



Surveillance study on honeybee colony losses

Portugal
Direção Geral de Alimentação e Veterinária
Direção de Serviços de Proteção Animal

Introduction

This surveillance study on honeybee colony losses is presented according to document ref^a SANCO/G2/PB/Ip (2011) 1045845. Portugal has surveillance systems implemented for bee health but this submission to the pilot surveillance study as proposed allows to have harmonized active surveillance procedures at an European level that should lead to the establishment of a consistent and robust set of epidemiological indicators, calculated following the same rules and protocols in all countries, and produced by comparable active surveillance procedures applied across comparable populations according to the climate constrains.

Contact point:

Direcção de Serviços de Proteção Animal – Direção Geral de Alimentação e Veterinária (DGAV)
Largo da Academia Nacional de Belas Artes, 2 1249-105 LISBOA
Tel: 213 239 651 - Fax: 213 239 644
secdsspa@dgav.pt
sofia.quintans@dgav.pt

A - Human and financial resources

Human resources

The programme will be implemented by trained personnel of the Regional Veterinary Services (DSAVR) of the National Veterinary Competent Authority (**DGAV**). The number of persons involved in the surveillance programme will be determined by Regional Services and based on the location of the targeted apiary sites to be visited.

As for the specific training of the personnel, DGAV organizes every year technical courses (bee health and bee products hygiene) that includes a practical approach (observation of an apiary and a sample collection demonstration) with the participation of personnel from the Regional Veterinary Services. We consider that this staff is prepared to be involved in the programme. As referred in the programme, standardized training sessions for personnel involved will be organized in order to ensure agreement on standardized operating procedures.

Financial resources

As current economic situation in Portugal is expected to be very restrictive during the period covered by the programme. The programme will have to be implemented with minimal financial resources.

B - Description of the epidemiological situation in honeybee hives

According to national legislation Decreto-Lei n^o 203/2005, of 25th November, bee notifiable diseases in Portugal are: Varroosis, nosemosis, american foulbrood, european foulbrood, acarapisosis, ascospheriosis, aethinosis (small hive beetle) and tropilaelaps.

Until 2006, the diagnosis of bee diseases was either clinical (visual exam) or laboratorial (see Table 1) and was done especially in cases of clinical suspicion.

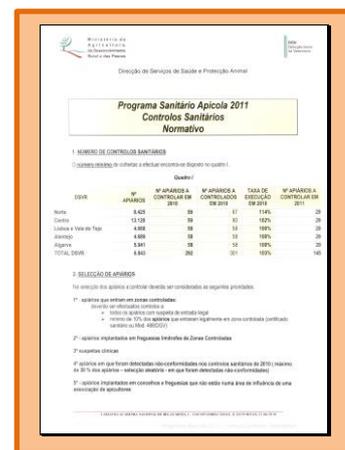
In 2006-2007 a national screening of bee diseases was organized by National Beekeeper Federation (FNAP), Veterinary Medicine School (FMV-UTL), beekeeper organizations, National Reference Laboratory (LNIV) and DGV (see laboratory results in Table I).

Table I

BEE DISEASE	Positive samples (sample=brood+bees)	
	% analyses of brood	% analysis of adult bee
Varroosis	18,31%	19,38%
Nosemosis		18,82%
Senotainosis		18,31%
Loque americana	18,31%	
Ascospferiose	3,65%	
Amebíase		2,25%

Since 2008, Portugal implemented an active surveillance with specific sanitary measures in order to determine sanitary status, supported in 2 types of programs:

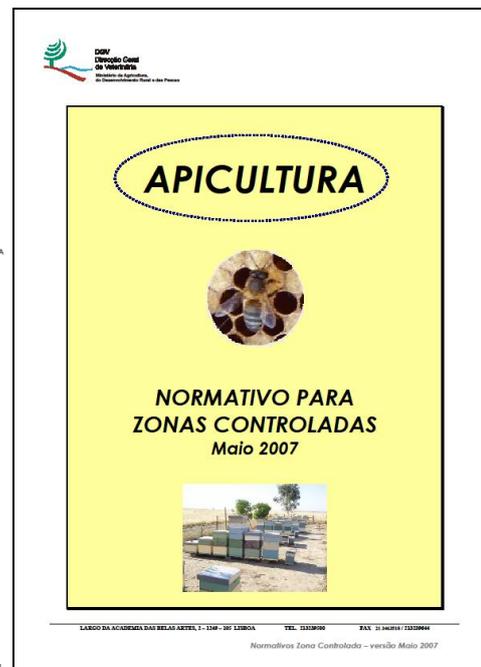
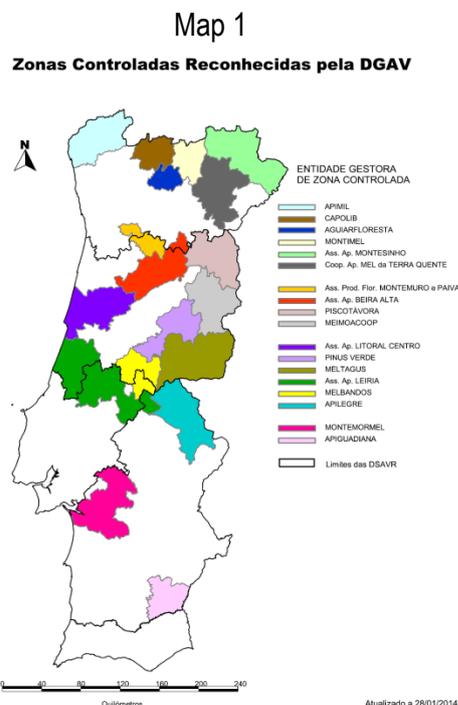
- ✓ **Official Annual Bee Health Programme (Programa Sanitário Apícola)** – implemented by the National Veterinary Competent Authority (**DGAV**) - This programme defines specific sanitary rules for bee health prevention as well as procedures to be used in controlled zones. It also includes specific procedures for official sanitary controls to apiaries.



- ✓ **Bee Health Programme of beekeepers organizations**– These programs are prepared and implemented by beekeepers organizations and validated by DGAV – measures considered in these programs are co-financed in Honey Program according to Regulation (CE) n.º 1234/2007 of 22 October and Regulation (CE) n.º 917/2004, of 29 April, and include visits to apiaries, collection of samples for laboratory analysis and varroosis treatment. These organizations also include those responsible for controlled zones.

In national legislation - *Decreto-Lei n.º 203/2005, of 25 November* - the concept of “**controlled zones**” is defined as geographical areas that have specific sanitary rules in order to have free disease status for bee diseases. Those controlled zones (see distribution in Map 1 and specific rules) are managed by beekeepers organizations and have trained staff that is very aware of the importance of training/supporting beekeepers *in loco*. In those zones, the main measures are:

- ✓ mandatory bee and brood analysis
- ✓ simultaneous treatment with the same products
- ✓ specific authorization is mandatory to get into those zones (pre-movement document and analysis are required).

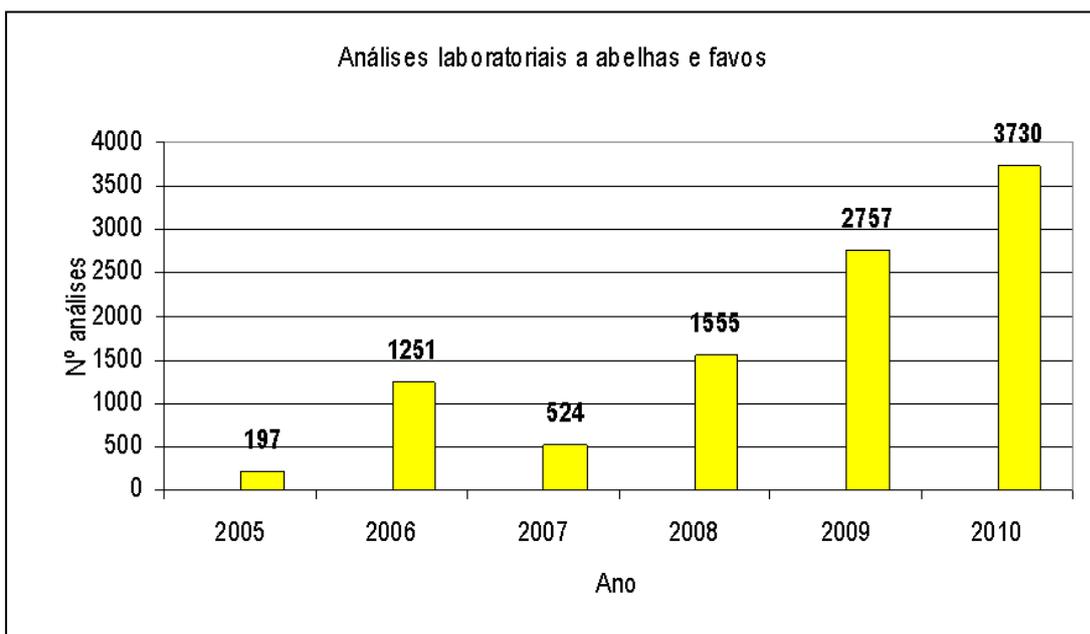


Collecting all data obtained from laboratorial results obtained from the two referred Programs, (Table II e Graphs 1 e 2) , allow us to confirm that there was an increase in the number of analysis in the last years. This increase is due to co-work of official entities (DGAV/National Reference Laboratory) and beekeepers organizations leading to the awareness of beekeepers to the importance of laboratory analysis for a correct diagnosis of bee diseases and for the importance of the implementation of controlled zones where analysis are mandatory for all epidemiological units (defined as all the apiaries from the same beekeeper that are located in the same parish) .

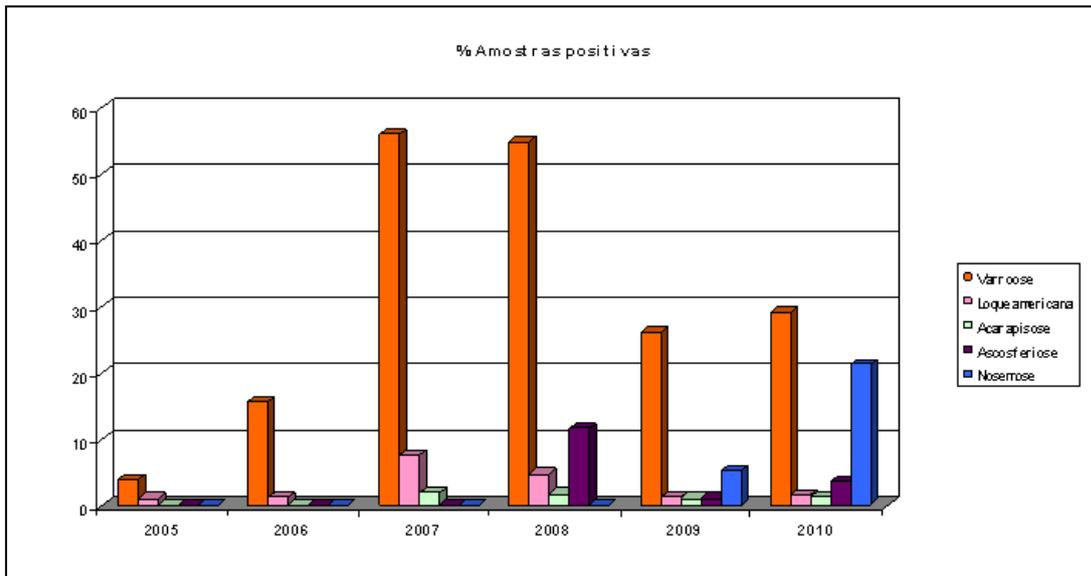
Table II

Disease	2005	2006	2007	2008	2009	2010
Varroosis	80	195	294	855	722	1098
American Foolbrood	20	15	40	73	34	59
Acarapiosis	+	+	10	27	30	46
Ascosteriosis	+	+	+	180	27	129
Nosemosis	+	+	+	+	143	793
Total analisis	197	1251	524	1555	2757	3730

Graph 1



Graph 2



C - Description of the surveillance systems already in place, if any, including number of dedicated staff, their level of expertise and supporting trainings

Passive surveillance

According to national legislation Decreto-Lei nº 203/2005, of 25 November, in Portugal bee diseases are notifiable diseases to the national veterinary competent authority (DGAV).

Active surveillance

As defined in point B, there are two types of active surveillance implemented according to national beehealth Programs.

Beekeepers organizations realize their sampling and disease control according to their specific programs approved by DGAV, and if they are responsible for controlled zones, collection of samples for laboratory analyses is mandatory for all epidemiological unit.

On the other hand, DGAV implements annually a bee health program that defines specific sanitary rules for bee health prevention and control as well as procedures for controlled zones. It also includes specific procedures for official sanitary controls to apiaries.

These official controls are performed by Regional Services of DGAV and are intended to verify the implementation of the national legislation, the sanitary status of the apiary and also to protect controlled zones. Those controls include a questionnaire and a sampling collection of brood and adult bees for laboratory analysis.

Apiary selection for official controls are based on the following risk criteria:

1. Apiaries entering controlled zones
2. Apiaries in the borders of controlled zones
3. Clinical suspicions
4. Apiaries where problems were detected in last year official control
5. Apiaries in areas not covered by beekeepers organizations

6. Apiaries from intra and extra-communitarian trade

These official controls began in 2008 and the number of controls was set according with an expected prevalence detection of 5% of bee diseases with 95% confidence interval in the susceptible population.

After 2 years of implementation, with an excellent execution rate and a good receptivity from beekeepers, those controls were maintained in 2011. Considering financial restriction and the increase of analysis done in controlled zones in the scope of co-financed Honey Programme, DGAV decided in 2011 to determine the number of controls according an expected prevalence detection of **10%** of bee diseases with 95% confidence interval in the susceptible population. After consulting epidemiological tables, we have obtained the number of sampling of 145 controls (29 controls per region for 5 regions).

Staff

Staff responsible for official controls (from Regional Services of DGAV) as well as staff from beekeepers organizations participate annually in DGAV technical training courses that includes a sample collection demonstration in the field.

D - Data regarding the number of beekeepers, the volume of honey production and of other bee products and the data on mortality

Number of beekeepers:

Registration of beekeepers and hives is mandatory in national legislation (*Decreto-Lei nº 203/2005, de 25 de Novembro*) as it is considered the support of health strategy and control actions and the first step to disease control.

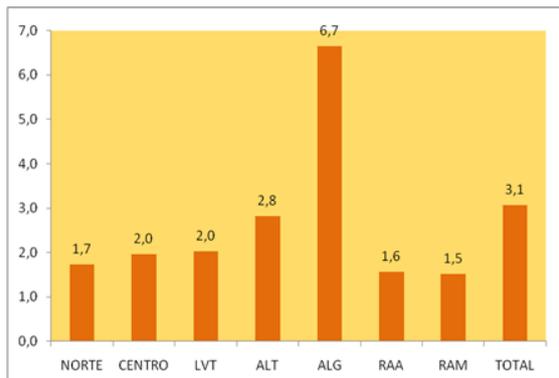
Data is collected in a database that gathers beekeeper information, apiary location and number of colonies (see table III and graphs 3 and 4).

Every year, in the month of June, all beekeepers must declare to DGAV (or other entities with a DGV protocol) the number of apiaries and bees. All the variation of this number (increase or decrease) must be also notified to DGAV.

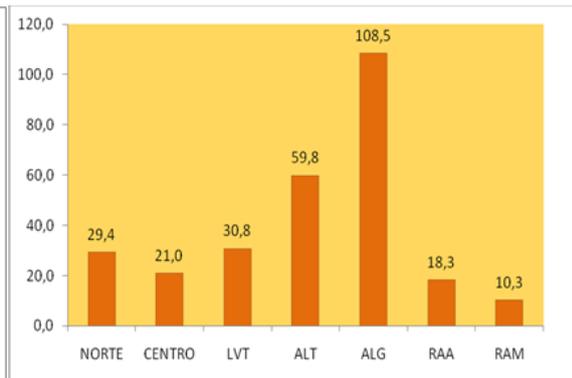
Table III

REGIONS DSVR	Beekeepers	Nº apiaries	Nº colonies	Nº apiaries/ beekeepers	Nº colonies/ beekeepers
Norte	4.854	8.425	142.628	1,74	29,4
Centro	6.684	13.120	140.579	1,96	21
Lisboa Vale Tejo	2.306	4.668	70.973	2,02	30,8
Alentejo	1.666	4.689	99.652	2,81	59,8
Algarve	893	5.941	96.925	6,65	108,5
Madeira	553	836	6.118	1,51	11,1
Açores	335	524	5.682	1,56	17
TOTAL	17.291	38.203	562.557	2,21	32,5

Graph 3 – Nº apiaries/beekeepers



Graph 4 – Nº colonies/beekeepers



Volume of honey production

The official data of honey production in Portugal is expressed in table IV.

Table IV

Year	Honey production (t); Annual
2010	7.426
2009	6.919
2008	6.654
2007	6.907
2006	5.978

Source - INE, Estatísticas da Produção Animal

Data on mortality

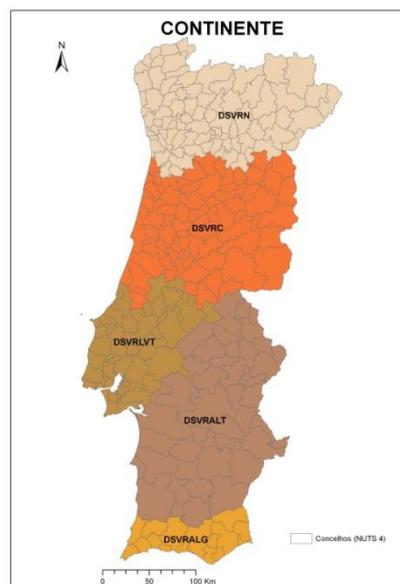
The available data on bee mortality in Portugal is considered normal mortality and has been related to climate conditions, summer fire and bee diseases.

E - Description and demarcation of the geographical and administrative areas in which the programme is to be applied

The programme will be applied in the national territory (mainland), coordinated by DGAV and implemented by Regional Services of DGAV (See Map 2):

- Direção de Serviços de Alimentação e Veterinária da Região Norte (DSAVRN)
- Direção de Serviços de Alimentação e Veterinária da Região Centro (DSAVRC)
- Direção de Serviços de Alimentação e Veterinária da Região Lisboa e Vale do Tejo (DSAVRLVT)
- Direção de Serviços de Alimentação e Veterinária da Região Alentejo (DSAVRALT)
- Direção de Serviços de Alimentação e Veterinária da Região Algarve (DSAVRALG)

Map 2



F - Measures to be applied and description of how the objective defined in Chapter 2 of the supporting document "Guidelines for a pilot surveillance project on honeybee colony losses"

The main measures needed for honeybee colony losses surveillance to be efficient, deal with following issues:

• **Target population:**

The sampling strategy is based in the total honeybee mainland population census: 38203 apiaries with 562557 colonies.

- **Nature of the targeted biological material to sample**

During the apiary inspection visits to be implemented, some biological material will be sampled as follows: one systematic sampling per examined hive during the first visit, additional symptomatic samplings in every hive with symptoms either on adult bees or on brood during visits 1, 2 and 3 .

- **Diseases and syndromes to be targeted**

The diseases to be targeted are national notifiable disease: varroosis, nosemosis, american foulbrood, european foulbrood, acarapisosis, ascospheriosis, aethinosis (small hive beetle) and tropilaelaps. For all these diseases (pathogens), specific records of clinical symptoms and/or presence of pest arthropods at the colony level will be established using the apiary inspection form. This form has to be systematically filled up by the specific person in charge of the apiary's visit.

- **Surveillance procedure to be implemented**

The procedure will follow the procedures indicated in chapter 2.2.4 and after selecting the sample of the honeybee population, 3 visits will be performed to the selected apiaries:

Apiary inspection visit 1: A first visit will be performed to collect data relating to beekeepers' practices, location of the beehives and environmental information, record of the number of living and healthy colonies and estimate the prevalence of some diseases (pathogens) (at least varroosis (*V. destructor*)). This visit will be implemented at the end of the season, before the wintering period (exact period to be defined). During this visit, a sample of house adult honeybees will be systematically collected from each colony irrespective of whether any symptoms are observed (i.e. from apparently healthy colonies as well as from any apparently infected/infested colonies). Additionally, specific symptomatic samples will be collected in colonies with any disease symptom

- Apiary inspection visit 2: A second visit to the same apiaries will take place at the end of the wintering honeybee season (to be defined) in order to objectively record any colony losses that have occurred during the winter. During this visit, only specific symptomatic samples (and/or dead bees) will be collected in colonies with any disease symptom and/or observed troubles (see Table 1). In order to ensure coordination between the COLOSS and the present survey, the beekeeper will be provided with the questionnaire from the COLOSS network during this visit and will be asked filling it up and sending it to COLOSS.

- Apiary inspection visit 3: A third and final visit is planned for the same apiaries during the honey production season in order to objectively estimate the number of lost/ weakened colonies.

Guidelines procedures will be available to staff according to chapter 2 recommendations.

- **Colony sampling method in the apiary**

There are specific instructions on the collection of bees and honey combs based on an epidemiological study (national screening 2006-2007 – see B).

Bees:

Approximately 50 to 70 live or recently death bees of the colony should be collected. Mention when bee are collected in the soil should be provided. In each apiary a number of colonies as defined in table V, should be collected, preferably of hives at the ends and center of the apiary. Bees taken from several colonies can be put in the same package, except in the case of suspected diseases on the colonies, that must be sampled individually.

There are special recommendations related to the fact that bees should never be sent in plastic bags neither with added honey or sugar and on the refrigeration (2-8 ° C) of samples until 78 hours after harvest or freezing for larger periods.

Brood

A fragment of honeycomb with brood (larvae and opercula), with about 12 cm x 12 cm should be collected. In each apiary a number of colonies as defined in table V, should be collected, preferably of hives at the ends and center of the apiary. Suspected weakened brood should always be sampled. (dead or smelly). There are special recommendations related to the fact that combs should never be sent neither with added honey and on the refrigeration (2-8 ° C) of samples until 78 hours after harvest or freezing for larger periods.

Identification and sending of samples

Samples of the same apiary should be identified with the same number and accompanied by an analysis request bulletin for bee diseases duly completed. All samples should be accompanied by laboratory requisition correctly completed. Preparation of samples and sample collections, by request, must be done in such a way as to avoid any leakage of the contents until arrival at the laboratory.

Table V

Number of colonies per apiary (range)	Number of colonies to be sampled per apiary
1 to 5	2
6 to 10	5
11 to 20	6
21 to 60	9
61 to 100 *	10

**According to national decree, the maximum number of colonies per apiary is 100 .*

• Data management, data treatment and interpretation

Data will be integrated in a database, then sent and centralized at both national and European levels to allow data treatment and analysis at these different levels.

• Training

National training sessions for personnel involved will take place before the beginning of the programme in order to follow Standard Operating Procedures.

• Communication

A report on the centralized global analyses will be provided by the Epidemiological team of EU RL every year to each participating MS. Extension and dissemination of the results of the project work will be divulgate and should be conceived as annual reports stemming from this global report. Additionally, a feedback procedure making obtained data and at least some discussions directly available to all



stakeholders and beekeepers involved into the programme will be clearly integrated into the project.

Beekeepers organizations and national federation will regularly be informed of the evolution and results of the program, in a cooperation standard.

• **Assessment and follow-up**

Key Performance Indicators (KPIs) developed in order to monitor the progress of the pilot surveillance programme on national and European levels will be analyzed and divulgate to the sector.

• **Institutional organization**

The programme will be under the responsibility of Animal Health Services (**DSPA**) of National Veterinary Competent Authority (**DGAV**) as coordinator and trainer. Trained staff from Regional Services will execute the visits to the apiaries.

G - The number of apiaries, colonies, visits, examinations and laboratory analyses based on the guidance of the supporting document

Analised the requirements for submitting the programme and considering epidemiological knowledge of bee diseases in the country and also actual financial restriction, we propose a strategy sampling based in an expected bee diseases prevalence of 10% with 95% confidence in the susceptible population (see prevalence table)

Prevalence table

TABLE:
(i) sample size required for detecting disease
(ii) confidence limits for number of positives

95%

population size (N)	(i) percentage of diseased animals in population (d/N) OR (ii) percentage sampled and found clean (n/N)											
	50%	40%	30%	25%	20%	15%	10%	5%	2%	1%	0,5%	0,1%
10	4	5	6	7	8	10	10	10	10	10	10	10
20	4	6	7	9	10	12	16	19	20	20	20	20
30	4	6	8	9	11	14	19	26	30	30	30	30
40	5	6	8	10	12	15	21	31	40	40	40	40
50	5	6	8	10	12	16	22	35	48	50	50	50
60	5	6	8	10	12	16	23	38	55	60	60	60
70	5	6	8	10	13	17	24	40	62	70	70	70
80	5	6	8	10	13	17	24	42	68	79	80	80
90	5	6	8	10	13	17	25	43	73	87	90	90
100	5	6	9	10	13	17	25	45	78	96	100	100
120	5	6	9	10	13	18	26	47	88	111	120	120
140	5	6	9	11	13	18	26	48	92	124	139	140
160	5	6	9	11	13	18	27	49	97	138	157	160
180	5	6	9	11	13	18	27	50	101	148	174	180
200	5	6	9	11	13	18	27	51	105	155	190	200
250	5	6	9	11	14	18	27	53	112	175	228	250
300	5	6	9	11	14	18	28	54	117	189	260	300
350	5	6	9	11	14	18	28	54	121	201	287	350
400	5	6	9	11	14	19	28	55	124	211	311	400
450	5	6	9	11	14	19	28	55	127	218	331	450
500	5	6	9	11	14	19	28	56	129	225	349	500
600	5	6	9	11	14	19	28	56	132	235	379	600
700	5	6	9	11	14	19	28	57	134	243	402	700
800	5	6	9	11	14	19	28	57	136	249	421	782
900	5	6	9	11	14	19	28	57	137	254	437	868
1000	5	6	9	11	14	19	29	57	138	258	450	960
1200	5	6	9	11	14	19	29	57	140	264	471	1102
1400	5	6	9	11	14	19	29	58	141	269	487	1238
1600	5	6	9	11	14	19	29	58	142	272	499	1364
1800	5	6	9	11	14	19	29	58	143	275	509	1489
2000	5	6	9	11	14	19	29	58	143	277	517	1553
3000	5	6	9	11	14	19	29	58	145	284	542	1895
4000	5	6	9	11	14	19	29	58	146	288	558	2108
5000	5	6	9	11	14	19	29	59	147	290	564	2253
6000	5	6	9	11	14	19	29	59	147	291	569	2358
7000	5	6	9	11	14	19	29	59	147	292	573	2437
8000	5	6	9	11	14	19	29	59	147	293	578	2495
9000	5	6	9	11	14	19	29	59	148	294	579	2548
10000	5	6	9	11	14	19	29	59	148	294	581	2588
=	5	6	9	11	14	19	29	59	149	299	598	2995

The table gives:

- (i) the sample size (n) required to be 95% certain of including at least one positive if the disease is present at the specified level
- (ii) the upper limit to the number (d) of diseased animals in a population given that the specified proportion were tested and found to be negative

Examples:

- (i) Expected proportion of positives is 2%.
The population size is 480 – use 500.
From the table, a sample of 129 is required to be 95% certain of detecting at least one positive.
- (ii) For a population of 1.000, a sample of 10% were all found to be negative. From the table, the 95% confidence limit for the number of positives is 29.

According to this expected prevalence and the number of apiaries per region (DSAVR) we propose to execute 3 visits in a total of 145 apiaries (29 per DSAVR) .- see table VI

Table VI

REGIONS (DSAVR)	Beekeepers	Nº apiaries	Nº apiaries to visit	Nº visits
Norte	4.854	8.425	29	87
Centro	6.684	13.120	29	87
Lisboa Vale Tejo	2.306	4.668	29	87
Alentejo	1.666	4.689	29	87
Algarve	893	5.941	29	87
TOTAL	17.291	38.203	145	435

H - Certification of the availability of qualified staff with adequate training in the diagnostic in the area of honey bee health to carry out the tasks

As DGAV has implemented since 2008 the official controls in apiaries, that required training and preparation of staff from regional services of DGAV, we considered that this staff is adequately qualified to perform visits to apiaries according to this programme as well as the diagnosing in the area of honey bee health. To reinforce the implementation of the programme, national training sessions for personnel involved will take place before the beginning of the programme in order to follow Standard Operating Procedures determined by European Commission.

The support laboratory for the analysis will be National Reference Veterinary Laboratory:

Laboratório Nacional de Investigação Veterinária
 Address: Estrada de Benfica, 701 1549-011 Lisboa
 Tel: +351 217115200
 Web site: <http://www.inrb.pt/lniv>

I - Equipment and products needed to carry out the tasks

The National Veterinary Competent Authority (DGAV) has already acquired specific equipment for official controls in apiaries; we consider that it will be sufficient to implement the programme.

