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HEALTH AND FOOD SAFETY DIRECTORATE-GENERAL

**Directorate G – Veterinary and International Affairs**  
Unit G5 Food chain and animal health expenditure

**SANTE/11645/2015**

**REPORT OF THE**

**“Brucellosis”**

**TASK FORCE SUB-GROUP**

**Meeting held in**

**Split**

**Croatia**

**17-18 June 2015**

**REPORT OF THE  
MEETING OF THE BRUCELLOSIS SUB-GROUP OF THE  
TASK FORCE FOR MONITORING DISEASE ERADICATION  
HELD IN SPLIT, Croatia, 17-18 JUNE 2015**

**PARTICIPANTS:** see Annex I

**AGENDA:** see Annex II

**LOCATION:** Croatian Chamber of Economy, Obala A. Trumbića 4, Split, Croatia.

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**INTRODUCTION - Objectives of the EU-Task Force Brucellosis sub-group**

The scope of the visit is to share information and experience of the expert members with the hosting colleagues as well as to give technical support if needed or requested by the visited country. After the visit a report is issued by the experts, based on the information provided on the spot by the country and on the findings verified directly by the experts themselves during the visit.

The main goal of the Task Force is to leave at disposal of the visited country the expertise of its expert members, in the light to give a contribution, with an external independent technical assessment, in the evaluation of strength and weaknesses of strategies and measures in place for the control and eradication of the disease concerned.

Conclusions and recommendations are formulated from a general point of view and are proposed in the report with the main scope to be a basis for the Veterinary Services of the visited country to reflect on the possible improvement of different aspects of the control and eradication programme for the disease concerned. The country visited may amend the programme according to what it is suggested by the Task Force, or it may choose other approaches, also in consideration of social and economic factors that may influence the success of the measures adopted, and which are not in the remit of the Task Force.

Conclusions and Recommendations are related to the picture of the situation as resulted during the visit based on the information provided by the country visited on the spot. Further developments of the country's situation may be the subject of a following visit aimed to get updated information and new feedback from the Veterinary Services.

The reports of the TF held in different countries in the last years are published on the following website: [http://ec.europa.eu/food/animal/diseases/index\\_en.htm](http://ec.europa.eu/food/animal/diseases/index_en.htm)

## DAY 1

### 1. Structure and organization of the Veterinary Services – Veterinary and Food Safety Directorate (VFSD).

*Dr. Tomislav Kiš, Head, Animal Health Protection Sector, Veterinary and Food Safety Directorate (VFSD), Ministry of Agriculture (MA).*

The speaker presented some general figures about the main domestic animal population in Croatia in 2015 (**Table 1**).

**Table 1. Animal Population in Croatia, 2015.**

<b>Animal Species</b>	<b>No of Heads</b>	<b>No of Farms</b>
<b>Cattle</b>	460,000	33,500
<b>Small ruminants</b>	700,000 <i>Sheep: 625,000</i> <i>Goats: 75,000</i>	22,000
<b>Pigs</b>	1,750,000	120,000
<b>Equidae</b>	24,000	4,300
<b>Poultry</b>	2.2 million laying hens 41 million broilers	

As far as the administrative subdivision are concerned (**Fig. 1**), Croatia is divided into five regions (Central Croatia, Eastern Croatia, Northern Littoral, Highland Croatia and Southern Littoral), with a total of 21 counties (including the City of Zagreb). Local governments are organized in 127 towns and 429 municipalities. The Country shares borders with Slovenia in the north-west, Hungary in the north-east, Serbia and Bosnia-Herzegovina in the south-east, and Montenegro in the south. The different components of the veterinary system in Croatia are shown in **Figure 2** (<http://croatia.eu>).

### **LEGAL FRAMEWORK**

- **Veterinary Act** (OG 82/13, 148/13)
- **Food Act** (OG 81/13, 14/14)
- **Animal Protection Act** (OG 135/06, 37/13, 125/13)
- **Act on Veterinary Medicinal Products** (OG 84/08, 56/13, 94/13, 15/15)
- **Act on Food Hygiene and Microbiological Criteria for Food** (OG 81/13, 14/14)
- **Act on official controls** performed in accordance with the requirements of food and feed law, animal health and animal welfare rules (OG 81/13, 14/14)
- Approximately 290 implementing ordinances

The implementation of acts/regulations/ordinances is the responsibility of both central and local levels. At central level, the implementation is carried out by the Ministry of Agriculture – Veterinary Food Safety Directorate (VFSD). At local level, the implementation is carried out by State Veterinary Inspectors (SVI), working in the regional offices and their branches, and by private veterinarians working for the Authorized Veterinary Organizations (AVOs).



Figure 1. Croatian territory, regions and borders. (<http://croatia.eu>)

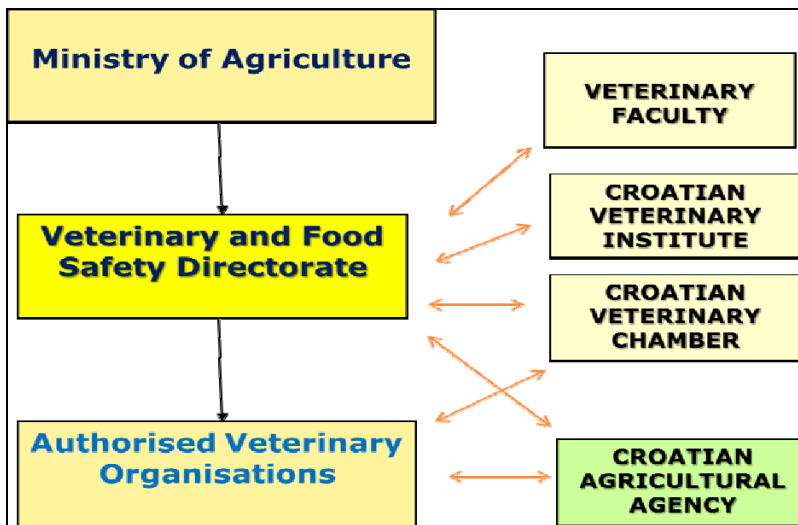


Figure 2. Structure of Veterinary Services in Croatia.

**Veterinary Faculty:** there is one veterinary faculty in the country, established in 1924 and located in Zagreb. The faculty hosts a total of 658 students, with an annual number of 50 graduates. Link: <http://www.vef.unizg.hr/>

**Croatian Veterinary Chamber:** established in 1992, is keeping the register of veterinarians. It includes 3,643 registered members and 2,267 active licences (1,580 men, average age 50 years, and 687 women, average age 44 years) Link: <http://www.hvk.hr/>

**Croatian Veterinary Institute (CVI):** the CVI is composed by the central veterinary laboratory in Zagreb and four regional veterinary laboratories located in Split, Vinkovci, Rijeka and Križevci. CVI provides laboratory services for animal health programs, official controls of animal health, food of animal origin and feed. The CVI and its regional laboratories are accredited according to ISO 17025 (34 laboratories and 173 methods). Link: [www.veinst.hr](http://www.veinst.hr)

**Authorised Veterinary Organisations (AVOs):** The AVOs are authorized by the Veterinary and Food Safety Directorate (VFSD), according to the provision included in the Veterinary Act, with a five-year contract. Currently, authorized AVOs are 129, employing 958 authorized veterinarians.

**Veterinary and Food Safety Directorate (VFSD):** within the Ministry of Agriculture, VFSD is the competent authority responsible for animal health, veterinary public health, food safety, veterinary/medical products, and animal welfare. The staff currently includes 73 people employed in the headquarters, 204 State Veterinary Inspectors, and 23 Border Veterinary Inspectors. The sectors of VFSD are four:

- a) **Animal Health Protection Sector**
- b) **Veterinary Public Health and Food Safety Sector**
- c) **Veterinary Inspection Sector**
- d) **Administrative, EU and Financial affairs Sector**

Link: [www.veterinarstvo.hr](http://www.veterinarstvo.hr) and [www.mps.hr](http://www.mps.hr)

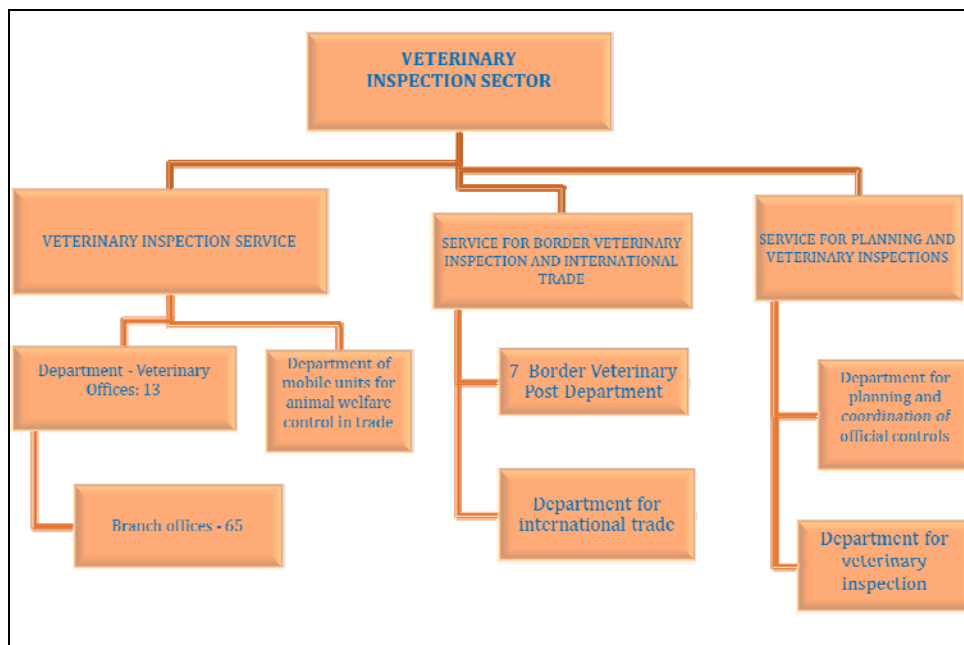
**a) Animal Health Protection Sector:** the activities performed by this Sector are the control and eradication of animal diseases (including zoonoses), contingency planning, crisis management, animal welfare, financing of measures on early detection and eradication of animal diseases, activities related to identification of animals and registration of movements.

It also performs tasks regarding the organization of veterinary service and development and maintenance of the Central Veterinary Information System (CVIS), designed to provide a unified system of all registers and software in the veterinary field.

**b) Veterinary Public Health and Food Safety Sector:** the Sector is competent for general food safety, safety of food of animal origin and feed, veterinary medicinal products (VMP) and veterinary medical devices, monitoring of residues, and animal by-products (ABP). The Sector coordinates RASFF activities at national level, manages activities related to the national residues control plan (NCRP), the residue programme for feed, and the monitoring programme for live bivalve molluscs (LBM). It is responsible also for keeping the different registers foreseen by the legislation (approved establishments, registered establishments, ABP business operators, VMPs operators, etc.).

**c) Veterinary Inspection Sector:** the Sector includes the following three departments (Fig. 3):

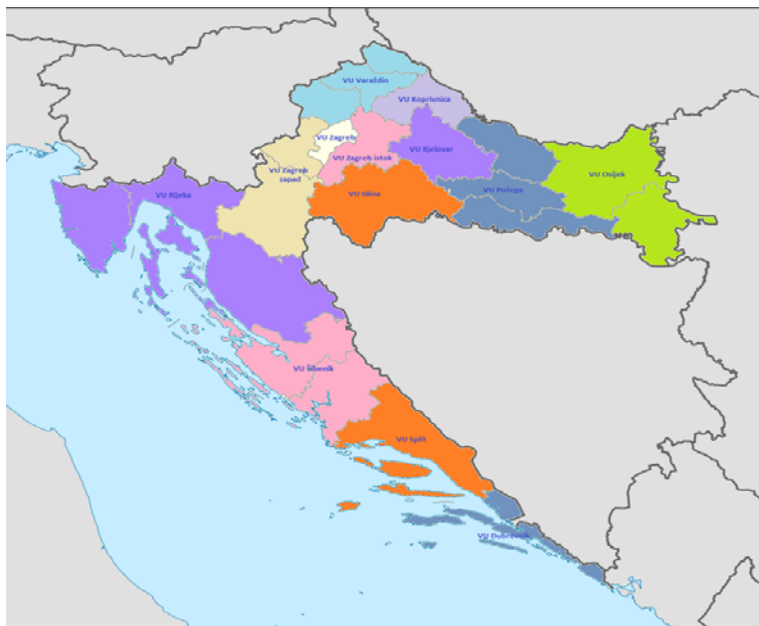
- c1) Veterinary Inspection Service
- c2) Service for Planning and Veterinary Inspection
- c3) Border Veterinary Inspection and International Trade Service



**Figure 3. Structure of the Veterinary Inspection Sector in Croatia.**

c1) The Veterinary Inspection Service is composed by 13 Regional Veterinary Inspection Departments (veterinary offices) located in City of Zagreb, Zagreb East, Zagreb West, Glina, Varaždin, Bjelovar, Koprivnica, Osijek, Slavonski Brod,

Šibenik, Rijeka, Dubrovnik and Split (**Fig. 4**), plus a mobile unit for control of animal welfare during transport. The 13 Regional Veterinary Inspection Departments have 65 branch offices. They are responsible for the implementation of official controls on animal health, animal welfare and production, and also on placing on the market of food and feed.



**Figure 4. The thirteen regional veterinary offices of Croatia.**

*c2) The Service for planning and veterinary inspection* consists of two departments:

- **Department for planning and coordination of official controls plans**, which coordinates and harmonizes legislation in the field of official controls; drafts guidelines, drafts annual and multiannual control plan, coordinates drafting of procedures for official controls; keeps the registry of official and reference laboratories; coordinates and communicates in the fields of food safety.
- **Veterinary inspections department**, which performs supervision of the work carried out by veterinary inspectors at local level with the aim of establishing the quality of the performed inspection, improving and making uniform the work of the inspection service. The department participates in drafting the annual and multiannual control plan, the written procedures and the check-lists for official controls. When needed, the department may also perform official controls; it also prepares all the documentation in relation to appeals.

*c3) Border Veterinary Inspection and International Trade Service* is organized in eight departments:

- seven border inspection posts (BIPs) departments, responsible for veterinary checks and controls at BIPs on consignments of animals, products of animal origin, feed of animal and non-animal origin and other objects that may transmit infectious or parasitic diseases or endanger human or animal health.
- One International Trade Department, responsible for legal and administrative activities in the field of international trade (import and Intra-Community trade).

**d) The administrative, European and Financial Affairs Sector** is responsible for veterinary expenditures, for preparing an annual budget plan for VFSD, for the monthly payments of veterinary measures taken by AVOs (when they are paid from state budget), for preparing solutions for compensation to the owners, for preparing the monthly fee calculations (invoices) for FBO (for the official controls carried out), for preparing the annual training plan (including organisation of education in the veterinary and food safety fields).

## **2. Structure and national distribution of bovine and sheep and goat livestock and the National Animal Identification and Registration System and Database.**

*Dr. Vladimir Čačinović, Veterinary and Food Safety Directorate (VFSD).*

The Animal Health Division of the VFSD is the Competent Authority for the implementation, coordination and surveillance of the Animal Identification System in Croatia. The Veterinary and Food Safety Directorate sets the requirements according to national legislation and delegates some tasks to Authorised private Veterinary Organisations (AVO) and to the Croatian Agriculture Agency (CAA).

The current EU legislation on animal identification and on databases is implemented in the Croatian legislation with the following Veterinary Acts:

- Ordinance on the conduction of obligatory identification and registration of bovine animals (OG 108/13);
- Ordinance on the conduction of obligatory identification and registration of ovine and caprine animals (OG 111/07);
- Ordinance on the obligatory identification and registration of porcine animals (OG 51/07);
- Ordinance on the equine identification and registration (OG 123/09).

All cattle, sheep and goats holdings are registered in the database with a unique holding code. The compulsory identification of cattle, sheep, goats and pigs is carried out by the



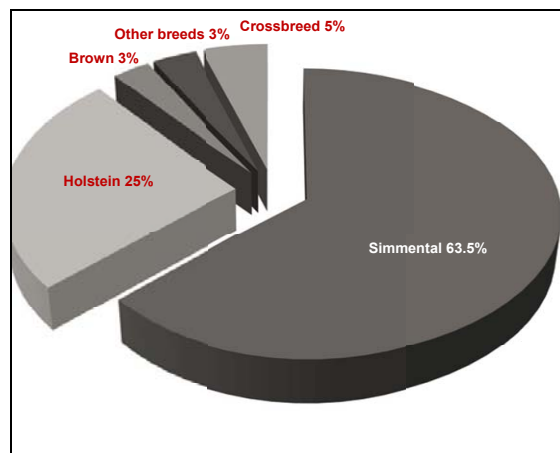
AVOs, the CAA and the farmer (animal keeper). AVOs are authorized also to carry out movement registrations and annual veterinary checks after specific training of the personnel and following clear procedures (check lists, leaflets, use of specific software). The farmer (animal keeper) can also carry out the animal identification in his own herd. This is normally applied in case of farms with a high number of animals. The animal keeper is responsible for the registration of the animals in the national database and in his own register kept on the farm. The updating of the database at animal level for each holding is done annually at the same time as the compulsory annual blood sampling.

### 2.1. Distribution of bovine livestock

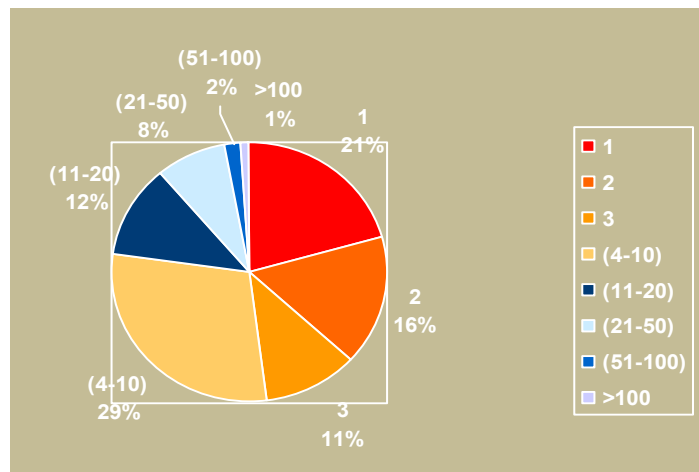
Cattle are identified in accordance with Regulation 1760/2000/EC, by means of two identical ear tags placed on both ears within 20 days after birth or, in any case, before leaving the original holding. A passport for each animal is issued after communication of birth. Movements of cattle are allowed only if they are accompanied by the individual passport. As foreseen in Regulation 1760/2000/EC, cattle introduced from Member States maintain their EU ear tag, while cattle coming from third countries are remarked. The movements of animals are registered in both holdings, *i.e.* in the register of the sending holding, as well as in the register of the receiving holding.

The total number of cattle in Croatia is around 450,000 animals. About 63.5 % of cattle population belongs to the Simmental breed, 25 % to Holstein and 3% to Brown Swiss, the remaining are crossbreed or other breeds (**Fig. 5**).

The herd size of cattle farms is very small, about 21% of farms own only one animal and only 1% of herds have more than 100 cattle (**Fig. 6**). Croatia follows the current trend seen in Europe that is a reduction of the number of herds and a parallel increase of herd size.



**Figure 5. Croatia, distribution of cattle breeds, 2015.**

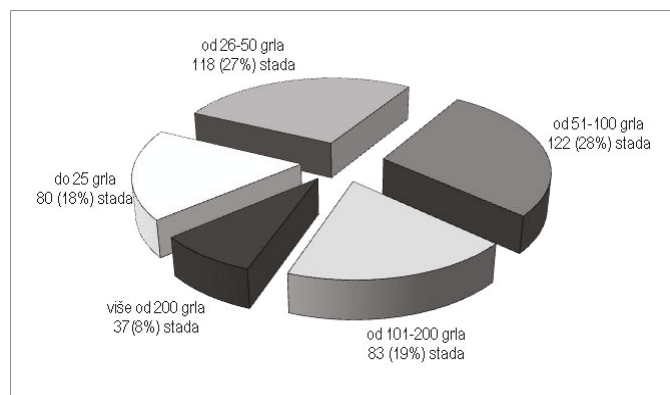


**Figure 6. Croatia, cattle herd size, 2015.**

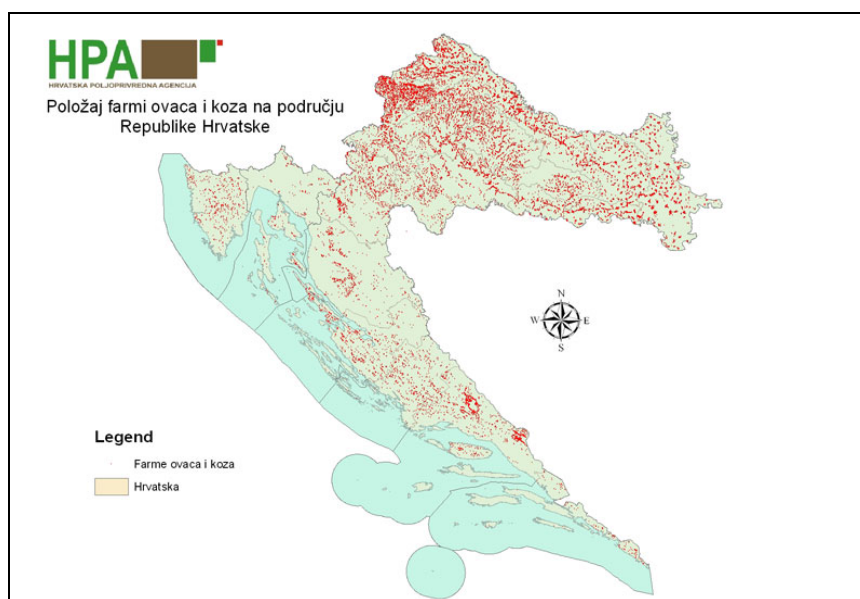
## 2.2. Distribution of sheep and goat livestock

The total population of sheep in Croatia is around 625,000 heads and around 74,000 heads for goats. Identification of sheep and goats is carried out in accordance to Regulation 21/2004/EC. Since 2009, all sheep and goats are identified with an electronic identification system in accordance with EU legislation. Sheep and goats are identified with one ruminal bolus (containing a microchip) with one ear tag in addition. Animals must be identified within a period of 6 month after birth and, in any case, before leaving the original holding. The receiving holding is responsible for movement notification. The movements of animals are registered in both holdings, *i.e.* in the register of the sending holding, as well as in the register of the receiving holding.

The main flock size is very small. More than 70% of flocks consist in less than 100 sheep (**Fig. 7**). Most of flocks are located on the mainland near to Slovenia and Serbia (**Fig. 8**).



**Figure 7. Croatia, size of sheep flocks (herdbook).**



**Figure 8. Croatia, distribution of sheep and goats farms.**

### **3. Bovine brucellosis eradication programme in Croatia: presentation of the national legislation and of the results of the brucellosis control programme implementation – Overview of the evolution, current situation and perspectives.**

*Dr. Martina Rubin, Veterinary and Food Safety Directorate (VFSD).*

The legal framework of the programme is the Veterinary Act (OG, 82/13, 148/13), which is further regulated by the following provisions:

- *Ordinance on measures for control and eradication of bovine brucellosis (Official Gazette, 112/13)*, that basically define suspect and confirmed cases of bovine brucellosis and lays down the measures to be implemented in case of disease suspicion or confirmation.
- *Ordinance on animal health requirements applicable for trade in cattle and swine (OG, 71/12)* - aligned with Directive 64/432/EEC, main framework for granting the status of bovine brucellosis free herds and territories (county based).
- *Annual Order on measures to protect animals from infectious and parasitic diseases and the financing thereof (OG)* – that establishes basic and main measures for the implementing year (screening for brucellosis, pre-movement testing, obligation of abortion notification).
- The Annual Programme, providing standard rule of implementation for the year of concern.

Since 2011, the programme has been aligned with 64/432/EEC directive and all breeding animals older than 12 months have been screened annually with RBT. All RBT positive samples are retested in NRL (CVI, Zagreb) with RBT (again), CFT, iELISA and cELISA. Milk ELISA was also used for OBF qualification in 2011 in dairy herds having more than 10 animals. Since 2000, pre-movement tests have been compulsory for all breeding cattle older than 12 months. From 2015 it is not mandatory any more for movements between OBF herds. Abortion notification is compulsory and all reported abortions are investigated in bacteriology. A suspect case is defined by a RBT positive result confirmed either by CFT or iELISA or cELISA. A confirmed case is defined by the isolation of *Brucella*. Suspect animals are either slaughtered and sampled for bacteriological investigation or retested after 35-45 days or tested in brucellin skin-test (BST). Decision is based on the status, history and situation in the herd, in accordance with the Ordinance. In case of abortion or unknown status of the herd, the positive animal is slaughtered.

The flowchart of decision making according to test results is shown on **Figure 9**.

Herd and animal control coverage in 2013 and 2014 and the proportion of OBF qualified herds from 2011 to 2014 are shown on **Figures 10** and **11**, respectively. For 2011 and 2012 data on eligible bovine population were not available, so herd and animal testing coverage in 2011 and 2012, which are 87 % and 90 % for herds, and 51% and 44% for animals, are compared to the total bovine population, including non-eligible herds and animals, so testing coverage is higher than previously mentioned. For 2013 and 2014 also, some proportion of non-eligible animals is included in the number of animals (animals in fattening units) which is compared to tested animals. Continued improvement of available data analysis is recognised as an important task and goal for VFSD.

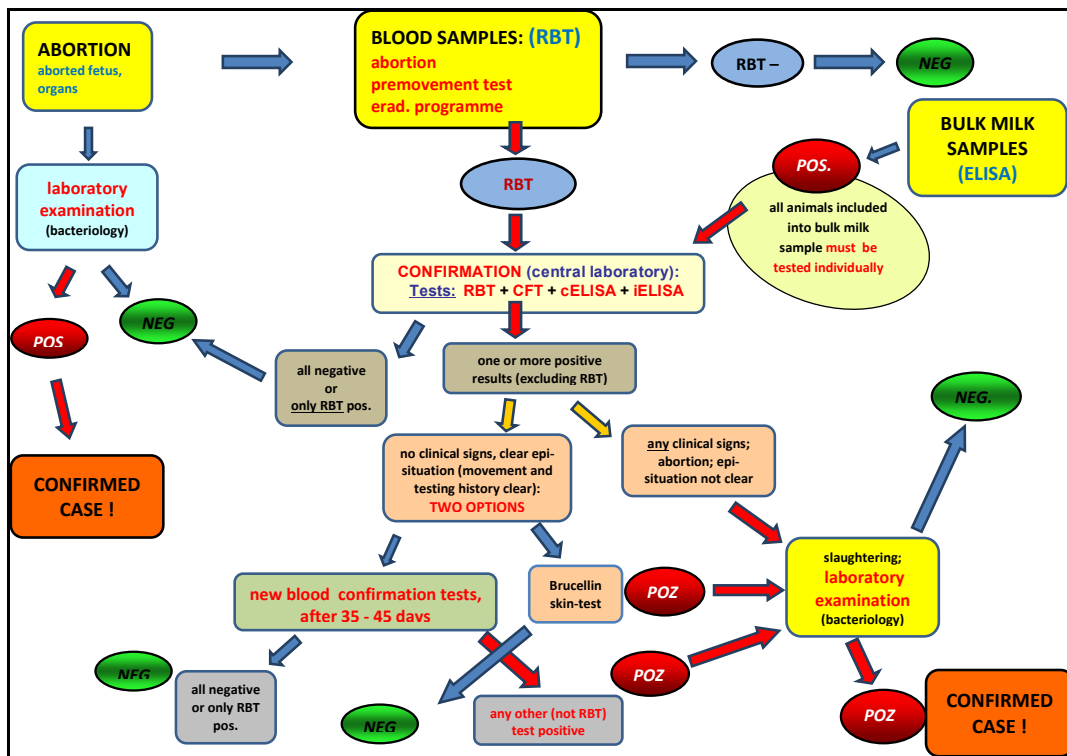


Figure 9. Flowchart of decision making according to test results.

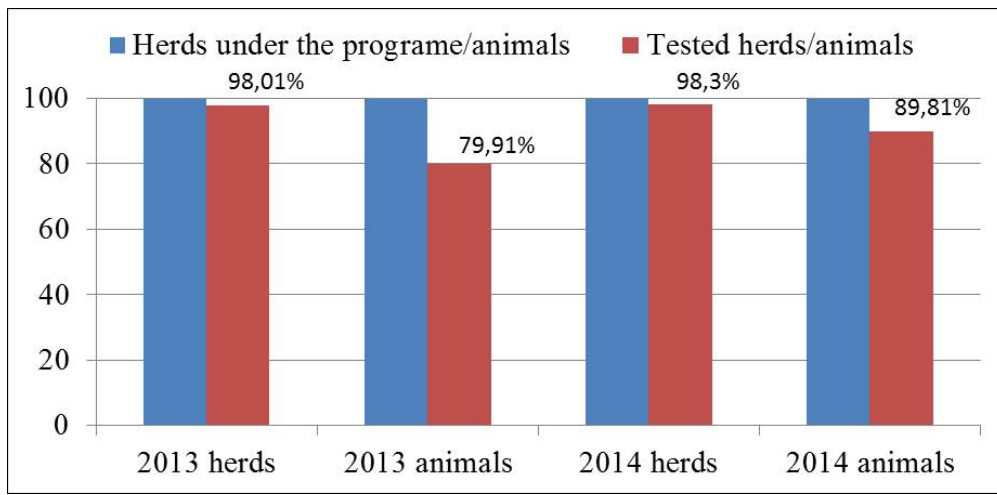


Figure 10. Bovine herd and animal control coverage in Croatia (2013-2014).

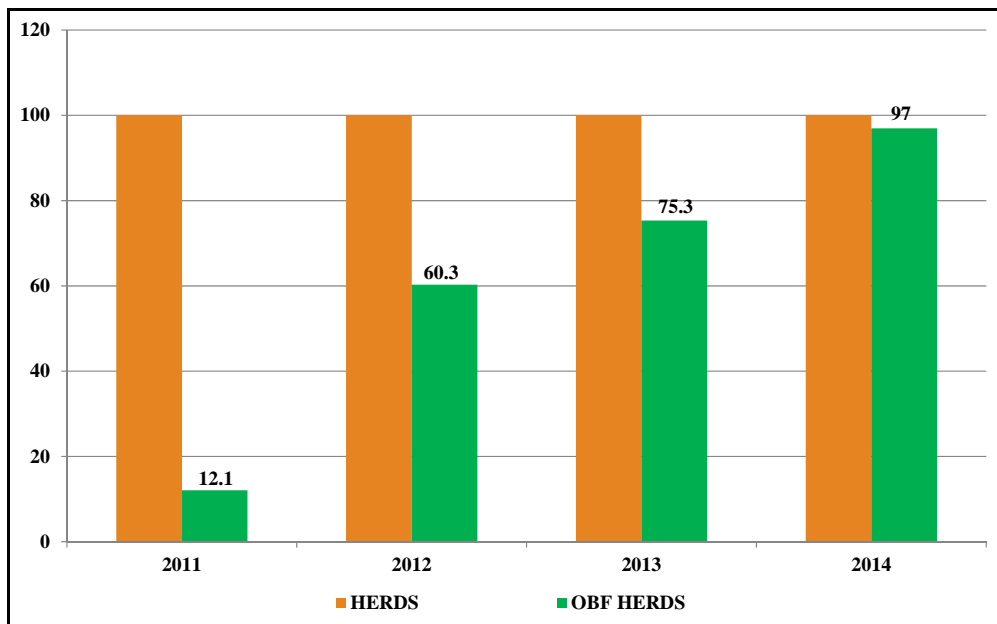


Figure 11. Proportion of OBF qualified herds in Croatia (2011-2014).

#### Suspect cases occurred in 2013-2015.

- 2013: one dairy herd in Istria County; 1 animal positive on RBT, CFT and cELISA among 167 animals tested. The positive animal was slaughtered and bacteriological examination resulted negative.
- 2014: 4 herds (8 animals) located in 3 counties (**Fig. 12a**).
- 2015: 14 herds (18 animals) located in 7 counties (**Fig. 12b**). All 18 herds were OBF herds with no previous cases of serologically positive animals or abortions.

Results of serological and bacteriological investigations allowed ruling out infection (Table 2).

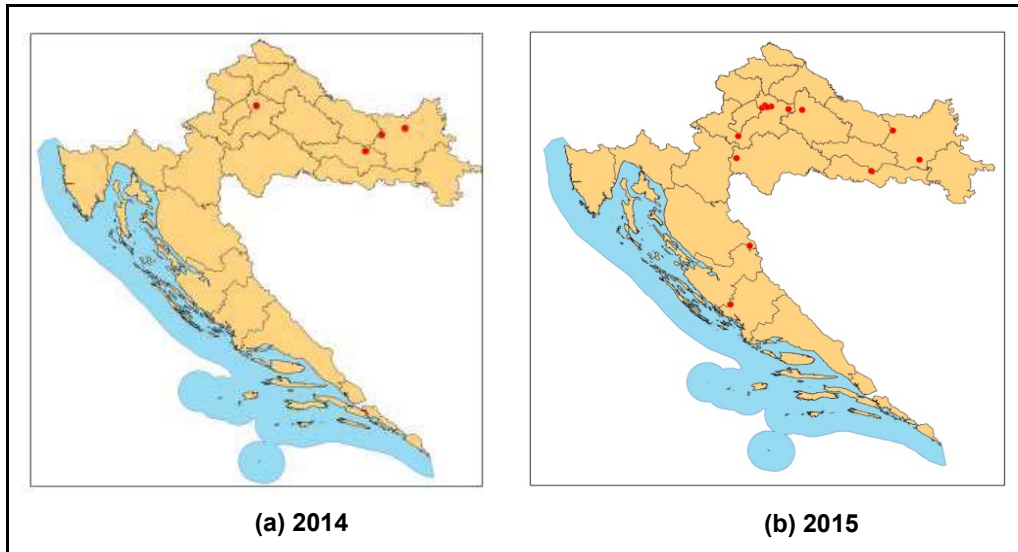


Figure 12. Bovine brucellosis suspect cases in 2014 and 2015 in Croatia.

**Abortions notified in 2008-2014**

All reported abortions were investigated in bacteriology during the period (Fig. 13); all were negative for *Brucella* spp. In order to improve the abortion notification, the VSFD has edited an educational leaflet, published on its website and which will be distributed to animal keepers. Until the end of 2015, the VSFD plans to increase efforts and resources in order to reach 99.8% OBF herds.

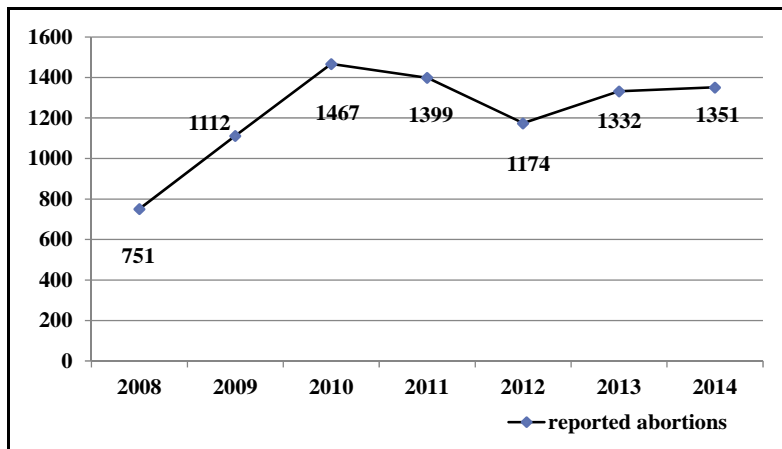


Figure 13. Abortions notified in 2008-2014 in Croatia.

Table 2. Bovine brucellosis suspect cases in 2014 and 2015 in Croatia. Tests performed to rule out *Brucella* spp. infection.

Year	County	Animals tested in the herd (No.)	Positive animals (No.)	Results
2014	Zagreb	118	2	Animals slaughtered, bacteriological investigations negative
	Požega slavonia	285	4	
	Osijek baranja	150	1	
		14	1	Animal retested after 45 days, negative
2015	Zagreb	4	2	Suspect animals and some seronegative animals subjected to brucellin skin test
		5	1	
		8	1	
		203	2	
		5	1	
		6	1	
		22	1	
	Lika senj	16	1	Brucellin skin test - all
	Osijek baranja	30	2	
		6	1	Animal slaughtered (positive on EBL)
	Požega slavonia	34	1	Suspect animals and some seronegative animals subjected to brucelin skin test
	Sisak moslavina	9	1	
	Bjelovar bilogora	45	1	
Šibenik knin	35	2	Brucellin skin test - 14 animals	



**4. Sheep and goat brucellosis eradication programme in Croatia: presentation of the national legislation and of the results of the brucellosis control programme implementation - Overview of the evolution, current situation and perspectives.**

*Dr. Martina Rubin, Veterinary and Food Safety Directorate (VFSD).*

The legal framework of the programme is the Veterinary Act (OG, 82/13, 148/13), which is further regulated by the following provisions:

- *Ordinance on measures for control and eradication of sheep and goat brucellosis* (Official Gazette, 114/14), that basically defined case of disease suspicion, case of disease confirmation and lays down the measures to be adopted.

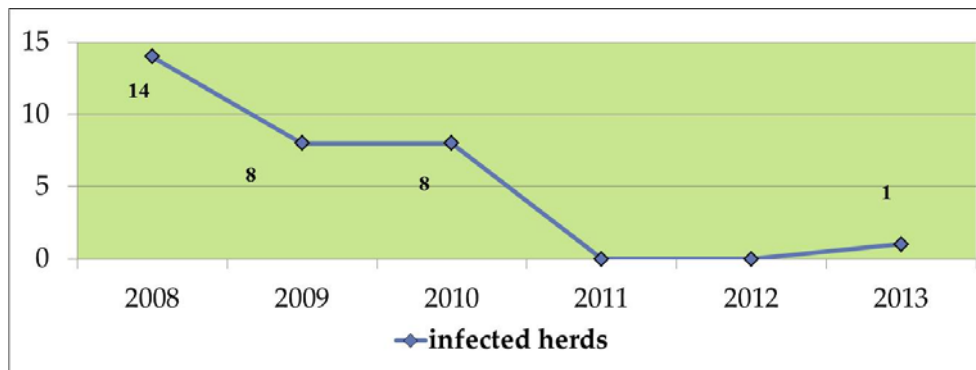
Actions taken in case of suspicious reactors: individual that results positive to CFT or animals of flocks epidemiologically linked to infected ones:

- All Rose Bengal positive samples are retested in NRL with CFT.
- In case more than 5% of animals in the flocks are RBT positive, the whole herd is retested with CFT.
- Suspected animals are culled.

A brucellosis case is confirmed when the isolation and identification of *B. melitensis* is carried out.

- *Ordinance on animal health requirements applicable for trade in sheep and goats* (OG, 51/09, 44/13) - aligned with Directive 91/68/EEC, main framework for granting the status of *B. melitensis* Free flocks and territories (county based).
- *Annual Order* on measures to protect animals from infectious and parasitic diseases and the financing thereof (OG) – that establishes basic and main measures for the implementing year (screening for brucellosis, pre-movement testing, compulsory notification of abortions).
- And, finally the Annual Programme providing standard rules of implementation.

The number of outbreaks detected in the past years is shown in **Figure 14**. The last outbreak was recorded in 2013.



**Figure 14. Number of sheep and goats brucellosis outbreaks reported in Croatia, 2008-2013.**

The active surveillance of the disease has been enlarged gradually to cover the whole sheep and goat population in the country. In the recent history, the programme can be divided into two main periods.

Period 2008-2011:

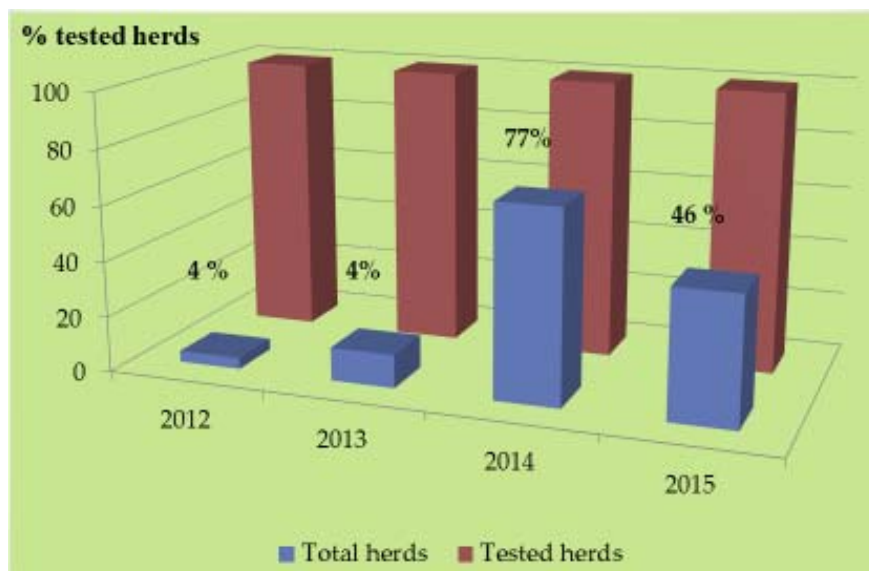
- Reporting all abortions in sheep and goats is mandatory - laboratory investigation.
- Mandatory testing of male animals before mating or introduction into the flocks.
- Pre-movement testing.
- Testing in high risk municipalities - following outbreaks: Karlovac county, Split Dalmatia, Šibenik knin county and Lika senj county.

Period 2012-2015:

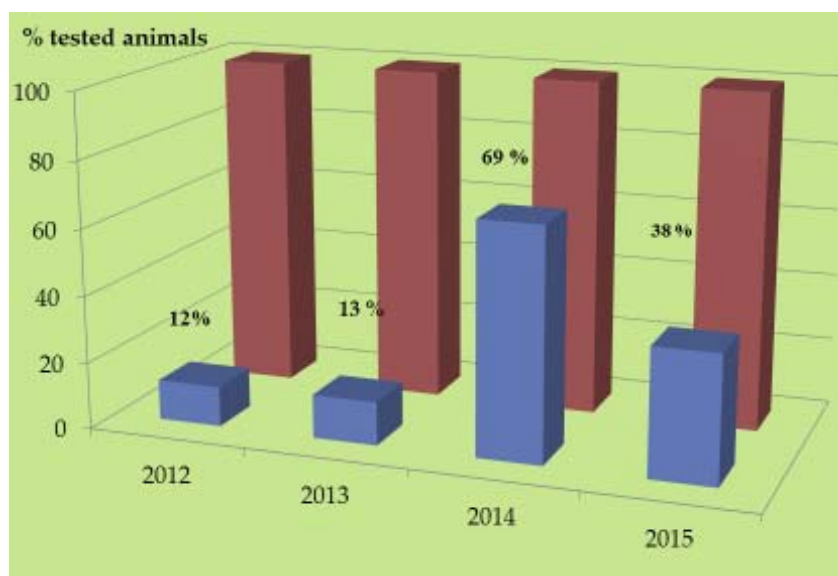
- Granting of officially free status to all dairy and mixed sheep and goat flocks (2012 and 2013).
- 2014 – Enlargement of testing to the whole sheep and goat population in Croatia and maintenance of the sanitary status in dairy and mixed herds.
- First check conducted in autumn 2014, and second one is currently ongoing.
- Testing regime supported by:
  - Pre-movement testing in the whole country– animals have to be tested 30 days prior movement (except between OBmF herds),
  - The laboratory investigation of all reported abortions,
  - Activities aiming to raise awareness among owners about the disease brucellosis and the importance of reporting abortions (distribution of educational leaflet to owners during testing in 2014).
- In 2014, 395 abortions were reported, 104 of which were bacteriologically investigated with negative results.

- By the end of 2014, 720 flocks were granted with the OBmF status (dairy herds); by the end of June 2015 the figure increases up to 2,134 flocks.

Regarding the level of programme implementation, there is a lack of coverage with marked differences among counties (**Fig. 15 and 16**); to address this issue, a third round of testing is foreseen, in order to cover as much flocks as possible. The objective is to have 70% of flocks qualified as OBmF by the end of the 2015.

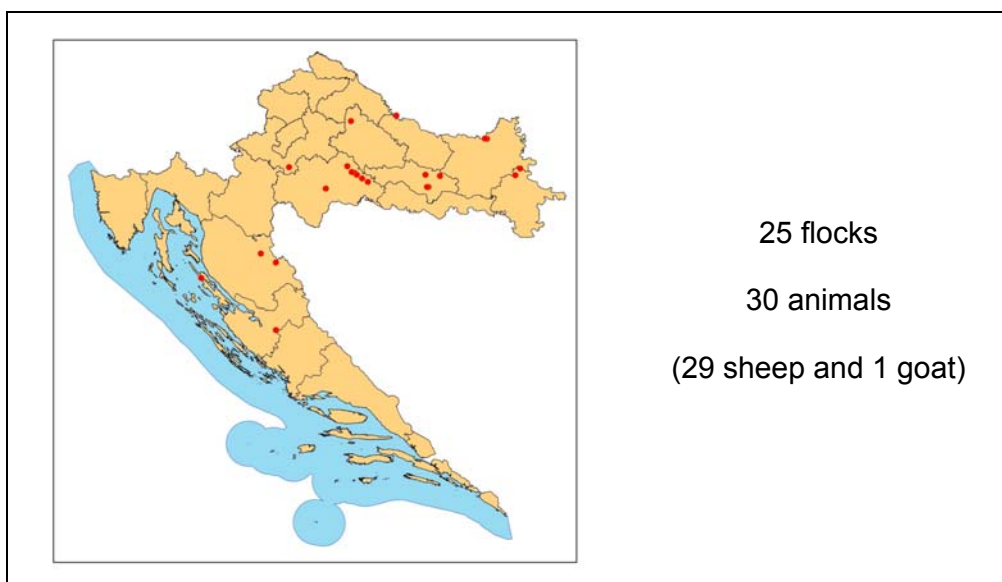


**Figure 15. Percentage of sheep and goats flocks tested in Croatia, 2012-2015. (Jan-June 2015)**



**Figure 16. Percentage of sheep and goats tested in Croatia, 2012-2015. (Jan-June 2015)**

Details on flocks experiencing False Positive Serological Reactions (FPSR) in 2014 are shown in **Figure 17** and **Table 3**. Veterinary services are working in the standardization of a protocol to deal with FPSR cases based on the use of skin test.



**Figure 17. Croatia, location of CFT positive flocks in 2014.**

**Table 3. Croatia, number of sheep and goats tested and number of sheep and goats resulted psitive to CFT in 2014.**

County	Number of animals tested in the herd	CFT positive animals	County	Number of animals tested in the herd	CFT positive animals
Sisak Moslavina	90	2	Zagreb county	97	1
	20	1		14	1
	28	1	Osijek baranja	414	2
	10	1		117	1
	15	1	Vukovar srijem	63	1
	5	1		10	1
	5	2		129	1
Lika senj county	76	1		85	1
	146	1	Virovitica podravina	114	2
	359	1			
Požega slavonia county	9	1	Bjelovar bilogora	41	1
	10	2			
	18	1	Zadar county	110	1
	6	1			

## 5. Brucellosis in humans.

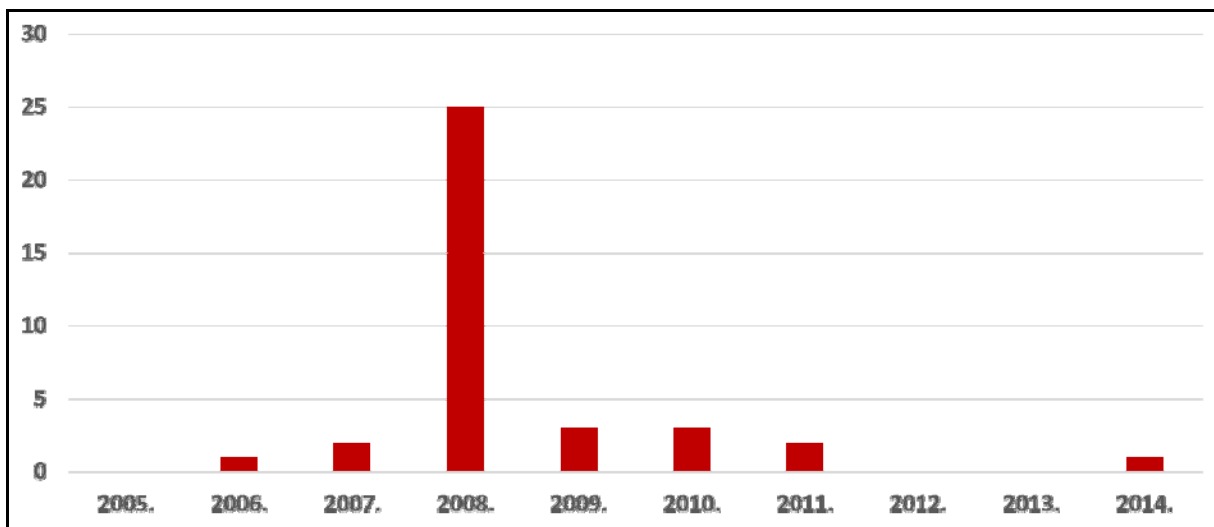
*Dr. Iva Pem-Novosel, Head, Zoonoses Section, Croatian Institute of Public Health).*

An overview of the disease characteristics in humans and of the national legislation framework on human brucellosis were presented. The following rules are currently in force in the Country:

- Act on protection of population on communicable diseases (OG/07, OG/09).
- List of mandatory notified communicable diseases (OG/2014).
- Ordinance of reporting on communicable diseases.
- Directive 2003/99/EU since 17 November, 2003 on Zoonosis surveillance and monitoring of zoonosis causative agents.

Every human case is investigated with a standardised questionnaire. In case of outbreak the medical sector has to notify the disease to the veterinary sector.

Data on human cases notified during the 2005-2014 period are shown in **Figure 18**.



**Figure 18. Number of human brucellosis cases notified in Croatia 2005-2014.**

In year 2008, 25 cases of human brucellosis were recorded in the Country, eight of which in Zagreb County and another eight in Karlovac County. The people infected found in Zagreb were immigrants from Kosovo where actually they got the infection. As far as the cases of Karlovac are concerned, the owner of a sheep flock and his family were affected. Also veterinarians got the infection during the procedures for cleaning placenta retention.

## **6. Veterinary inspection service organisation, official controls related to cattle and sheep and goat holdings in case of brucellosis suspicion/outbreaks.**

*Dr. Plamenka Kovač Levantin, Split Regional Veterinary Inspection Office (MA-VFSD).*

The VFSD is the Competent Authority and is responsible for the overall design, follow-up and evaluation of the programme, as well as for adopting corrective measures if needed. In the different territories the VFSD is supported by the Veterinary Inspection Department through 13 Veterinary Offices (sections) covering 21 counties.

Veterinary inspections are carried out according to a legal basis, the Veterinary Act (82/2013), establishing the general framework, and other pieces of national legislation transposing the Community *acquis*.

In addition, an Annual Order (3/2015) establishes tasks and responsibilities of stakeholders (owners, authorized veterinary associations, inspector, veterinary services) involved in the protection of livestock against infectious diseases, and describes in details all the aspects that should be subjected of control by the inspectors with regards to infectious diseases, including the veterinary checks on the implementation of brucellosis programmes (cattle, sheep and goats, swine). The speakers reviewed the requirements for granting the OBmF status, maintenance of it, suspicion and withdrawal, movement controls, etc. This annual order also establishes the financial aspects and penalties in case of non-compliance.

Every field activity, including identification, registration and entering of information in the databases, are responsibility of AVOs. These veterinary associations sign a 5-year contract with the CA, and are responsible for the recruitment and for the good performance of private veterinarians. The contract is based on fees, annually revised and published on the website of the Ministry of Agriculture. The fees consist in fixed amounts per sampled animal (approximately 6 euros per cattle, and 3 euros per sheep or goat; it includes sampling material and travel expenses).

The audit and inspection of field duties are carried out by 61 veterinary inspectors belonging to the Veterinary Inspection Department. This service is also responsible for the implementation of all activities in case of disease outbreak (restriction of movements, blood sampling and specimens sampling for bacteriology investigation, slaughtering, cleaning and disinfection activities, epidemiological investigation, etc.).

One of these Veterinary Offices is based in Split, where the speaker works. This office accounts for the county of Split-Dalmatia and accounts for 16 inspectors working in 3 different Veterinary Divisions (Split, Sinj and Imotski).

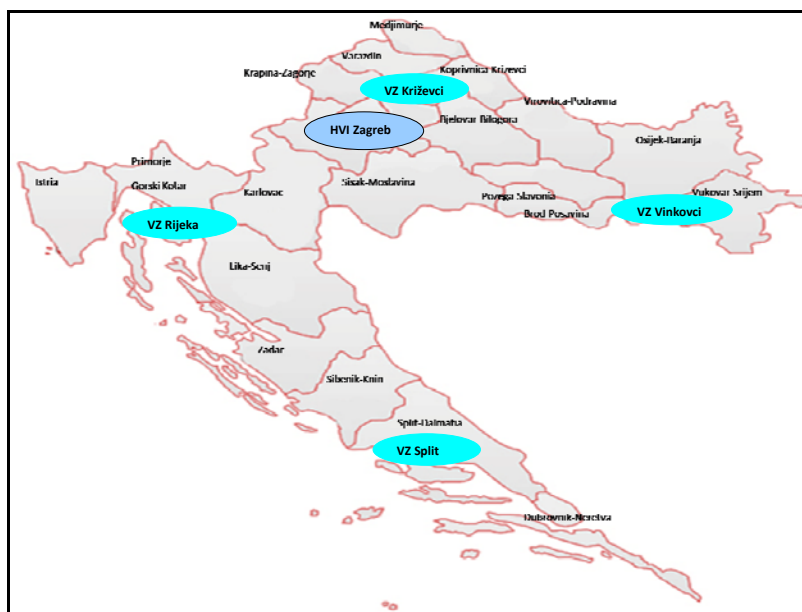
The Veterinary Division of Split is responsible for the veterinary inspections and official controls according to an annual plan establishing objectives, targets, methodologies, type of controls, frequency etc. This annual plan includes both inspections programmed in advance according to risk-based sampling scheme and also on-spot visits when there are suspicions of non-compliance.

For example, the sampling size of checks carried out to verify compliance with identification and registration rules is 5% of cattle holdings, 3% of sheep holdings and 3% of goat holdings. During the visits, inspectors conduct a comprehensive check on all aspects involved in the programme: identification and registration, documentation, movements, verification of the sampling scheme, results of testing, etc. The report on the veterinary checks is incorporated in the database for the management of I&R and veterinary aspects.

## **7. The National Reference Laboratory for brucellosis – Laboratory investigation of brucellosis.**

*Dr. Silvio Špičić, NRL for Brucellosis, Croatian Veterinary Institute (CVI), Zagreb.*

The first diagnostic activities in brucellosis started in 1946, when the first case of sheep and goat brucellosis was identified in Istria County. The epidemiology department of the laboratory was founded in 1949 and the serology department in 1951. From 1951 to 2007 the department in charge of brucellosis laboratory activities bore different names and is now the “Laboratory for bacterial zoonoses and molecular diagnostics of bacterial diseases” of the Croatian Veterinary Institute (CVI). The CVI has its headquarters in Zagreb together with central reference laboratories and includes 4 regional veterinary institutes (Križevci, Vinkovci, Split and Rijeka), that serve as official laboratories where the RBT screening is performed (**Fig. 19**).



**Figure 19. The CVI Zagreb and its 4 regional veterinary institutes.**

The Croatian Veterinary Institute (CVI), NRL for brucellosis, and the regional veterinary laboratories are all accredited according to ISO 17025 for the RBT. The reagents used in the CVI have been controlled by the brucellosis EURL and the CVI has been participating to the proficiency tests organised by the EURL since the beginning. Proficiency ring trials as well as trainings are organized annually by the NRL at national level.

The NRL performs all the serological tests (RBT, CFT, iELISA and cELISA) for confirmation of RBT positive results obtained in the regional veterinary laboratories.

The NRL is also in charge of all bacteriological examinations. The tests performed in 2014 by the CVI in cattle and sheep and goats are shown in **Table 4**.

**Table 4. Tests performed in cattle and in sheep and goats by the CVI in 2014.**

Tests	Cattle		Sheep and goats	
	Tests performed (No.)	Tests positive (No.)	Tests performed (No.)	Tests positive (No.)
RBT	237,009	108	469,393	431
CFT	83	7	1,134	39
iELISA	84	9	N/A	N/A
cELISA	83	6	N/A	N/A
Bacteriology	268	0	90	0



The CVI has also contributed to the investigations performed in the past outbreaks in 2004, 2005, 2008, 2010 and 2013.

The NRL is also in charge of investigations concerning ovine epididymitis (*B. ovis* infection) as well as porcine, canine and marine mammal brucellosis:

- In 2014, 327 rams were *B. ovis* CFT positive in 17 counties.
- As regards porcine brucellosis, *B. suis* biovar 2 is the predominant biovar present in Croatia (as in the rest of the EU). Studies performed in 2001-2011 resulted in isolation of *B. suis* biovar 2 from outdoor ranged pigs and wild boars as well as a specific “Croatian” biovar 3 in pigs, wild boars and horses (**Table 5; Fig. 20**). The Brucellin skin-test is used as an additional confirmatory tool in suspect pig farms due to the lack of specificity of serological tests in pigs.

**Table 2. *B. suis* strains isolated in Croatia (2000-2011).**

Year	Host	<i>B. suis</i> biovar	Positive flocks	County(*)	References(**)
2000-2004	pigs	2		SM, OB, BP, VS, PS	Cvetnić <i>et al.</i> 2009
		3	1	OB	
2001-2002	wild boar	2		SM, OB, BP, VS, ZG	Cvetnić <i>et al.</i> 2004
	horse	3	1	SM	Cvetnić <i>et al.</i> 2005
2003-2004	wild boar	2		SM, OB, VS	Cvetnić <i>et al.</i> 2009
		3			
2008	pigs	2	3	SM, OB	Špičić <i>et al.</i> 2010
2009	pigs	2	3	SM, ZG	Špičić <i>et al.</i> 2013
2010	pigs	2	4	VS	Špičić <i>et al.</i> 2013
2011	pigs	2	5	SM, BB, VS, ZG	Špičić <i>et al.</i> 2013
	wild boar	2		OB	Špičić <i>et al.</i> 2013

(\*) Counties: SM - Sisak-Moslavina; OB - Osijek-Baranja; BP - Brod-Posavina; VS – Vukovar-Srijem; PS – Požega-Slavonija; ZG – Zagreb County; BB - Bjelovar-Bilogora

(\*\*) References:

Cvetnić Ž, Tončić J, Špičić S, Lojkić M, Terzić S, Jemersić L, Humski A, Čurić S, Mitak M, Habrun B, Brstilo B, Očepek M, Krt B, 2004. Brucellosis in wild boars (*Sus scrofa*) in the Republic of Croatia. *Vet Med (Praha)* (2003) 49:115-122.

Cvetnić Ž, Špičić S, Čurić S, Jukić B, Lojkić M, Albert D, Thiébaud M, Garin-Bastuji B. Isolation of *Brucella suis* biovar 3 from horses in Croatia. *Vet Rec* (2005), 156:584-585.

Cvetnić Ž, Špičić S, Tončić J, Majnarić D, Benić M, Albert D, Thiébaud M, Garin-Bastuji B. *Brucella suis* infection in domestic pigs and wild boar in Croatia. *Rev Sci Tech OIE* (2009), 28:1057-1067.

Špičić S, Zdelar-Tuk M, Račić I, Duvnjak S, Cvetnić Ž. Serološka, bakteriološka i molekularna dijagnostika bruceloze u domaćih životinja u Hrvatskoj. *Croat Med J* (2010), 51:320-326.

Špičić S, Zdelar-Tuk M, Račić I, Vujnović A; Benić M, Duvnjak S, Cvetnić, Željko. Sensitivity of actual laboratory diagnostic methods used for surveillance of swine brucellosis in Croatia. *Int J Appl Res Vet Med* (2013), 11:167-173.



Figure 20. Geographical distribution of *B. suis* strains isolated in Croatia (2000-2011).

### 8. Case report on two *B. melitensis* outbreaks in Split (Dalmatia County) in 2010 and Lika (Senj County) in 2013.

*Dr. Ivana Lohman Janković, Head, Vet-Epi Service Animal Health Protection Sector (MA-VFSD).*

#### CASE REPORT 1 – year 2010

The farm is located in the Dalmatia County, near Split. The holding was a mixed holding with 398 animals, mostly goats for meat production (384), dairy cows (8), donkeys (2) and dogs (4). Animals were housed on the farm during night, while grazed on common pastures near the farm during the day, together with animals from other holdings of the village. Sometimes animals of unknown origin were also found on the common pasture. The holding was regularly registered in the database, the register was regularly updated. Animals were properly identified except dogs. The holding was regularly tested for Brucellosis in 2009 by the AVO with negative result. Tests performed for Brucellosis in the previous years were also negative. No clinical signs or abortions were reported during these years. On 12 May 2010 the annual compulsory sampling of the holding was carried out. Five animals out of the 193 animals sampled for Brucellosis tested positive in RBT,

four animals tested positive in CFT, and one animal showed an anti-complementary reaction. No clinical signs of disease and no abortions were reported. The positivity was properly notified by the AVO to the local Veterinary Inspector. The holding was put under restrictions, the local public health authorities were informed and an epidemiological investigation was carried out. All positive animals were culled.

The epidemiological investigation revealed that in December 2008 a total of 24 goats were temporary conferred to another holding that in 2009 was found *B. melitensis* infected. Within a range of five kilometres, also two neighbouring holdings with susceptible animal species had a history of previous positivity. In these holdings *B. melitensis* cases were found in 2005 and 2009 respectively. In addition, according to the statement of the owner, 25 animals disappeared (not recorded properly). All contact holdings as well as the holdings using the common pasture were tested (twice within 3 months).

On 27 July 2010, again, one animal was found positive. On the first of October 2010 out of 193 tested goats, 95 were RBT positive and out of them 87 were CFT positive. The donkeys and the dogs tested negative. Dogs were not vaccinated against rabies. Total depopulation of the holding (383 animals, including donkeys and dogs) was carried out.

Conclusion: it was not possible to identify exactly the source of infection. It is suspected that it could have been an animal with unknown status on the commune pastures or the reintroduction of one or more animals from the above mentioned positive flock. Today at the holding only cattle are kept and no goats any more.

### CASE REPORT 2 – year 2013

On 26 September 2013, the public health institute informed the local veterinary inspector of a confirmed case of Brucellosis in a 16 year old boy in Lika Senja county, near the border to Bosnia and Hercegovina (B-H), whose family owned sheep. . The holding consisted of 104 breeding sheep, three rams, 16 lambs and two dogs. The animal register was not updated regularly; also animal movements were not reported properly. The holding was regularly registered in the database. A total of 34 sheep were found not identified. The animals had no contact on pasture with animals of other flocks. The owner had frequent contacts with relatives in B-H, which were also farmers. No clinical signs of disease were found. The annual testing was not carried out by the AVO in 2012. A person was found positive for Brucellosis. This person was working in 3 other sheep flocks (helping in unloading animals). All animals were immediately tested at farm level. The movement of animals and animal products from the holding was restricted. A total of 107 animals were blood sampled. On 04 October 2013, out of the 107 samples tested, 73 were

positive to RBT and 79 to CFT positive. Because of these findings, the flock was depopulated; all animals were culled and destroyed, including dogs also. On 24 October 2013 the positivity for Brucellosis was confirmed by bacteriological isolation. All contact flocks (n=10) were tested for Brucellosis (a total of 1,251 animals) twice within a 3 month period with negative results. The epidemiological investigation was carried out on all holdings within a 3 km radius (303 animals in 20 holdings); they were tested with negative results.

In conclusion, the source of infection was attributed to a probable illegal introduction of animals from B-H from flocks with unknown brucellosis status.

#### **9. The national veterinary information system.**

*Dr. Tomislav Kiš, Head, Animal Health Protection Sector, Veterinary and Food Safety Directorate (VFSD), Ministry of Agriculture (MA).*

The veterinary information system is an important tool for managing and recording the activities carried out in animal health either by the Official Veterinarians or by the AVO. Also public administrations authorised animal owners (for the purpose of animal identification) and public laboratories have access to the Information system. Link: [www.hpa.hr](http://www.hpa.hr). The first version of the information system was available in 2009. In 2011, specially designed modules have been developed for the Bovine Brucellosis and Enzootic Bovine Leucosis eradication programmes. In 2014, the Brucellosis sheep and goats eradication programme was covered as well as the Aujeszky's disease control programme in pigs. In 2015, the Information System will include the Bluetongue vaccination programme. The system is based on several data such as the holding number, the ear-tag numbers of animals present in the holding, the name and address of the owner, and furthermore. The software package is also dedicated to the activities to be performed in the framework of the existing eradication and control programmes. It contains also the current status of the herd for the above mentioned diseases, the date of achievement of the status and other relevant information. The testing results entered by the laboratories are available to the official veterinarians and the AVO. The system is designed to store information on vaccination, *i.e.* for Bluetongue.

## **DAY 2**

### **10. Field Visit, Sheep and goat farm (Village MUĆ).**

*Dr. Plamenka Kovač Levantin, Dr. Marijo Vučković, Split Regional Veterinary inspection office (VFSD).*

The TF Brucellosis subgroup visited a sheep flock accompanied by the colleagues of the Croatian Veterinary Services and the authorized veterinarian responsible for the implementation of the programme in the farm. A 46-heads flock was visited, reared under extensive system for meat production. Lambs are sold to the market while the low milk production is kept for self-consumption (not pasteurized) as small cheeses. This year the production has been around 39 lambs; 15% average was kept in the holding as replacement of old animals. The farm also raised pig and poultry for self-consumption as well. The flock is settled in an area where other 10 flocks are reared. The system of husbandry is based on daily moving to pastures but in split areas were flocks do not mix. Possible contacts may occur in pathways and roads. Males are shared with neighbours for mating as a tradition. The breed sheep was the *Dalmatinska pramenka*, an autochthonous animal breed well adapted to that particular ecosystem. Animals are not supplemented with feedstuff except in winter. No preventive treatments are administered as anti-parasitic treatment or vaccination. The flock has been vaccinated twice against Bluetongue this year. Currently, it was undergoing qualification as OBmF with two tests carried out, the first with single positive reactors, and the second with negative results.

### **11. Field Visit, cattle farm (Village BRNAZE).**

*Dr. Plamenka Kovač Levantin, Dr. Marijo Vučković, Split Regional Veterinary inspection office (VFSD).*

A second farm was visited in the morning of day 2. The holding was located in the village of Brnaze, about 33 km from Split, not far away from the border with Bosnia and Herzegovina. The TF Brucellosis subgroup visited the cattle holding (reg. number JIBG 50071394) accompanied by the colleagues of the Croatian Veterinary Services and the veterinarian from the AVO, responsible for the implementation of the programme and the identification in the holding. It was a bovine fattening herd with 41 heads, most Simmental breed. The system of husbandry is based on housing the animals in boxes (groups of 6-10 animals). Restocking is achieved by buying young calves from farmers in the area or a dealer. Adult bovines are sold for slaughter to the local market with a weight of about 500 kg. The farm raised also milk production with one Simmental cow (and her calf) in a

separate facility about 25 meters away from the fattening unit. Fattening animals are supplemented with feedstuff. Preventive anti-parasitic treatment is carried out regularly. No other similar holdings are in the closed area, only some holdings with one or a few cattle. All animals in the holding visited were properly identified; passports for each bovine were shown and register was regularly actualized. The farm keeps cattle only since 2008. Annual compulsory blood sampling was carried out; no historical background with Brucellosis recorded. In December and April 2014, compulsory blood sampling for Brucellosis was implemented; four female heads older than 12 months were tested with negative results. The national Ordinance prescribes testing of bovines older than 12 months for Brucellosis. Three bovines have been vaccinated on 27 February and on 25 March 2015 against Bluetongue. The owner plans to increase the production in the near future significantly.

## **CONCLUSIONS**

1. The eradication programs for Bovine (BB) and Sheep and Goats (S&GB) brucellosis have been well designed by the country. The last case of brucellosis due to *B. abortus* was recorded in 1965, while the last case of *B. melitensis* was recorded in sheep in 2013. Control measures are fully in line with the relevant EU legislation. The Veterinary and Food Safety Directorate (VFSD) in the MoA and the Authorized Veterinary Organizations (AVO) are responsible of the program implementation.
2. Data provided about testing coverage were not complete, giving the impression that there is no sufficient coverage to ensure the absence of *B. abortus* or *B. melitensis* in the country. The herd/flock coverage has been low in the past years but clearly improved in 2014-2015.
3. Illegal movement of animals and direct contact with infected herds/flocks in common pastures crossing borders with neighbouring countries have been identified as main risk factors for the spread of both BB and S&GB.
4. The Croatian Veterinary Institute (CVI), NRL for brucellosis, and the local veterinary laboratories are all accredited according to ISO 17025. The reagents used in the lab have been controlled by the EU Reference Lab for brucellosis. Proficiency ring trials are organized by the NRL at national level. However, not all brucellosis analytical lines are accredited.
5. Compulsory total depopulation is applied in confirmed infected herds/flocks. Positive animals are investigated in bacteriology.
6. The management of FPSR is foreseen in the programme with clear rules, including the use of brucellin skin test on reactors. However, clear and harmonized protocols are not available at national level.
7. The rules for animal identification and herds/flocks registration in Croatia are in line with current EU legislation, as all livestock holdings have to be identified by a unique holding number and registered on a central computer database; for sheep and goats the means of animal identification as depicted in Regulation (EC) 21/2004 are in force (i.e. double identification).
8. Biosecurity measures seem to be not sufficiently implemented.

## **RECOMMENDATIONS**

1. The very good interaction between the VFSD, the labs and the AVOs should be maintained in order to achieve rapidly the OBF/OBmF status.
2. Testing coverage should continue to be improved both at herd/flock and at animal level to reach the objective of 100% coverage. The reasons for insufficient coverage should be investigated, including an intensification of veterinary inspections, where necessary. Corrective actions, including incentives for sampling, should be implemented in the respective areas.
3. Reinforcement of movement controls and trace-back and trace-forward of outbreaks may contribute to reducing the risk of new brucellosis outbreak occurrences. These reinforced measures should be maintained even after reaching the OBF/OBmF status. Epidemiological investigation of suspect cases should be maintained including cross-border collaboration.
4. It is advisable for the NRL to get accreditation on all brucellosis analytical lines, including bacteriology.
5. The group recommends continuing the policy of compulsory application of total depopulation in confirmed brucellosis outbreaks. Adequate means should be kept to maintain a good level of bacteriological investigation in suspected cases. Protocols for bacteriological investigation of suspect cases in cattle should be set down similar to the one in force for S&G.
6. Guidelines for the management of FPSR should be produced and harmonized at national level. The Brucellin skin test should not be applied only on seropositive animals but also on a certain number of negative animals in the same lot of (proportion to be formalized).
7. The group recommends that the animal identification and notification of movements are maintained and verified.
8. Special attention should be given to issues related to biosecurity at all levels. Reinforcement of biosecurity awareness at all level might be necessary.

The Working Document SANCO/6095/2009 should be taken into due consideration when designing, planning and implementing the measures foreseen by the programme. The document can be found at the following web address:

[http://ec.europa.eu/food/animal/diseases/eradication/eradication\\_bovine\\_sheep\\_goats\\_brucellosis\\_en.pdf](http://ec.europa.eu/food/animal/diseases/eradication/eradication_bovine_sheep_goats_brucellosis_en.pdf)



**A warm thank you is extended to the Croatian hosts for their great hospitality and willingness to share information about the details of the programme. The effort of arranging this meeting is greatly appreciated.**

## Annex I

**MEETING OF THE BRUCELLOSIS SUB-GROUP OF  
THE TASK FORCE FOR MONITORING DISEASE ERADICATION  
HELD IN SPLIT, CROATIA, 17-18 JUNE 2015**

### **PARTICIPANTS**

#### **Task Force Brucellosis Sub-Group - members**

- Fabrizio DE MASSIS, Chairman                      Italy
- Manuel DURAN-FERRER                              Spain
- Bruno GARIN-BASTUJI                                France
- Ernst STIFTER    Italy

#### **European Commission**

- Valentina PIAZZA                                      Head of Sector :Veterinary, Unit G5, food chain and animal health expenditure (DG SANTE)
- Nicolas KRIEGER                                        Unit G5 (DG SANTE)

#### **Country Representatives (main list)**

<b>Name</b>	<b>Position</b>
Tomislav Kiš	Head, Animal Health Protection Sector, Veterinary and Food Safety Directorate (VFSD), Ministry of Agriculture (MA)
Ivana Lohman Janković	Head, Vet-Epi Service Animal Health Protection Sector (MA-VFSD)
Iva Pem-Novosel	Head, Zoonoses Section, Croatian Institute of Public Health
Martina Rubin	Associate, MA-VFSD
Vladimir Čačinović	MA-VFSD
Silvio Špičić	NRL For Brucellosis, Croatian Veterinary Institute (CVI)
Eddy Listes	Split Regional Veterinary Institute, CVI
Plamenka Kovač Levantin	Split Regional Veterinary Inspection Office (MA-VFSD)
Marijo Vučković	Split Regional Veterinary Inspection Office (MA-VFSD)

## Annex II

**MEETING OF THE BRUCELLOSIS SUB-GROUP OF  
THE TASK FORCE FOR MONITORING DISEASE ERADICATION  
HELD IN SPLIT, CROATIA, 17-18 JUNE 2015  
AGENDA**

#	<i>Timing</i>	<i>Item</i>	<i>Presenters/ Rapporteur</i>
<b>DAY ONE</b>		<b>Split, Croatian Chamber of Economy</b>	
	09:00	Welcome and introduction	
	09:15	Presentation of the subgroup on brucellosis Task-Force – European Commission - and overview on the scope of his mission	Dr. Fabrizio De Massis
1	09:30	Presentation of the Structure and Organisation of Veterinary Services	Dr. Tomislav Kiš
2	10:00	Presentation of structure and national distribution of bovine and ovi-caprine livestock	Dr. Vladimir Čačinović
3	10.30	Presentation of the National Animal Identification and Registration System and Database	Dr. Vladimir Čačinović
	11:00	<i>Coffee Break</i>	
4	11:15	Bovine brucellosis eradication programme in Croatia: presentation of the national legislation and of the results of the brucellosis control programme implementation – Overview of the evolution, current situation and perspectives.	Dr. Martina Rubin
5	12:00	Sheep and goat brucellosis eradication programme in Croatia: presentation of the national legislation and of the results of the brucellosis control programme implementation - Overview of the evolution, current situation and perspectives.	Dr. Martina Rubin
	12:45	End of Session I – Panel Discussion.	All Participants
	13:00	<i>Lunch</i>	
6	14:00	Brucellosis in humans	Dr. Iva Pem-Novosel
7	14:30	Veterinary inspection service organisation, official controls related to cattle and sheep and goat holdings in case of brucellosis suspicion/outbreaks	Dr. Plamenka Kovač Levantin
	15:00	<i>Coffee Break</i>	
8	15:15	Presentation of the National Reference Laboratory for brucellosis – Laboratory investigation of brucellosis	Dr. Silvio Špičić
9	16:00	Case report on two <i>B. melitensis</i> outbreaks in Split (Dalmatia county) in 2010 and Lika (Senj County) in 2013	Dr. Ivana Lohman Janković
10	17:00	Presentation of the national veterinary information system	Dr. Tomislav Kiš
	17:30	End of Session II – Panel Discussion.	All Participants

18:00	<i>Closure of the first day of meeting</i>	
<b>DAY TWO Field Visit - Villages MUĆ and BRNAZE</b>		
09:00	Meeting at the Hotel and travel to BRNAZE and MUĆ	Dr. Plamenka Kovač Levantin, Dr. Marijo Vučković
09:30	Field visit - sheep farm (village MUĆ)	
11:00	Field visit - cattle farm (village BRNAZE)	
12:30	Travel back to Split	
13:00	<i>Lunch</i>	
14:00	EU Task Force Brucellosis subgroup meeting.	TF BRC Subgroup
16:00	Presentations of final conclusions and recommendations by the Group - Final opportunity for questions and discussions.	All Participants
17:30	<i>Closure of the meeting</i>	