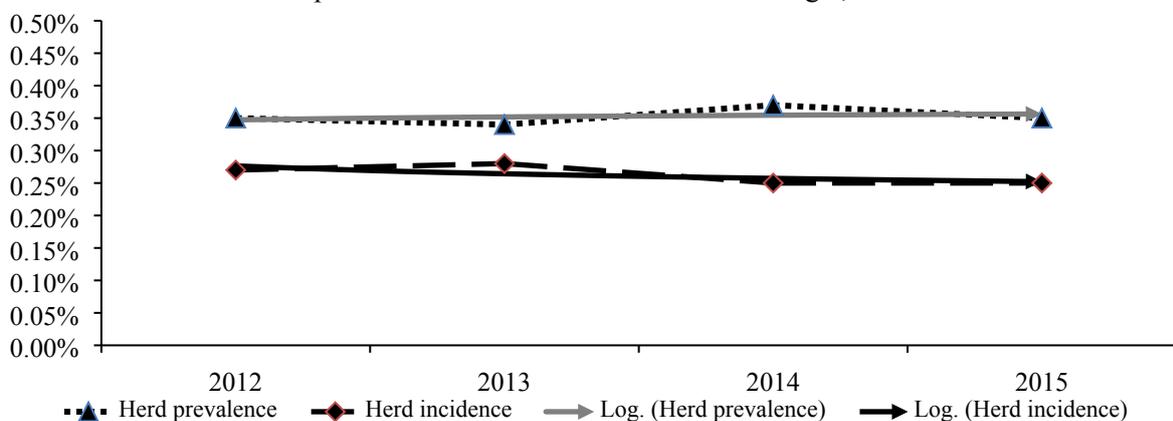
*Graph 1*

Between herd prevalence and incidence of bB in Portugal, from 2012 to 2015



*Graph 2*

Between herd prevalence and incidence of bTB in Portugal, from 2012 to 2015



There is a regional difference in the burden of both diseases. In 2015, the highest apparent between herd prevalence of bB was found on one island of the autonomus region of Azores (i.e. Sao Miguel, bB prevalence of 1.06%), followed by Alentejo, the region on mainland Portugal with the highest cattle density (apparent bB prevalence of 0.54%). In 2015, the apparent between herd prevalence of bTB in Alentejo was also the highest in the country (bTB prevalence of 1.34%).

The previous DG Health and Food Safety audit<sup>3</sup>, on the same subject, was performed in 2009. At the time of this present audit, one of the fifteen audit recommendations from 2009 remained open. The open recommendation concerns registration of pastures for cattle grazing during summer, located in mountain areas and controls of cattle movements, as required by Commission Decision 2001/672/EC. The other recommendations were related to the proportion of cattle population covered by the eradication Programme (including the inclusion of testing fattening herds, the categories of animals excluded from bTB and bB testing), the marking of vaccinated and reactor animals, the strengthening of epidemiological investigations and correct application of post-mortem examination at slaughterhouses.

## **5 FINDINGS AND CONCLUSIONS**

### **5.1 PLANNING OF THE IMPLEMENTATION OF THE bTB AND bB PROGRAMMES MEASURES**

#### **Legal requirements**

Point 4.2 of the Programme for the eradication of bB for 2015 (bB Programme), and point 4.2 of the Programme for the eradication of bTB in Portugal for 2015 (bTB Programme) approved by Grant Decision approving national programmes and associated funding of 30 January 2015 (SANTE/VP/2015/ES/SI2.700826).

#### **Findings**

1. There is organisation and supervision of the eradication activities as mentioned in point 4.2 of both the bB and bTB Programmes. The role of all bodies involved in the implementation of the Programmes is also in line with the approved Programmes.
2. DGAV is responsible for setting the bB and bTB policy objectives and targets. It designs the eradication strategy, coordinates its implementation and monitors the effectiveness of the Programmes measures at the national level. At regional level, Regional Directorates for Food and Veterinary Services (DSAVR) coordinate, supervise and monitor the implementation of the Programmes. In mainland Portugal the Programmes are implemented by veterinarians from Livestock Producers Organisations (OPPs). This includes tuberculin testing, blood sampling, vaccination when applicable, animal identification and general animal health surveillance. In the Autonomous region of Azores, the bB and bTB Programmes are implemented by the official veterinary services

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<sup>3</sup> Audit report DG(SANCO)2009/8248, [http://ec.europa.eu/food/fvo/audit\\_reports/details.cfm?rep\\_id=2356](http://ec.europa.eu/food/fvo/audit_reports/details.cfm?rep_id=2356)

and private veterinarians. The capacity for bTB testing has significantly improved since 2014 due to involvement of private veterinarians, in this Autonomous region.

3. The DGAV has established effective communication and coordination with DSAVRs and effectively communicates with interested bodies, such as OPPs, as required in Article 4(3) of Regulation (EEC) No. 882/2004.
4. Each OPP sets, for the area of their responsibility, the plan for the implementation of the annual bB and bTB Programmes. They draft their annual plan following the technical guidelines set by the DGAV. The plan contains a quantitative description of testing and sampling of cattle. It is subject to verification and approval by the regional CA.
5. The CCA has developed documented procedures for the implementation of the Programmes. This includes manuals of instructions/written procedures (including forms/instructions on testing requirements/frequency, vaccination, sampling, notification of disease suspicion, official protocols/check-lists) for official staff, veterinarians and other bodies.
6. The CCA has established comprehensive information systems that support collection and management of data related to implementation of the bB and bTB Programmes. An animal health database (PISA.Net) records all sanitary activities (testing, sampling, vaccination, animals subject to slaughter, etc) and official decisions made as regards the implementation of animal health programmes. It also provides for reporting according to EU Commission's predefined templates.
7. An animal movement database (SNIRA/iDigital) supports registration of animal movements and issuing of the electronic movement documents. There is interoperability between these two databases allowing the health status of cattle herds as regards bTB and bB to be communicated from the PISA.Net before issue of movement documents. The CCA has also established a comprehensive system for animal health collection of data at slaughterhouses. Each detection of a disease suspicion during the post-mortem inspection, as well as detailed information of bTB suggestive lesions detected, is registered in a central database managing the information regarding food establishments, including veterinary inspection at slaughterhouses (SIPACE).
8. At the opening meeting, the CCA presented the targets for reduction of prevalence of both bB and bTB in 2016 and 2017. According to these targets set, by the end of 2017, the prevalence of both diseases is expected to be below 0.2%.
9. The audit team noted that there is a good level of involvement and support from farmers to the bB and bTB Programmes objectives. According to the farmers representatives met on mainland Portugal, bB and bTB represent a burden due to the cost of both testing and restrictions associated with outbreaks of the diseases. Farmers are willing to continue to support the CCA efforts and, some of their representatives met during the audit, expect elimination of the two cattle diseases to be achieved by 2020.

### **Conclusions on planning of the implementation of the bTB and bB Programme measures**

10. The CCA adequately coordinates and make use of veterinary services (both public and private) to ensure implementation of the bTB and bB eradication Programmes.
11. A good level of support and agreement with farmers in addition to regular communication with them, ensures access to herds subject to implementation of bTB and bB eradication measures and justifies farmers clear and ambitious expectations regarding the eradication of these two cattle diseases.
12. Comprehensive and functional data management systems provide for consistent collection and organisation of data on implementation of the Programmes. It also supports planning, controls, supervision as well as availability of data for analysing the progress of the bTB and bB eradication efforts.

## **5.2 IMPLEMENTATION OF bTB AND bB ERADICATION PROGRAMMES**

### **Legal requirements**

Article 11 and Annex A(I) and A(II), Annex B(2) and Annex C(1)(2) to Directive 64/432/EEC; points 3, 4.4.1, 4.4.2, 4.4.4 to 4.4.10 of the bTB Programme for 2015 and points 4.4.4 to 4.4.10 of the bB Programme for 2015, approved by Grant Decision approving national Programmes and associated funding, of 30 January 2015 (SANTE/VP/2015/ES/SI2.700826), Articles 6(1), 7, 8(1), 14(1), 15 and 16(1) of Directive 78/52/EC.

### **Findings**

#### *5.2.1 On farm measures to detect bTB and bB*

##### *5.2.1.1 Tuberculin testing*

13. The CCA applies an intradermal comparative tuberculin test (ICTT) in accordance with point 2.2.5.3.2 of Annex B of Directive 64/432/EEC, for establishment and maintenance of officially bTB free status (OTF) of a herd. The CCA indicated that this testing scheme is applied to avoid slaughtering of false positive animals.
14. In all regions visited, the CCA had provided the OPP veterinarians performing ICTT (from now on referred to as “field testers”), with detailed instructions on how to perform tuberculin testing. In the Autonomous region visited, each field tester was also informed by official veterinarians on how to perform the ICTT and when to repeat the application of tuberculin (e.g. if an intradermal bulb is not formed).
15. In one region visited, the audit team noted that, in order to increase ability to detect bTB positive animals, the local CA decided in some situations (e.g. in high risk herds), to

interpret the results of the ICTT based on reaction to bovine tuberculin, irrespective of the reaction to avian tuberculin.

16. Regional CAs carry out general, unannounced, on-the-spot control of OPP veterinarians implementing the bTB and bBT Programmes, according to the annual official plan. The controls were implemented according to written procedures (“check list”, Model 897/DEV). In terms of tuberculin testing, the controls include storage of tuberculin, instruments used to measure the skin thickness, preparation of the place for inoculation and inoculation of tuberculin, reading of the skin reaction, etc.

#### *5.2.1.2 Use of Gamma-interferon assay*

17. Gamma-interferon assay, envisaged in points 3 and 4.4.6 of the 2015 of the bTB Programme, was not used in 2015 to increase the proportion of bTB reactors correctly identified by field testing. The CCA explained that gamma-interferon assay is not accredited. Additionally, the demanding logistics required for this test (e.g. samples must be processed the same day as collected in order not to lose the test performance) limited the use of this assay in 2015. The CCA also mentioned that under the envisaged use of this test (as outlined in the 2015 bTB Programme) and based on experience from previous years, the contribution of gamma-interferon assay in detecting the reactor animals was lower than expected.

#### *5.2.1.3 Quality control of work of field testers*

18. Although not specifically mentioned in the 2015 bTB Programme, the CCA carries out supervision of the work of field testers. According to the CCA plan, 5% of field testers (selected at random or on a targeted basis) should be supervised annually. The targets for such controls were largely associated with resources.
19. Over the past two years, official veterinary services in all regions visited have increased the frequency of on-the-spot supervision of field testers. The results provided by the CCA indicate that 11% (58/532) of field testers were supervised in 2014. Irregularities were detected in one supervised field tester.
20. In one region visited, targeted selection was based on factors such as, suspicion of disease during post-mortem inspection in animals with a negative result from the ICTT, odd succession of “zero” in change of skin thickness, a new team being involved in the testing, new positive herds, re-occurrence of bTB reactor animals in a herd.
21. In two regions visited, official veterinarians supervised implementation of the ICTT carried out following the notification of slaughterhouse bTB suspicions, as part of an official investigation.
22. In the Autonomous region visited, tuberculin testing was implemented by private veterinarians working for farmers associations. The audit team noted that official veterinarians carried out repeated on-the-spot supervision of implementation of the ICTT by field testers. This included three consecutive supervisions of all field testers,

until the official veterinarian was satisfied with improvement in their performance (e.g. until no technical deficiencies such as reading and interpretation of ICTT result were detected). Documented evidence (individual tester reports and final report, altogether 25 individual and three final reports) on supervision was presented to the audit team. The initial non-conformities identified during the supervision included: keeping the tuberculin for more than two days at ambient temperature, no tuberculin found in storage, no record keeping on tuberculin storage temperature, no registrations of quantities of tuberculin received/used, errors in inoculation of the tuberculin, late reading of the skin reaction.

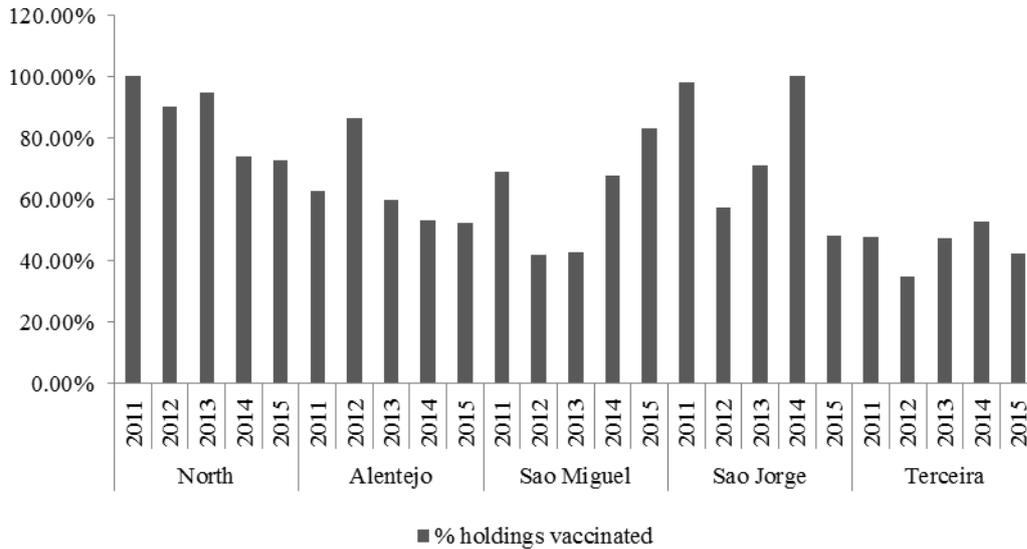
23. In one region visited with a high prevalence of bTB, the OPP coordinator stated that in 2015, 20 out of 90 veterinarians (working for that OPP), were subject to control by the OPP coordinating veterinarian. The audit team noted that this controls included ICTT and sampling for bB. According to the OPP coordinator, no underperformers were identified in 2015.
24. The CCA indicated that an increased frequency of on-the-spot supervision of the work of field testers will continue in 2016. This is because they consider (e.g. based on rate of detection of bTB suggestive lesions at slaughterhouses) that the quality of their work can/needs to be improved.

#### *5.2.1.4 Vaccination against bovine brucellosis*

25. In two regions on mainland Portugal and on three islands of the Autonomous region of Azores, young cattle and/or adult females are vaccinated with the RB51 vaccine, in line with the 2015 Programme. The CCA acquires the RB51 vaccine and distributes it to regions.
26. In many areas, vaccination coverage of herds is lower than expected from the Programme for 2015 (*Graph 3*). The improvement in achieving the vaccination targets among herds is evident on Sao Miguel.

*Graph 3*

Percentage of vaccinated herds out of the expected according to the approved programme, from 2011 to 2015



27. The CCA is concerned about the persistence of brucellosis in several areas in one regional unit. The CCA stated that infection always manages to find new susceptible animals in neighbouring herds in those areas. Documentation shows the regional CA did not always identify underlying factors for such persistence. In that region, vaccination of cattle is performed mainly in infected herds. The CCA mentioned that non-affected farmers in those areas are reluctant to accept vaccination of their herds as this limits access of their animals to the EU market.
28. The CAs did not carry out on-the-spot verification of OPP veterinarians' sampling and vaccinating cattle against bB, since 2013 in the region where OPP were used. The regional CA explained that official resources were focused on on-the-spot control of the work of field bTB testers. The regional CA also mentioned that bovine herds in some areas in this region had been officially brucellosis free (OBF) for more than twenty years. The CA emphasised that the measures implemented so far continue to be effective in stopping the spread of the infection outside the affected areas.

### 5.2.2 Slaughterhouse surveillance

29. Notification and investigation of suspected bTB lesions detected at the slaughterhouse is implemented according to points 3 and 4.4.1 of the 2015 bTB Programme.
30. The CCA has established a comprehensive system, guidelines and procedure for notification and sampling of bTB suspected cases at a slaughterhouse. If animals were sent for regular slaughter and they present lesions, the notification of bTB suspicion has to be sent to the local CA of the area of origin of the animals. This is also the case for animals with lesions that belonged to restricted herds and reacted negatively to the skin test. When lesions are found in bTB reactor animals, there is no requirement to send the notification to the regional CA in the place of origin of the bTB reactor animal, because the holding has already been detected as positive.

31. Official procedures require bTB suspect lesions detected during routine slaughter to undergo laboratory investigation to confirm or rule out the infection. Animals with post-mortem lesions which belong to restricted herds where the infection has already been confirmed, are immediately considered infected without further need for laboratory investigation. CCA procedures require ICTT positive animals to have selected organs submitted for histopathology and bacteriological examination.
32. The CCA has established a system and associated documentation, to supervise official veterinarians' tasks at the slaughterhouse. It is based on observation during post-mortem inspection and verification of record keeping/entry into the database. In one slaughterhouse visited, the audit team observed that official verification was carried out by the regional CA and included supervision of post mortem inspections, detection and notification of bTB suspect lesions, registration of lesions in SIPACE and sampling.
33. The CCA monitors submission rates per slaughterhouse regarding notification of bTB suspicions and bTB suspect lesions. According to data provided for the period from 2012 to 2015 (*Table 1*), there is a decrease in the number of bTB suspicions detected at slaughterhouses and an increase in the percentage of slaughterhouses which did not notify any bTB suspicion.

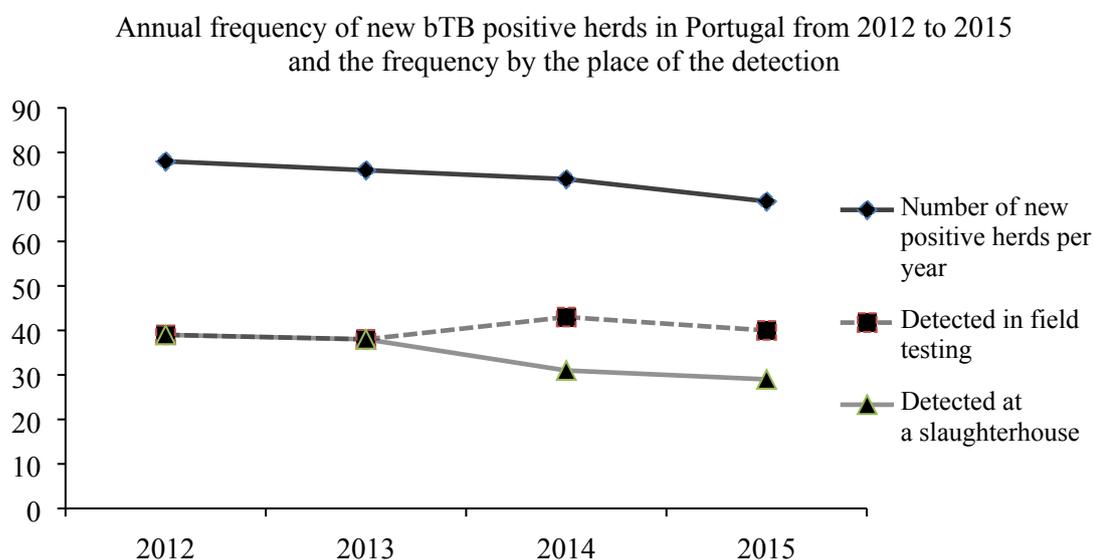
*Table 1.* Frequency of bTB suspicions detected in slaughterhouses and related notifications, from 2012 to 2015

<b>Year</b>	<b>Average number of bTB suspicions per 1000 cattle slaughtered (range)</b>	<b>% of slaughterhouses with no notifications of bTB suspicion</b>
<b>2012</b>	0.35 (0 to 0.96)	42.5
<b>2013</b>	0.22 (0 to 0.83)	55.0
<b>2014</b>	0.21 (0 to 1.11)	52.5
<b>2015</b>	0.17 (0 to 1.02)	69.2

34. According to the data provided by the CCA, the majority of slaughterhouses which did not submit any notification of bTB suspicion were slaughtering less than 1,000 cattle a year.
35. The CCA did not investigate if there are factors (other than annual number of cattle slaughtered) that can explain the differences in rates of detection of bTB suggestive lesions among slaughterhouses (assuming that notification of bTB suspicion always followed the detection of bTB suggestive lesions).
36. Data indicate a continuously high number of lesions detected in animals originating from regions with the highest prevalence of bTB. However, none of the slaughterhouses situated in the region with the highest bTB prevalence in the country, notified a bTB suspicion in 2015. The regional CA indicated that the majority of positive animals from the region with the highest bTB prevalence were slaughtered in other regions.

37. The rate of new bTB positive herds detected at slaughterhouse continues to be very high, considering the current bTB prevalence (*Graph 4*). For example, 42% of new bTB positive herds in 2015, have been detected at a slaughterhouse.

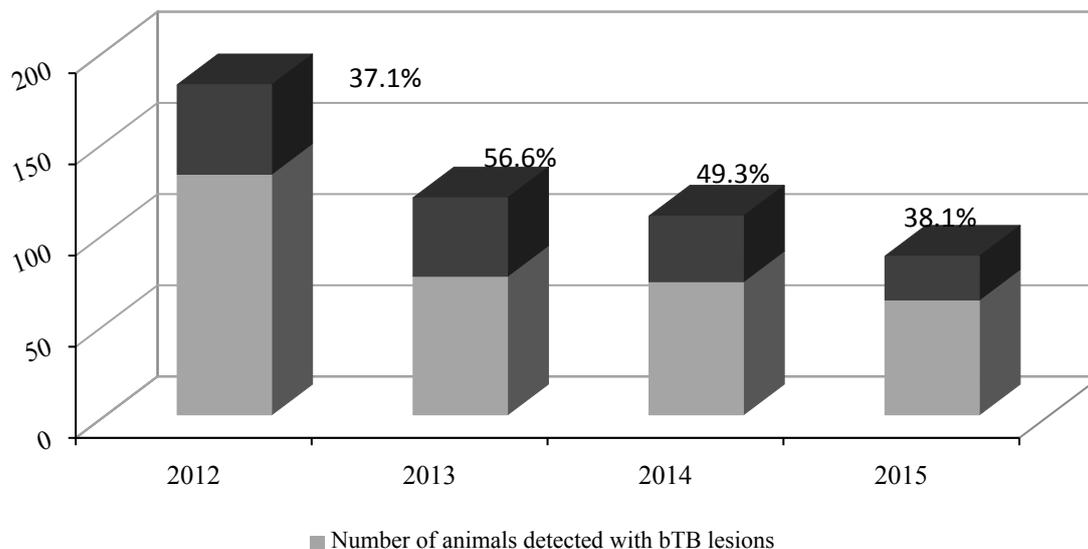
*Graph 4*



38. The audit team was informed in the national reference laboratory (NRL) that calcified lesions are frequently submitted from slaughterhouses. Although the calcified lesions are not always of mycobacterial origin, the NRL pointed out that it is difficult to detect mycobacteria from such lesions. The CCA did not investigate to which extent these difficulties may have impacted the decreasing trend of confirmation of bTB at the country level, since 2013, as shown in *Graph 5*. Also there is no investigation on if/how possible difference in submission of calcified lesions among the slaughterhouses/regions impact the regional differences in bTB confirmation rate. For example, in the region with the highest prevalence of bTB in the country, out of 26 notifications of detection of bTB lesions at a slaughterhouse in 2015, *M. bovis* was isolated in 14 animals with bTB lesions (54%), which is higher than in the country average.

*Graph 5*

Detection of cattle with bTB suspect lesions at a slaughterhouse and the number and percentage of cattle with bTB suspect lesions, confirmed bTB positive, from 2012 to



39. From the carcasses condemned due to detection of bTB suspect lesions in 2015 (0.2% of all bovines slaughtered) 76% were cattle sent for sanitary slaughter while 26% were cattle sent for regular slaughter. In the same year, 0.5% (2/407) of cattle sent for slaughter due to brucellosis were detected as having brucellosis suspect lesions at slaughterhouses.
40. The CCA monitors age distribution of cattle with bTB suspect lesions in which the disease was subsequently confirmed either by isolation of *Mycobacterium tuberculosis complex* (including *M. bovis*, *M. tuberculosis* and *M. caprae*) during culture or by histopathological analysis.
41. According to data provided by the CCA, bTB suggestive lesions were detected in cattle of all age categories, including fattening animals (e.g. those slaughtered at age up to 2 years) from 2012 to 2015. According to the CCA animals with bTB suspect lesions were originating from both classified and non-classified holdings.

### 5.2.3 Measures after slaughterhouse suspicion, detection of bTB reactor or bB positive animals

#### 5.2.3.1 Movement restrictions and official investigations

42. In case of suspicion of bB and bTB, the CCA applies movement restrictions in line with Articles 6(1) and 14(1) of Directive 78/52/EEC, but with significant delays in some cases.
43. When a notification of a slaughterhouse suspicion and when the ICTT or a serological test in the case of bB revealed a positive result, the CCA downgrades the health status of a herd in PISA.Net (for herds that are OTF, OBF and brucellosis free (BF)). When regional CAs downgrade the bB or bTB status in PISA.Net, this automatically updates

the SNIRA/iDigital database, and consequently blocks the issuing of movement documents from the system by an animal keeper (i.e. person responsible for animals).

44. National instructions do not specify a time frame to impose movement restrictions (downgrading the status). In the region with the highest bTB prevalence in Portugal, the audit team checked several cases and movement restrictions were not imposed after the notification of bTB suspicion from the slaughterhouse but only after a positive bacteriological examination. In one case, this was 20 days and in another 49 days after the notification of bTB suspicion from a slaughterhouse. The regional CA explained that these were exceptional situations and that the time before taking action was prolonged due to official staff being overburdened with other tasks. The information provided from the local CA for 2015, in the region concerned, indicates that it took on average 10 days (minimum 0, maximum 49) to implement movement restrictions after notification of a bTB suspicion. The official veterinarian explained to the audit team that in one situation he had the opinion that the slaughterhouse finding was a false alarm as in a particular herd animals were subjected to the ICTT two and a half months before the detection of lesions, with negative results.
45. The CCA allows movement of cattle from bTB and bB infected and other restricted herds to fattening herds specifically approved by the regional CA, called E2 fattening herds. These herds are required to have strict biosecurity measures in place (including fencing). The data provided by the CCA at the time of this audit, indicate that there are 19 E2 fattening herds on mainland Portugal. The CCA requires pre-movement testing of all cattle from restricted herds moving to E2 fattening herds. From 2010, the regional CA issues movement documents for animals leaving restricted herds.

*Table 2: Number of E2 fattening herds, authorised to receive animals from restricted herds, respective number of movements and number of animals moved to E2 fattening herds in 2015.*

<b>DSAVR</b>	<b>E2 fattening herds</b>	<b>Number of movements to E2 fattening herds</b>	<b>Number of animals moved from restricted to E2 fattening herds</b>
<b>Center</b>	1	9	293
<b>Lisbon and Tagus Valley</b>	12	83	1265
<b>Alentejo</b>	7	82	1251

46. The audit team observed that regional CAs have strict controls on E2 fattening herds. These controls include desktop (quarterly) and on-the-spot (twice a year) verification of movements of animals into and from E2 fattening herds. In two regions visited, the audit team observed that on-the-spot checks are done on a sample of 120 animals. For those, they included verification of animal movements, health status of the herd of origin, pre-movement testing of animals coming from restricted herds and place of destination. In

one region visited, the official veterinarian mentioned that no major non-compliances were detected as a result of these checks in the previous five years.

#### 5.2.3.2 *Slaughter of bB and bTB positive animals*

47. In line with Articles 7 and 15 of Directive 78/52/EEC, the CCA requires keepers to slaughter bB and bTB positive animals within 30 days after they are notified of test results. In one region, the data received indicate that it took on average 24 (range 13 to 36) days to officially notify the animal keeper about positive bB/bTB test results. On average, 71% (range 16% to 97%) of bB/bTB positive animals were slaughtered within 30 days.

#### 5.2.3.3 *Slaughter of all susceptible animals from an infected herd (depopulation)*

48. Both bB and bTB Programmes for 2015 indicate when depopulation of all susceptible animals in an infected herd can be implemented. This includes the possibility of depopulating herds which had no improvements in the last 12 months and herds where *Brucella spp.* or *Mycobacterium* was isolated.

49. The number of depopulated herds was: one in 2015, five in 2014, four in 2013 and six in 2012.

#### 5.2.3.4 *Cleaning and disinfection*

50. In most cases, cleaning and disinfection of bB or bTB positive herds, after slaughter of positive animals, was not carried out under official supervision nor in accordance with the instructions given by the official veterinarian. This is not in line with Articles 8(1) and 16(1) of Directive 78/52/EEC.

51. In all regions visited, cleaning and disinfection is the responsibility of the keeper. Keepers receive advice from private veterinarians on disinfectants to be used. In one OPP visited, the audit team was informed that a veterinarian instructs the keeper to disinfect the area where positive animals were kept and which disinfectant to use. There was no official verification if/how effectively cleaning and disinfection on positive herds (bTB and bB) is carried out.

52. In the Autonomous region visited, the audit team checked the official letter issued by the regional CA following the finding of a bB positive animal. The letter specified the measures to be implemented (such as isolation of positive and suspect animals, compulsory slaughter of positive animals, restrictions on movements, notification of abortions). The keeper was also advised that movement restrictions are lifted after two negative serological tests. The letter contained no reference to cleaning and disinfection nor that this should be carried out under official supervision, in accordance with the instructions given by the official veterinarian. The local CA agreed to immediately include this information regarding cleaning and disinfection for bB and bTB positive herds.

53. In one region visited, official staff indicated that in the case of depopulation of cattle in a positive herd, the OPP veterinarians are required to produce documented evidence on cleaning and disinfection after the depopulation.
54. In the Autonomous region, the audit team visited a herd where one bB seropositive animal was detected in 2015. The keeper was fully aware of the measures to be implemented following the detection of bB positive animals. The keeper indicated that he had received advice on cleaning and disinfection of premises (including the disinfectant to be used) from the private veterinarian. The cleaning and disinfection was not officially supervised which is contrary to Article 8(1) of Directive 78/52/EEC.

#### 5.2.3.5 *Epidemiological investigation*

55. The CCA has established procedures (including a structured questionnaire) which are used by the regional CA when carrying out epidemiological investigations. In a region visited, epidemiological investigations (of both bTB and bB) were carried out by a regional official veterinarian who had not received specific epidemiologic training for this. Official staff in the same region visited pointed out that they are experienced in dealing with bTB but have little time to fully investigate all bTB outbreaks. Official staff in another region visited informed the audit team that 85% of work related to the epidemiological investigation of bTB (e.g. investigation of movements, testing and the disease history in a herd) is desk-top based (e.g. carried out in the office by using the PISA.Net and SNIRA/iDigital).
56. The data provided by the CCA indicates that epidemiological investigations are not always carried out following the detection of bTB positive animals in an OTF herd, which is not in line with point 4.4.9 of the 2015 bTB Programme. The CCA pointed out that the epidemiological investigation is always carried out in case of bacteriological or histological confirmation of the infection.
57. Regarding bB, an epidemiological investigation is carried out when the presence of brucellosis is confirmed through isolation of *Brucella abortus* in a bacteriological examination as envisaged in point 3 of the 2015 bB Programme. In the Autonomous region visited, the audit team noted that the epidemiological investigation was also carried out in cases of detection of bB seropositive animals. The data provided by the CCA show an increasing trend of epidemiological investigations on bB positive holdings, but the percentage remains low (around 60% of positives are investigated).
58. The procedures do not require an epidemiological investigation in a restricted herd in cases of new positive animals detected during the repeat testing.
59. The team reviewed several epidemiological investigations. In some cases, epidemiological investigations stopped before identifying the source of infection and conclusions were made with weak consideration all known risks for the introduction and/or spread of the infection.

60. The CCA described the frequency of factors (identified during the epidemiological investigation) which may possibly explain the occurrence of bB or bTB in OTF, OBF or BF herds. According to the data provided by the CCA, in 2015 direct contact with ruminants from other herds seemed to be the most frequent risk factor for bB infection (identified in 75% of investigated herds), followed by the common pastures (identified in 50% of herds) and introduction of animals (identified in 47% of investigated herds). In a high percentage of investigated bB positive herds in 2015, a possible epidemiological link to infection was not established (i.e. described as “other” in the epidemiological investigation).
61. Regarding bTB in 2015, epidemiological investigations indicated possible contacts with wild animals (due to sharing common feeding and watering points in dry season) as a source of infection for 26% of investigated bTB positive herds. For 20% of investigated bTB positive herds, the possible epidemiological link to the infection was described as “other”. Direct contact with ruminants from other herds as a source of contamination of OTF herds decreased to a third, from 2014 to 2015.
62. In 2015, bTB re-occurred in 15.5% of OTF herds (i.e. herds that had the disease during the period from 2012 to 2014 and had gained the officially free status) while bB re-occurred in 11.7% of OBF or BF herds.
63. Point 4.4.9 of the 2015 bTB Programme indicates that holdings linked to a positive holding are investigated and the regional CA makes recommendations regarding a herd status, biosecurity measures and, if necessary, ICTT testing. In one region visited, herds epidemiologically linked with a positive herd were subject to a review of their history of bTB testing, but they were only systematically tested if they had experienced bTB in the past. In another region visited, animals in all herds with established epidemiological links were tested.
64. In the Autonomous region visited, the audit team reviewed two epidemiological investigations in bB seropositive herds. They did not identify the source of the infection. Illegal movements and introduction of a bB seropositive male animal, were identified as possible factors for the introduction of the infection. The local CA mentioned that there also was also the possibility of picking up the infection on a pasture or that the test result was a false positive because the testing of cattle in all neighbouring herds revealed negative results.

#### *5.2.4 Classification and maintenance of herd status*

65. The audit team verified that DSAVR classify the status of cattle herds regarding bTB and bB (including upgrading, downgrading (e.g. suspension or withdrawal) or maintaining OTF, OBF or BF status according to the criteria set in the Programmes for 2015.
66. In case of bTB, the status of a herd is changed or maintained based on the results of the field (i.e. the ICTT performed according to Annex B(2) to Directive 64/432/EEC) or

laboratory examination (bacteriological or histological), as well as following the slaughterhouse suspicion. For brucellosis, a change of a herd status is made based on the results of laboratory tests performed according to Annex C to Directive 64/432/EEC.

67. According to the data provided by the CCA, in 2015 there were 25600 OBF, 1839 BF and 27202 OTF herds on mainland Portugal (except the Algarve which is recognised as an officially free region as regards bTB and bB). On the Autonomous region of Azores (islands other than those recognised officially free from bTB and bB) there were four OBF and 5,337 BF, while all cattle herds (5,341) were classified as OTF, in 2015.
68. The CCA reduced the frequency of testing in areas where prevalence of bTB is lower than 0.2%. In such areas only animals older than 24 months are tested for bTB, as envisaged in point 3 of the 2015 Programme.
69. In 2014, the CA evaluated in 11 positive bB and bTB herds, if required time intervals for repeated testing were observed. In total, 31 testing intervals had been checked and non-conformities were detected in 15 cases (48%).

#### *5.2.4.1 Herd status as regards bTB*

70. The CCA implements the testing regime according to Annex A(I)(1)(c) to Directive 64/432/EEC to classify a cattle herd as an OTF.
71. In 2015, all male animals were systematically excluded from bTB testing for the purpose of maintenance of OTF herd status in some parts of the country (e.g. where the prevalence of bTB is between 0.2% and 1%). This was not in compliance with the requirements of points 3 and 4.4.2 of the 2015 bTB Programme nor with Annex A(I)(2)(c) to Directive 64/432/EEC. For the purpose of maintenance of OTF herd status, the 2015 national operational instructions on bTB testing requested, for these areas, to test only female animals older than 6 weeks.
72. The 2016 bTB Programme modified this point. It requires testing of males not intended for fattening and females older than 6 weeks (for the maintenance of OTF herd status in areas where herd prevalence is between 0.2% and 1%). This measure is not fully in line with the requirements of Annex A(I)(2)(c) to Directive 64/432/EEC. For these areas, the 2016 operational instructions on bTB testing instruct that males for breeding older than 6 weeks should also be tested. In one region visited, the OPP coordinating veterinarian stated that only male animals that will, according to the keeper, be kept for breeding will be subject to bTB testing.
73. Most fattening herds are excluded from bTB testing (they are called by the CA "E1 non-classified" fattening herds). This is not in line with point 2 of the 2015 bTB Programme, which indicates "all animals over 6 weeks of age, in herds that are not OTF, undergo ICTT testing until the herd achieves the disease-free status". Neither of the Programmes (2015 or 2016) mentioned the term "E1 non-classified fattening herds" (e.g. herds of unknown status as regards bTB and bB).

74. According to the data provided by the CCA, there were 36,848 cattle herds in the mainland in 2015 and 75% of them have been under the bTB eradication Programme in 2015. In 25% of herds there were no cattle for reproduction (e.g. fattening herds) or no animals eligible for bTB testing; almost half of these herds are in the northern part of the country (*Table 3*).
75. Most animals from OTF herds intended for fattening are generally not tested for bTB during their whole lifespan. This is because these animals were not tested even if present on a holding during the annual whole herd testing in a herd of origin nor in the fattening herd, the majority of which are excluded from any bTB testing. Many fattening herds keep animals free range, often in contact with animals from other herds or wildlife, and they are not an isolated epidemiological unit contrary to what is required in Annex A(I)(2)(c) to Directive 64/432/EEC.
76. At the time of this audit, there were 12 fattening herds subject to annual bTB test, and classified according to Annex A(I)(1)(c) to Directive 64/432/EEC, for the purpose of EU trade. These are called “E1 classified” fattening herds, or “officially free fattening herds” in the bTB eradication Programme for 2016.

*Table 3: Number of herds excluded from the bTB and bB eradication Programmes, per regions in the mainland and 3 Islands of the Autonomous region of Azores.*

Year	DSAVR	Tuberculosis		Brucellosis	
		Non breeding herds	Without eligible animals	Non breeding herds	Without eligible animals
2012	North	6644	847	6644	1052
	Center	3706	559	3706	640
	Lisbon and Tagus Valley	2042	14	2042	26
	Alentejo	930	0	930	34
	Sao Miguel	0	283	0	879
	Terceira	0	506	0	828
	Sao Jorge	0	306	0	33
	<b>TOTAL</b>	<b>13322</b>	<b>2515</b>	<b>13322</b>	<b>3492</b>
2013	North	3976	928	3976	1119
	Center	2375	476	2375	565
	Lisbon and Tagus Valley	1393	44	1393	55
	Alentejo	941	0	941	56
	Sao Miguel	0	327	0	676
	Terceira	0	328	0	619
	Sao Jorge	0	282	0	54
	<b>TOTAL</b>	<b>8685</b>	<b>2385</b>	<b>8685</b>	<b>3144</b>
2014	North	4623	847	4623	1003
	Center	2738	378	2738	299
	Lisbon and Tagus Valley	1149	13	1149	22

	Alentejo	746	0	746	35
	Sao Miguel	0	385	0	887
	Terceira	0	669	0	399
	Sao Jorge	0	298	0	38
	<b>TOTAL</b>	<b>9256</b>	<b>2590</b>	<b>9256</b>	<b>2683</b>

#### 5.2.4.2 Herd status as regards bB

77. To classify a cattle herd as OBF or BF, the CCA implements the testing regime according to Annex A(II)(1)(c) to Directive 64/432/EEC.
78. In 2015, 75% of herds have been under the bB eradication Programme. Males for fattening were excluded from testing, provided they were, at the completion of a fattening period, sent directly to slaughter, in line with Annex A(II) to Directive 64/432/EEC.
79. The audit team noted that in dairy herds, milk indirect ELISA (Enzyme-linked immunosorbent assay) is used for testing for the purpose of maintaining OBF and BF herd status, in compliance with point 3 of the 2015 Programme. In the Autonomous region visited, the audit team noted that pooled milk samples were taken by technicians and tested in the regional laboratory according to the sampling protocol established by the NRL. This sampling protocol does not state that pooled milk samples should only be taken if at least 30% of dairy cows are in milk, contrary to Annex (C)(2.2.3.4) to Directive 64/432/EEC. The local CA did not verify whether this legal requirement was followed before indirect an ELISA test was used on a pooled milk sample.
80. The CCA has a system in place to downgrade the holding status (e.g. triggered by a positive serological test result, when testing scheme/frequency was not observed or when epidemiological investigation raised suspicion). In the Autonomous region visited, the audit team checked the suspension of a BF herd status, implemented by the regional CA. The procedure and frequency of testing until the herd restored the BF status, had been implemented according to point 4.4.4 of 2015 bB Programme and Annex A(II)(6A) to Directive 64/432/EEC. When the presence of *Brucella* was confirmed by bacteriological examination, the BF herd status was withdrawn and there was a follow-up testing to restore the status, in line with Annex A(II)(6B) to Directive 64/432/EEC. The audit team verified that status of herds, belonging to the same epidemiological unit as a positive herd, were suspended and animals in these herds were also subject to the same frequency of bB testing as a positive herd.

#### 5.2.5 Control of movements of animals within the country

81. Cattle moving within the country are accompanied by a cattle passport and movement document (i.e. declaration of movement or permit of movement). The declaration of movement is issued by the keeper in cases of OTF, OBF and BF herds as well as E1

fattening herds. According to the national procedures, the permit for movement is issued by the regional CA in cases of herds under restrictions (e.g. suspended, infected).

82. In August 2015, the CCA introduced a system to issue the movement documents electronically. Whenever a movement document is issued, PISA.Net sends up-to-date information on a herd status to SNIRA/iDigital. This means that a movement document cannot be generated from the system unless the health status of a herd regarding bTB and bB is verified.
83. The CCA applies pre-movement testing according to points 4.4.4 to 4.4.6 of the 2015 bB and bTB Programmes. Cattle older than 12 months entering OTF, OBF and BB herds must be tested for bTB and bB within 30 days prior to movement. According to the CCA, pre-movement testing does not significantly impact the detection of new positive herds.
84. Animals from E2 fattening herds are only allowed to be moved directly to a slaughterhouse within the country. Since August 2015, the system does not allow the issuing of movement documents if the notified place of destination for animals from E2 fattening herds is not a slaughterhouse. The computer system also flags a warning if an animal arrives from E2 fattening herds.
85. The audit team noted that there is an improvement regarding the registration of pastures and movements to pastures compared to the previous Commission audit on the same subject, e.g. out of 181 pastures notified to the CCA, 164 (91%) are registered in SNIRA/iDigital. The CCA still does not fully comply with the requirements in Article 2 of Commission Decision 2001/672/EC, regarding data on cattle movements to common pastures.
86. Cattle over 12 months of age moving to common pastures must be tested within 30 days before the movement. In one region visited, the regional CA has detected irregularities in PISA.Net as regards the movement of cattle to common pastures (e.g. incomplete registration of movement in SNIRA/iDigital). The local CA organised a meeting with the farmers concerned and raised the issue of movement of cattle to common pastures and related pre-movement testing.
87. The CCA explained that incomplete registration of movements to common pasture might be partly due to the change in the movement registration data management system (e.g. from 10 August 2015 to 01 January 2016 all cattle herds were moved to the new system (SNIRA/iDigital). During that period some herds faced difficulties regarding registration of departures from common pastures in the new system.

#### *5.2.6 Re-occurrence of bTB and bB*

88. According to the data provided by the CCA, the percentage of OTF herds, with a previous history of bTB reactors, increased: there were 15.5% in 2015 compared with 6.9% in 2014 and 6.1% in 2013.

89. The CCA stated that, in many cases, re-occurrence of bTB was explained by the *Mycobacterium* contaminated environment shared by cattle and wildlife.
90. Reoccurrence of bB on OBF or BF herds that had a history of bB cases, also increased in 2015 compared to 2014 and 2013 (11.7%, 7.4% and 8%, respectively).

#### 5.2.7 Measures to detect the bTB infection in wildlife and to prevent its spread

91. The CCA has identified the risk areas for tuberculosis in large game (deer and wild boar). These high risk areas are mainly situated along the border with Spain. The CCA pointed out that overpopulation of large game plays an important role in the transmission of *Mycobacterium*. The CCA also identified high mixing patterns between cattle and large game as well as high contact rates during the dry season (when animals more frequently share feeding and watering points). This increases the likelihood of transmission of infection among animals (domestic and wild).
92. For two affected regions along the border with Spain, data provided by the CCA for the hunting season October 2014 to March 2015, indicate that 6.2% of hunted deer and 8.9% of wild boar had visible lesions. These were sampled by a veterinarian and *Mycobacterium bovis* was isolated in 81.7% and 66.2% of samples taken from deer and wild boar, respectively.
93. In two affected regions visited with a high burden of *Mycobacterium* infection in wildlife, the local CA intensified communication with hunters. The audit team noted that the CCA requires notification of hunting and on-the-spot inspection, by a trained veterinarian, of hunted animals. The veterinarian should take samples when he detects tuberculosis suggestive lesions in hunted deer and wild boar. Hunters must ensure that by-products of animals with lesions are safely disposed of in order to decrease environmental contamination. In one region visited, the audit team was informed that the number of large game with lesions in some areas has decreased, compared to the time before compulsory safe disposal of animal-by products from large game with tuberculosis suggestive lesions was introduced.
94. The CCA is exploring possibilities (e.g. fencing of feeding or watering points for cattle) to reduce the risk of transmission of *Mycobacterium* between wild and domestic animals and aims to initiate a research project to continue to study the *Mycobacterium* transmission dynamics.

#### 5.2.8 Training of field testers, slaughterhouse inspectors/workers, hunters

95. The last series of intensive training of all field testers on bTB testing (including practical training on the tuberculin injection technique and reading the test results) was implemented in 2010/2011.
96. In two regions, training of OPP veterinarians on bTB was carried out in 2012. In one region visited, training of sanitary inspectors on checks at the slaughterhouse was provided in 2013. The CCA informed the audit team that due to a lack of financial

resources, training of field testers, slaughterhouse inspectors/workers and official veterinarians was discontinued between 2013 and 2015. The CCA plans a new series of training on bTB testing (with practical exercises) in 2016.

97. In the Autonomous region visited, training of private veterinarians on bTB testing was organised at the beginning of their engagement in bTB testing in 2014. This was followed by multiple official supervision visit to their work on-the-spot, as described earlier in this report.

### Conclusions on implementation of bTB and bB control and eradication measures

98. Current measures to detect and eliminate both bTB and bB are effective in keeping the disease pressure low. Official procedures and instructions for all bodies involved in the implementation of the Programmes provide for a consistent approach and promote compliance in delivering the Programmes' measures.
99. The bTB testing strategy based on ICTT increases the proportion of non-infected animals that test negative, and consequently avoids removing too many healthy animals (e.g. false positives) and most likely results in missing a number of infected animals (false negatives). The insufficient use of other diagnostic tests in 2015 to supplement ICTT based diagnosis was a missed opportunity to improve the ability to identify and eliminate bTB infected animals as early as possible, and consequently to reduce the number of newly infected animals.
100. Exclusion of male animals from testing in some areas on mainland Portugal may have compromised the maintenance of the OTF herds in these areas in 2015. Also, exclusion of 25% of herds on the mainland from field testing, decreases the ability of the system to detect and eliminate the infected animals as early as possible. As a consequence, the time window for the infection to find another susceptible animal is prolonged as well as the time to finally eliminate the infection. This to some extent explains the continuous persistence of *Mycobacterium* infection in some areas. This approach decreases the confidence in the true level of bTB infection in cattle population on mainland Portugal and most likely increases the overall long term cost of the eradication efforts. Although animals from fattening herds are subject to slaughterhouse surveillance, due to its limitations, a high number of infected animals may not be detected at the slaughterhouse<sup>4</sup>.
101. The comprehensive procedures/requirements in place for notification of lesions detected at post-mortem inspection, allow the CA in the region of origin of cattle to be promptly aware and initiate the required actions. However, the delayed initiation of follow-up actions (in particular imposition of movement restrictions) seen, may have led to unnecessary spread of infection.
102. The high percentage of new bTB outbreaks detected at slaughterhouses proves the sensitivity and value of slaughterhouse surveillance in both protection of public health and detection of new bTB outbreaks. On the other hand, it also indicates that the infection goes undetected in the field, due to factors such as the ability of the test to correctly identify bTB infected animals, the quality of implementation of the ICTT, the absence of testing of male animals.
103. Current measures to combat bB on the mainland prevented the spread of the infection outside the affected areas. Measures did not solve the problem of long term persistence

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<sup>4</sup> Studies such as "Corner L. A. (1994). Post mortem diagnosis of *Mycobacterium bovis* infection in cattle. [Vet Microbiol.](#) 1994 May;40(1-2):53-63, indicate that up to 53% of infected cattle may go undetected at this stage.

of bB in some affected areas. The current approach of vaccinating mainly infected herds in affected areas in one region on the mainland, undermines the effectiveness of the vaccination to stop the spread of the *Brucella abortus* infection within the same epidemiological units.

104. The level of supervision of bTB testing provides for additional confidence in the reliability of implementation of the ICTT. However, its true impact on the ability of detection of reactor animals is uncertain, in particular when considering the number of animals with bTB lesions from OTF herds detected in slaughterhouses.
105. The system of issuing of movement documents electronically (since August 2015), largely provides for effective controls over cattle movements. In terms of E2 fattening herds this provides additional guarantees that the risk to other herds is reduced to negligible.
106. The fact that there is no systematic official supervision on implementation of cleaning and disinfection decreases confidence in the elimination of the infection in the contaminated environment.
107. Although data collected during epidemiological investigation are used in defining the measures on positive/contact herds, the restriction of these investigations to the point in time when the herd was first found infected, reduces the ability to generate additional data/information on possibly changing risks/exposure patterns, adverse practices or to verify already collected information.
108. The re-occurrence of positive animals in OTF, OBF and BF herds raise doubts regarding the completeness/quality or adequacy of the applied testing schemes. It also raises a question on the effectiveness of the measures implemented to eliminate the residual infection in such herds or continuous exposure to an unknown or undetected external source of infection.
109. As there is no assurance that indirect ELISA is used on a sample of milk taken from the milk collected from a farm with at least 30% of dairy cows in milk, there is no confidence in the representativeness of a herd by the milk sample taken for testing. As a consequence, the CCA cannot fully guarantee that non-compliance with this criteria has not resulted in reduced confidence in the quality/reliability of testing for the maintenance of a herd status as regards bB.

### **5.3 LABORATORY DIAGNOSTIC**

#### **Legal Requirements**

Article 4(2)(c) and 12 of the Regulation (EC) No 882/2004 and Annex C to Council Directive 91/68/EEC.

#### **Findings**

110. The CCA has an adequate laboratory capacity for bB and bTB testing, as required by Article 4(2)(c) of the Regulation (EC) No 882/2004.
111. The NRL for bTB and one regional laboratory for brucellosis are not accredited, contrary to what is required by Article 12 of the Regulation (EC) No 882/2004. The NRL for brucellosis and ten more laboratories in the country involved in brucellosis testing are accredited according to EN ISO/IEC 17025 on “General requirements for the competence of testing and calibration laboratories”.
112. The audit team noted that verification of the quality and consistency of the results produced by the NRL for bTB is ensured for molecular methods but there are no checks on the proficiency of culture-testing procedures nor for the serological method.
113. The NRL for tuberculosis participated in the European Union Reference Laboratory (EURL) for bTB ring trials for molecular methods. Laboratory staff also participated in training on polymerase chain reaction based method for detection and typing of *Mycobacterium* (so called spoligotyping) organised by the EURL for bTB. The NRL indicated that the spoligotyping was recently carried out only for scientific purposes. The CCA has not yet used the results on strains identified in 2015 to support the understanding of the epidemiology of the *Mycobacterium* infection. The CCA pointed out that they recognise the usefulness of spoligotyping (due to its rapidity in detecting the causative *Mycobacterium* and in providing epidemiological information about the strain identified) but financial constraints have prevented wider use of the method in recent years.
114. The bacteriological examination in the NRL for bTB is carried out according to Chapter 2.4.7. Bovine tuberculosis<sup>5</sup>, of the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (2009) of the World Organization for Animal Health. The audit team was informed by the laboratory staff, that for bacteriological examination both liquid and solid culture systems are used simultaneously. The incubation period for liquid culture system is six weeks. In case when liquid culture is positive within the six weeks, incubation of solid culture is discontinued. In cases where there is no growth in liquid culture during the six weeks, incubation of solid culture continues for six more weeks.
115. The staff in the NRL for bTB indicated that the gamma-interferon assay was intensively used from 2005 to 2014 but not in 2015.
116. According to the regional CA, the regional veterinary laboratory of the Azores is accredited for histopathology procedures.
117. The audit team noted that tuberculin is purchased centrally (from one producer) and distributed to the OPPs under conditions which ensure maintenance of the cold chain. The CCA fully relies on controls of the tuberculin preparation batches (such as sterility, safety, sensitising effect, potency, specificity, stability, etc.) carried out by the producer.

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<sup>5</sup> [http://www.oie.int/fileadmin/Home/eng/Health\\_standards/tahm/2.04.07\\_BOVINE\\_TB.pdf](http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.04.07_BOVINE_TB.pdf)

No verification of the product characteristics is carried out by the CCA, after its import or before use of the product in the field.

118. According to the CCA, from 2012 to 2015, the NRL for brucellosis did not organise inter-laboratory trials for other official laboratories carrying out testing within the framework of the bB Programme. The CCA also indicated that regional laboratories participated in an external proficiency testing schemes for serological tests used (e.g. PT0015 *Brucella abortus* CFT; PT0020 *Brucella abortus* RBT; PT0018 *Brucella abortus*, milk). According to the CCA, the NRL for brucellosis continues to have access to the results of the performance of the regional laboratories in the proficiency trials organised by an external laboratory. This is how they verify the quality of their work as regards serological testing.

#### **Conclusions on diagnostic support**

119. The absence of the accreditation or other external quality control to verify the NRL's performance/maintenance of the quality of the culture-testing and the serological method, does not contribute to building confidence in the quality and reliability of the test results it delivers, within the framework of the bTB eradication Programme.
120. For brucellosis, the current insight of the NRL into the performance of regional laboratories in proficiency testing schemes organised by the external laboratories, increases the confidence in the quality of the results of tests they perform. However, it cannot fully replace its obligation to organise inter-laboratory tests to add to an objective evaluation of the quality of work of the regional laboratories and to ensure that they continue to deliver results whose quality is acceptable to the EURL.

## **5.4 VERIFICATION OF IMPLEMENTATION, EVALUATION OF EFFECTIVENESS AND ADJUSTMENT OF MEASURES**

### **Legal requirements**

Point 4.4.11 of the bB and bTB Programmes for 2015, approved by Grant Decision approving national Programmes and associated funding, of 30 January 2015 (SANTE/VP/2015/ES/SI2.700826).

### **Findings**

121. The CCA has established mechanisms to monitor implementation of the bB and bTB Programmes as mentioned in point 4.4.11 of the 2015 bB and bTB Programmes.
122. The CCA analyses the performance (e.g. prevalence, incidence) and activity indicators (e.g. implementation rates) of the success of the implementation of the bB and bTB Programmes. The analysis of the implementation and progress of the Programmes is carried out at local, regional and central levels and they issue technical quarterly reports on the progress of coverage rates (at herd/animal levels) and on the disease evolution.

123. The regional CA carry out quantitative desk-top monitoring of the implementation of the Programme (e.g. checks in the PISA.Net if bTB testing and bB sampling and vaccination, when appropriate, were implemented, time intervals between the tests, time to slaughter of positive animals) aiming at detecting underperforming OPPs or areas/herds where the Programmes measures had not been implemented according to the plan.
124. There are on-the-spot official checks to evaluate the work of OPP veterinarians (less frequent than the desk-top verification of their work). In one region visited, the audit team noted that checks on OPPs in 2014 included control of the work of an OPP coordinating veterinarian, coordination, communication, guidelines provided to field veterinarians, record keeping, data uploading/entering to PISA.Net, equipment for ICTT, sampling, etc. In another region, no such control was carried out in 2015.
125. The internal audit department carries out planned audits to evaluate the effectiveness of the implementation of the bTB and bB eradication Programmes. Internal audits were carried out according to the "*Manual of Procedures: Internal Audits*" (which follows guidelines laid down in Decision 2006/677/EC and ISO 19011/2003 norm) and the "*Manual of Good Practices for the Auditing Services*". The most recent internal audit (2014/2015) evaluated performance of central and regional CA in a view of their duties within the framework of the bTB and bB eradication Programme. The internal audit 2014/2015 found that, since 2010, performance of both Programmes has significantly improved.
126. The internal audit 2014/2015 issued a number of recommendations to the CCA including: revision of "*Manual for the collection of samples under the brucellosis eradication Programmes*" and "*Written procedures for implementation of the bB and bTB eradication Programmes*"; verification of compliance with time between two testings; identifying the technicians working for the bTB and bB Programme; strengthening controls at slaughterhouses; more involvement of the CCA in the epidemiological investigations; development of instructions to carry out such an investigation; adoption of procedures for official controls and testing in fattening herds that participate in the intra-EU trade. According to the information provided by the CCA, the majority of the recommendations issued by the internal audit 2014/2015 have been satisfactorily addressed at the time of this audit of the Commission services.
127. The audit team noted that data such as results of ICTT and laboratory testing for individual animals and slaughterhouse surveillance are collected and stored/available in an electronic format. These data have not been sufficiently used (analysed) to identify gaps/additional measures or better target official actions to ensure improvement. The audit team noted that there was no wider, critical, objective analysis of reasons for the slowing down of progress in the eradication of bTB and bB.
128. The CCA has not evaluated the fitness for purpose of the use of the ICTT under local circumstances (e.g. what is gained and what is lost in terms of the removing/missing of

infected animals, will this strategy result in detection and elimination of the majority of infected animals in the short term and does it contribute to the acceleration of the eradication). The CCA has not also analysed the impact of excluding the majority of animals intended for fattening from bTB testing, on the persistence of infection.

129. The CAs did not carry out analysis aimed at explaining re-occurrence of new positives in officially OTF, OBF or BF herds. Similarly, they did not assess the expected and obtained sample submission rates per slaughterhouse.

130. There is no analysis of slaughterhouse cases with their bTB testing history, herds/environments of origin or comprehensive analysis of epidemiological data. Molecular epidemiology (used in the past as part of a scientific project) was not used in 2015 (some typing of *Mycobacterium* was carried out for the research purposes, but data were not used by the CCA) for understanding the transmission dynamics of *Mycobacterium* in cattle and wildlife (e.g. large game).

**Conclusions on verification of implementation, evaluation of effectiveness and adjustment of measures**

131. Tools currently used to verify implementation of the bTB and bB eradication Programmes (e.g. desk-top verification in PISA.Net) provide monitoring to ensure quantitative evidence as to if and when the Programmes' activities and measures have been implemented as required. This also promotes compliance with coverage of herds/animals and the deadlines for the implementation of the Programme measures.

132. Internal audits provided for identification of weaknesses and gaps where improvement was needed to ensure the Programmes' arrangements were implemented as planned. The smooth response of the CCA to addressing the recommendations of the internal audit, proves its flexibility and readiness to work towards improvement.

133. In the absence of identification and objective analysis of the true impact of factors preventing a faster progress, identification of tailor-based corrective actions, measures and tools as well as subsequent adjustments to the Programmes, the time needed to eliminate bB and bTB in the country will most likely continue to be uncertain.

**5.5 FOLLOW-UP**

The table below summarizes the follow-up to the relevant recommendation (not closed in the meantime) made in report DG(SANCO)2009-8248.

No	Recommendation	Assessment
1	To register pastures for cattle grazing during summer located in mountain areas and to control cattle movements there as required by Commission Decision 2001/672/EC.	Partly addressed. See finding No 85.

## 6 OVERALL CONCLUSIONS

The competent authorities implemented many of the measures contained in the 2015 eradication Programmes. They availed of comprehensive and functional data management systems which were used to support planning and controls. The systems provide data to analyse the progress of the tuberculosis and bovine brucellosis eradication efforts.

The central competent authority considered epidemiological indicators and knowledge gathered during the monitoring and evaluation of the effectiveness of the bovine tuberculosis and bovine brucellosis Programmes when setting the Programmes' objectives and annual targets. The measures in place to detect and eliminate both bovine tuberculosis and bovine brucellosis are effective in keeping the diseases prevalence low.

The weaknesses observed include insufficient use of epidemiological data, lack of assessment of fitness for purpose of the current bovine tuberculosis testing scheme, absence of knowledge regarding the impact of not testing animals in fattening herds, absence of analysis of the high rate of new bovine tuberculosis positive herds detected at slaughterhouses. Insufficient analysis and learning from the data prevented objective identification of the most effective and efficient measures to achieve the objectives of the Programmes and to accelerate the eradication of bovine tuberculosis and bovine brucellosis in the country. Also, the exclusion of male animals from testing in some areas in the mainland of Portugal, may have compromised the maintenance of the officially tuberculosis free herd status in these areas during 2015.

Acceleration of the eradication of bovine tuberculosis and bovine brucellosis can hardly be expected without critical and objective analysis of the factors that substantiate the persistence and spread of the bovine tuberculosis/bovine brucellosis infection and those that may explain why progress in the elimination of the diseases has slowed down in the recent years. This in particular concerns: (i) the ability of the current field testing scheme to detect the majority of positive animals, (ii) the impact of not testing non-breeding male animals in breeding herds and the exclusion of more than 10000 fattening herds from bovine tuberculosis field testing, (iii) the impact of individual performance and reliability of the work of the field testers, (iv) the impact of non-vaccination of all at risk herds in a bovine brucellosis infected area, (v) the speed and completeness of implementation of risk reduction measures in positive herds. The absence of such analysis will continue to affect understanding of which measures are the most appropriate to achieve the objective of eradication of bovine tuberculosis and bovine brucellosis in Portugal within the established target period.

## 7 CLOSING MEETING

The closing meeting was held in Lisbon on March 9, 2016. The audit team presented to the CCA the main findings and preliminary conclusions of the audit. During the meeting the

CCA did not expressed major disagreement with majority of presented findings and preliminary conclusions.

## 8 RECOMMENDATIONS

No.	Recommendation
1.	<p>To ensure that, when used, the derogation for testing males for tuberculosis is applied in accordance with Annex A (I)(2)(c) to Directive 64/432/EEC, and in particular that derogations for testing male animals for fattening are only applied to those coming from officially tuberculosis-free herds, located within an isolated epidemiological unit and that will go directly for slaughter, for regions where the average — determined on 31 December of each year — of the annual percentages of bovine herds confirmed as infected with tuberculosis is not more than 1 % of <b>all</b> herds within the defined area during the two most recent annual supervisory periods.</p> <p><i>Recommendation based on conclusion No.:100.</i> <i>Associated findings No.:71, 72.</i></p>
2.	<p>To ensure that after the slaughter of animals, in which presence of brucellosis or tuberculosis, has been officially established, sheds and other herd quarters, and all containers, equipment and other articles used for the animals are cleaned and disinfected under official supervision, in accordance with the instructions given by the official veterinarian, as required in Article 8(1) and 16(1) of Directive 78/52/EEC.</p> <p><i>Recommendation based on conclusions No.:106, 108.</i> <i>Associated findings No.:50, 54.</i></p>
3.	<p>To ensure that the ELISA test is used on a sample of milk taken from the milk collected from a farm with at least 30% of dairy cows in milk, as required in Annex (C)(2.2.3.4) to Directive 64/432/EEC.</p> <p><i>Recommendation based on conclusion No.:109.</i> <i>Associated finding No.:79.</i></p>
4.	<p>To ensure that laboratories that carry out the analysis of samples taken during official controls are designated, assessed and accredited by the CA as required by Article 12 of Regulation (EC) No. 882/2004.</p> <p><i>Recommendation based on conclusion No.:119.</i> <i>Associated finding No.:111.</i></p>

### ANNEX 1 LEGAL REFERENCES

[This table be will be added separately via the audit properties]

The competent authority's response to the recommendations can be found at:

[http://ec.europa.eu/food/fvo/rep\\_details\\_en.cfm?rep\\_inspection\\_ref=2016-8773](http://ec.europa.eu/food/fvo/rep_details_en.cfm?rep_inspection_ref=2016-8773)

## ANNEX 1 – LEGAL REFERENCES

Legal Reference	Official Journal	Title
Reg. 882/2004	OJ L 165, 30.4.2004, p. 1, Corrected and re-published in OJ L 191, 28.5.2004, p. 1	Regulation (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules
Reg. 652/2014	OJ L 189, 27.06.2014, p. 1-32	Regulation (EU) No 652/2014 of the European Parliament and of the Council of 15 May 2014 laying down provisions for the management of expenditure relating to the food chain, animal health and animal welfare, and relating to plant health and plant reproductive material, amending Council Directives 98/56/EC, 2000/29/EC and 2008/90/EC, Regulations (EC) No 178/2002, (EC) No 882/2004 and (EC) No 396/2005 of the European Parliament and of the Council, Directive 2009/128/EC of the European Parliament and of the Council and Regulation (EC) No 1107/2009 of the European Parliament and of the Council and repealing Council Decisions 66/399/EEC, 76/894/EEC and 2009/470/EC
Dir. 64/432/EEC	OJ 121, 29.7.1964, p. 1977-2012	Council Directive 64/432/EEC of 26 June 1964 on animal health problems affecting intra-Community trade in bovine animals and swine
Dir. 78/52/EEC	OJ L 15, 19.1.1978, p. 34-41	Council Directive 78/52/EEC of 13 December 1977 establishing the Community criteria for national plans for the accelerated eradication of brucellosis, tuberculosis and enzootic leukosis in cattle
Dir. 77/391/EEC	OJ L 145, 13.6.1977, p. 44-47	Council Directive 77/391/EEC of 17 May 1977 introducing Community measures for the eradication of brucellosis, tuberculosis and leucosis in cattle
Dec. 2008/341/EC	OJ L 115, 29.4.2008, p. 44-46	2008/341/EC: Commission Decision of 25 April 2008 laying down Community criteria for national programmes for the eradication, control and monitoring of certain animal diseases and zoonoses

Dec. 2001/672/EC	OJ L 235, 4.9.2001, p. 23-25	2001/672/EC: Commission Decision of 20 August 2001 laying down special rules applicable to movements of bovine animals when put out to summer grazing in mountain areas
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