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REPORT OF THE

"SHEEP & GOATS BRUCELLOSIS"

TASK FORCE SUBGROUP

Meeting held in Valladolid, Spain, on 23-24 June 2009

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TASK FORCE SUBGROUPS MEETING HELD IN VALLADOLID, SPAIN, ON 23-24 JUNE 2009

1 AGENDA

See Annex 1.

2 PARTICIPANTS

See Annex 2.

3 INTRODUCTION

The meeting was held in Valladolid, in the Hotel Felipe IV.

The presentations and discussions were either in English or Spanish and a simultaneous translation service was provided. Copies of all the presentations were given to the experts.

4 FIRST DAY

- **Welcome & introduction.**

  Dr. Baudilio Fernandez-Mardomingo Barriuso

  Dr. Lucio I. Carbajo Goñi

  Dr. Olga Mínguez González

  Dr. Agapito Portillo Sánchez

- **Presentation of the Expert Sub-group & Introduction.**

  Dr. B. Garin-Bastuji and experts

Dr. Baudilio Fernández –Mardomingo, General Director of Livestock Productions, Autonomous Community of Castilla y León, chaired the opening session. As representative of the region hosting the meeting, he gave the welcome to the Task Force Subgroup, to the Spanish experts, and to the Official Veterinary Services participating in the meeting, as well (Central Veterinary Office, Autonomous Community of Castilla y León, and Autonomous Community of Castilla La Mancha). He highlighted the strategic importance of sheep and goats sector in the agriculture of the region, and the effort made by his Administration in combating Brucellosis during the past decades.

Dr José Luis Paramio Lucas, as representative of the Chief Veterinary Officer, thanked everybody for participation and wished a fruitful meeting.

Dr Olga Mínguez, Chief of the Regional Veterinary Office of the Autonomous Region of Castilla-León, welcomed everybody and chaired the first working session.

Dr Bruno Garin-Bastuji, chairman of the Sheep and Goats Brucellosis Subgroup, thanked all the speakers and remind the scope of the meeting, as an action to advice the Spanish Administration in improving the Spanish National Eradication Programme.

- **Structure and organization of the Official Veterinary Services in Spain. Other veterinary services in the implementation of the sheep and goats brucellosis programme.**

  José Luis Paramio Lucas. Subdirección General de Sanidad de la Producción Primaria. Ministry for Environment, Rural and Marine Affairs (MARM).

The former Ministry of Agriculture, Fisheries and Food (MAPA) has been recently modified according to a new political design.

The current Ministry for Environment, Rural and Marine Affairs (MARM) is responsible for public policies related to livestock production, including animal health issues: basically, strategic design, planning, programming, co-ordination and monitoring of actions and activities (Central Competent Authority). The implementation of animal health policies at regional level is under the responsibility of the Autonomous Communities.

The Subdirección General de Sanidad de la Producción Primaria (General sub-direction of Safety the Primary Production) is the body of MARM with responsibilities in Animal Health issues (Chief Veterinary Officer):

- Design and co-ordination of Animal Health Policies.
- Co-ordination and supervision of intra-community trade and trade with Third Countries of animal and non-food animal products.
- Co-ordination of information systems and management of National Databases and Rapid Alert Systems.
- Co-ordination of National Reference Laboratories.
- Co-ordination of Animal Hygiene Actions and Official Controls at production level.
- Co-operation with Autonomous Regions in developing regional policies.
- Co-ordination and co-operation with extra-national bodies: European Union, OIE, WHO, FAO etc.

Regarding the responsibilities of the Spanish Veterinary Services in the context of the Spanish Sheep and Goats Brucellosis Eradication Programme 2009-2010, a number of items could be highlighted:

a) Competent Central Authority is responsible for the supervision and co-ordination of the Programme, and for reporting to European Commission as well: Subdirección General de Sanidad de la Producción Primaria (Spanish CVO).

   Since 2001, there is in operation a National Committee (Comité del Sistema de Alerta Sanitaria Veterinaria) chaired by the Spanish CVO and with the participation of all the Regional Veterinary Services. This Committee is on the top of the decision-making process, and gathers and evaluates all the relevant sanitary and epidemiological information and decides about corrective measures, if necessary.

b) Regional Competent Authorities are organized following a common pattern, although some specific differences may exist by operative reasons:
   - Chief of the Regional Animal Health Service
   - Chief of the Local Animal Health Service (Provincia)
   - Local Veterinary Units (LVU) (Comarca). The Veterinary Officers of LVU are responsible for the implementation and control of the programme at the local level.

Field teams could be integrated by Veterinarian of Public Enterprises, or in that areas with a solid development of Farmers Sanitary Associations (ADS) by authorized Veterinarians working for them.

The Regional Veterinary Services implement the programme according to operational manuals that include activities of inspections and audits of all actors.

Conclusions

The organization of the Official Veterinary Services in Spain is consistent with the political design of the State, and in general terms, adequate to face with success a complex action like the Spanish Sheep and Goats Brucellosis Eradication Programme. Given that the implementation of the programme is an exclusive responsibility of the Autonomous Regions, extra-effort is made regarding co-ordination at all levels of the Administration (Central Level, Regional Level, Province Level, UVL).

The role of Public Enterprises at local level is considered of paramount importance in order to speed up the implementation of the programme and increase its efficacy: coverage of checks, reduction of the time period diagnosis-animal culling, vaccination coverage, etc.

- **Evolution of the National Eradication Programme on sheep and goats brucellosis in Spain. Epidemiological situation. The National Eradication Programme 2008-2010.**
  Dr. José Luís Sáez Llorente. Subdirección General de Sanidad de la Producción Primaria (MARM).

Introduction.

Spanish sheep and goats population not only represent a high relative weight in the Spanish livestock but also is one of the biggest in the EU due to its census (23,895,607 in 2007), number of holdings (124,758 in 2007) and production. In 2007, 117,672 holdings and 19,504,820 animals where included in the Eradication Programme. Currently the coverage of the Programme is closed to 95 % of holdings and 87 % of heads. This shows the great effort made by the Spanish Administration when the figures are compared with the outcome from the beginning of the 90s' (41,000 holdings and 2 million animals checked).

General objectives and master lines of the Programme.

The final goal of the Programme is to reach the epidemiological requirements of EU legislation to declare Spain Brucellosis Officially Free (up to 99.8% BmOF flocks, absence of clinical signs of Brucellosis and Bacteriological isolation during the last 5 years and withdrawal of the vaccine in the last 3 years).

The master lines could be summarized as follows:

- Regional strategies adopted according to epidemiological situation and the various managements systems of small ruminants.
- Test-and-slaughter of positive animals and compensation of farmers.
- Vaccination of animals in regions or areas according to the epidemiological risk.
- Stamping out in almost free areas.

Current situation.

Geography in Spain is very diverse and, accordingly, are small ruminants breeds and management systems. This determines brucellosis epidemiology and must be taken into account when defining eradicating strategies. Roughly, Spain could be divided into a Dry Spain (Center and South), a Humid Spain (North), a Mediterranean Spain and, finally, the Islands.

Canary Islands are BmOF and the objective there is to keep this status according to Directive 91/68/EC.

In Balearic islands, Asturias, Navarra and Bask country, with a 0% flock prevalence, the target is to reach the BmOF status in 2010. The main strategy is to increase checks, and stamping-out measures. Vaccination is forbidden, except in emergency cases.

Aragón, Cantabria, Castilla La Mancha, Castilla León, Extremadura, Galicia and La Rioja have reached a flock prevalence lower than 2.5%. The target is to reach the eradication conditions in 2010 by speeding up the qualification of holdings. Vaccination is forbidden in M3 (BmF) and M4 (BmOF) "Local Veterinary Units" (UVL) (except when an epidemiological risk do exist), and compulsory in the other areas. Stamping-out is implemented in M3 and M4 areas and test-and-slaughter in the other areas (every 3 months).

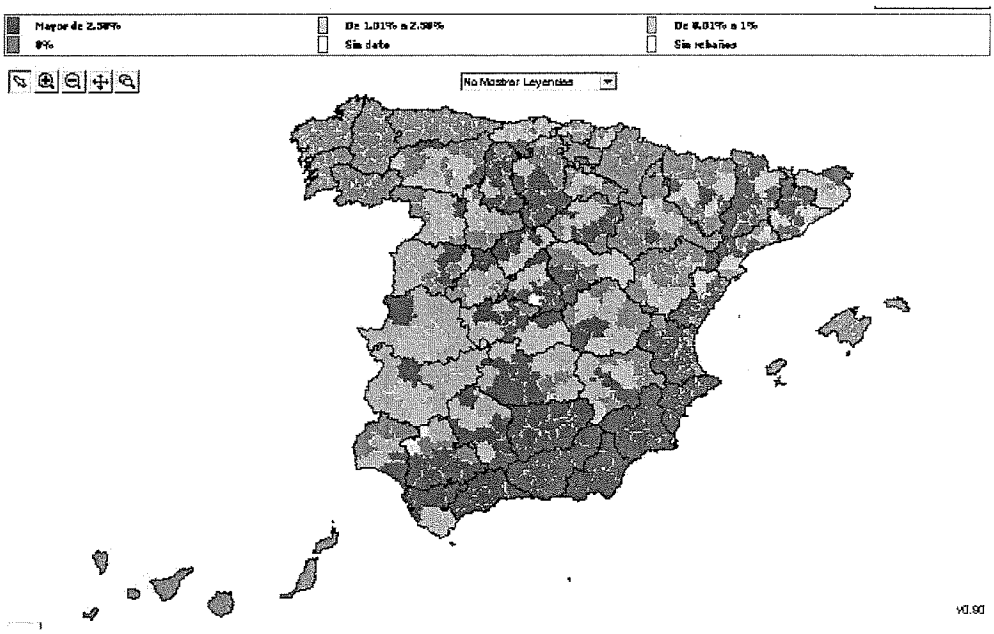
Finally, the flock prevalence is above 2.5% in Andalucía, Cataluña, Madrid, Murcia and Valencia. All these regions belong to Mediterranean Basin. The target is to reach pre-eradication conditions in 2010 (prevalence less than 0.5%) by an effective control of the disease in highly infected areas with vaccination. The programme is here based on vaccination of replacement, test-and-slaughter and qualification of holdings. Mass vaccination is foreseen in the Programme for highly infected UVL (prevalence > 4.42%) but is not currently implemented.

The huge reduction of Brucellosis Human cases (8,500 cases in the 80s' vs. 246 cases in 2007) with a solid decreasing trend in the last decades gives an idea of the success of the programme.

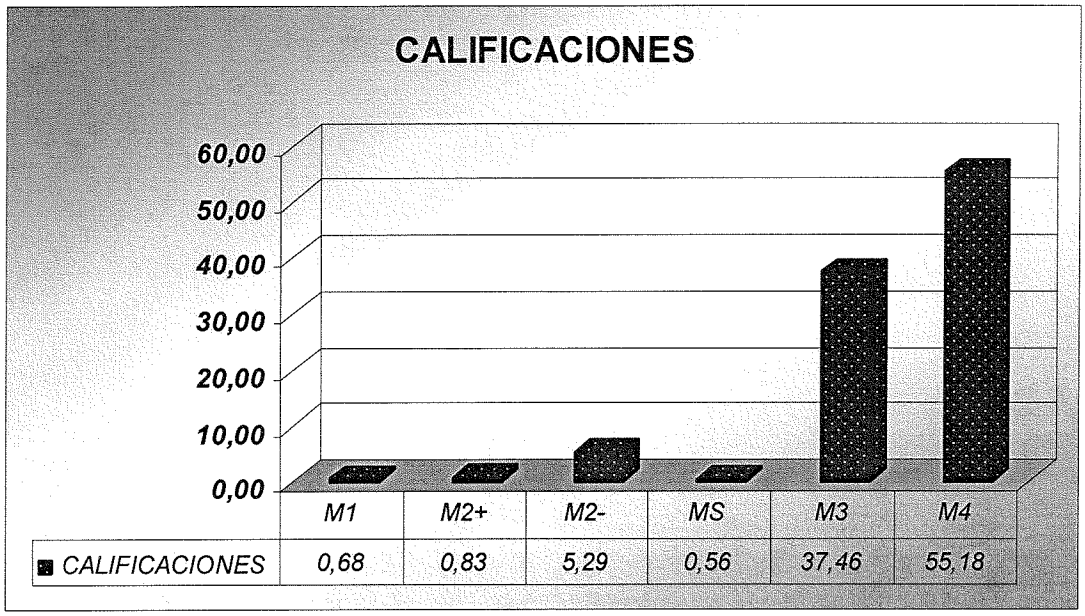
The evolution of flock prevalence and incidence (flocks) as well as the geographical distribution of the disease could be summarized in the following tables and figure:

CCAA	EVOLUCIÓN DE LA PREVALENCIA DE REBAÑO (EN %)							
	2001	2002	2003	2004	2005	2006	2007	2008
ANDALUCÍA	27,11	21,62	12,72	12,97	13,76	11,56	10,39	8,55
ARAGÓN	32,30	15,14	11,83	5,97	4,18	1,59	1,52	0,70
ASTURIAS	0,16	0,05	0,01	0,00	0,00	0,00	0,00	0,00
BALEARES	0,20	0,00	0,00	0,00	0,00	0,00	0,00	0,00
CANARIAS	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
CANTABRIA	3,71	2,63	1,90	1,05	0,55	0,49	1,39	0,78
CASTILLA LA MANCHA	8,93	7,96	6,58	7,52	6,09	3,55	2,10	2,11
CASTILLA Y LEÓN	15,28	9,60	6,95	6,37	3,72	1,97	1,80	1,51
CATALUÑA	27,15	19,09	20,69	17,51	14,06	9,53	5,44	3,14
EXTREMADURA	5,60	4,34	3,07	3,68	3,18	2,22	1,84	0,95
GALICIA	0,29	0,18	0,08	0,03	0,04	0,01	0,01	0,00
LA RIOJA	6,73	9,42	10,00	8,50	2,54	1,11	1,61	0,70
MADRID	15,03	5,10	7,89	5,60	4,69	6,44	3,42	4,01
MURCIA	18,22	0,14	8,02	6,15	4,71	3,96	8,70	7,00
NAVARRA	1,08	1,30	0,40	0,13	0,09	0,00	0,00	0,00
PAÍS VASCO	0,09	0,42	0,44	0,31	0,08	0,12	0,00	0,15
VALENCIA	39,48	26,44	22,35	15,42	15,13	8,10	3,74	7,58
TOTAL	**11,97**	**7,18**	**5,58**	**5,12**	**4,43**	**3,20**	**2,79**	**2,13**

CCAA	EVOLUCIÓN DE LA INCIDENCIA DE REBAÑO (EN %)							
	2001	2002	2003	2004	2005	2006	2007	2008
ANDALUCÍA			3,25	4,00	4,67	3,38	6.36	7,25
ARAGÓN			2,13	1,17	1,53	0,37	0.35	0,42
ASTURIAS			0,01	0,00	0,00	0,00	0.00	0,00
BALEARES			0,00	0,00	0,00	0,00	0.00	0,00
CANARIAS			0,00	0,00	0,00	0,00	0.00	0,00
CANTABRIA			1,62	0,81	0,52	0,34	1.36	0,59
CASTILLA LA MANCHA			0,00	2,90	3,77	1,32	0.52	0,89
CASTILLA Y LEÓN			3,88	3,85	2,13	0,81	1.46	1,28
CATALUÑA			5,84	6,81	3,97	4,11	2.06	1,49
EXTREMADURA			1,04	1,23	0,90	0,16	0.07	0,24
GALICIA			0,05	0,02	0,03	0,01	0.01	0,00
LA RIOJA			6,62	3,44	0,63	0,89	1.38	0,70
MADRID			3,28	2,64	2,34	5,39	1.58	2,09
MURCIA			5,01	3,74	2,98	1,69	3.83	3,96
NAVARRA			0,00	0,09	0,09	0,00	0.00	0,00
PAÍS VASCO			0,44	0,31	0,00	0,00	0.00	0,15
VALENCIA			4,56	5,05	3,22	4.70	2,27	1,61
TOTAL			1,68	1,95	1,69	1,02	1,46	1,51



Another remarkable outcome of the Programme is the number of holdings already qualified BmOF or BmF as shown in the following figure:



Measures recently introduced to improve the programme that proved efficient are:

- Reduction of the time period between laboratory diagnosis and slaughter of positive reactors to less than 15 days;
- The offspring of positive reactors is compulsorily slaughtered, when it is possible to trace it;
- Promotion of bacteriological diagnosis (sampling procedures, laboratory investment in high containment facilities or equipments, training of personnel, standardization of protocols);
- Control of movement to common pastures. The various small ruminants management systems in Spain are based on a daily or periodical movement of flocks for grazing, most of the time to communal lands. The introduction of a rationale in the movement of the flocks has revealed very effective in areas where the infection rate is low.
- Pre-movement test of 100 % flocks 30 days before the transportation to other holdings or prior to the transhumance period.
- Implementation of epidemiological investigations in all flocks with suspended status for a trace-back / trace-forward of infection, and also in endemic areas to identify risk factors. A group of expert at central level has worked in a Manual of Procedures and also in a template to validate the epidemiological inquires on the web-site. The system will be operating during this year 2009.
- Re-enforcement of vaccination policy for controlling the disease in highly infected areas. The minimum period of administration of the vaccine in a flock is 5 years. Mass vaccination in foreseen in emergency cases although it has not been applied in the last years. It is considered that, despite the improvement, more effort has to be made in this particular item.
- Monitoring of brucellosis in wildlife. There has been conducted some collaborative studies amongst some Autonomous Communities, Universities and other Research Organisations, to estimate the epidemiological role of wildlife in potential maintaining the disease in domestic animals.
- Periodical audits of field teams.

Conclusions

During year 2008 the positive trend in reducing prevalence and incidence of the disease is maintained.

There is a significant number (5) of Spanish communities that are in the position of obtaining the qualification of BmOF Region if the effort is maintained or improved.

In some regions with not so favourable figures it is necessary to evaluate the problems encountered in applying test-and-slaughter policy and vaccination schemes.

In general, it is urgent to solve the problem related to the suspicion of False Sero-Positive Reactors by combining different serological tests and to improve the bacteriological protocols to study the disease. The solid recognition of free areas will allow focusing efforts and resources in the problematic areas.

- **The eradication programme of Sheep and Goats brucellosis in the autonomous Community of Castilla y León. Evaluation of the epidemiological situation and implementing measures.**
  Dr. Olga Mínguez González. Head of Animal Health Service. Autonomous Community of Castilla y León.

The Autonomous Region

Castilla Y León Autonomous Community (CyL AC) has a territory of 94,224 Km^2, 31 % of which above 1000 m altitude and a population of 2,557,330 inhabitants. Agriculture is the main economic activity but climate and geography play important roles in the success of production systems. Animal production is 44.85 % of the value of agriculture production of the region (10 points above national average) which is over 4,450 million €. In the animal production value, small ruminant milk is about 13 % and meat is almost 9 %.

Small ruminant production

This AC has almost 20 % of reproductive female sheep of Spain and is the most important AC in terms of animal production. CyL has about 4,353,000 sheep in 13,700 farms. It is also the region with the most important sheep milk production, with 1,380,700 females and 3,570 farms.

Comparing CyL with the national numbers, this AC has 62 % of sheep kept for meat production and 38 % of those kept for milk production.

In the last years, a slight tendency of decreasing of number of farms has been noticed but not on the number of animals, favouring an increase of animals per farm (about 300 reproductive females per farm).

Most milking animals are from local breeds as Churra, Castellana, Manchega and some from Assaf and Lacaune. Meat breeds are Merino, Landchaf, Merino Precoz and crosses.

Caprine production comprises only 177,000 animals in 2,000 farms.

Veterinary Services

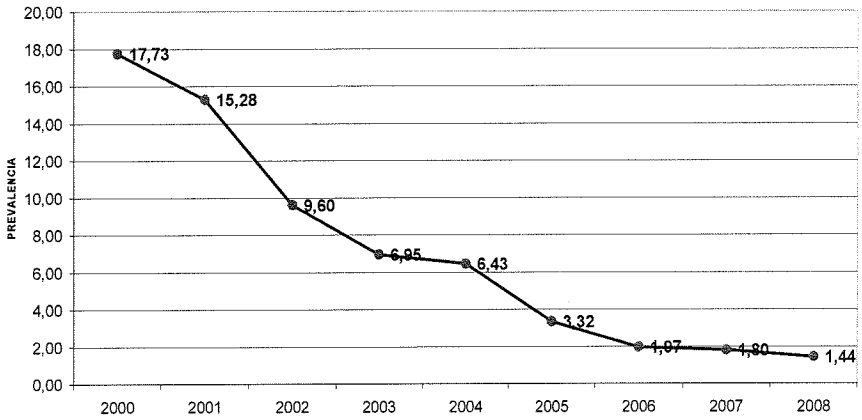
The Veterinary Services of CyL are within the General Directorate of Agricultural Production of the AC, with 3 important Services "Animal Health", "Resources for Livestock Production" and "Planning and Livestock Sanitary Structure" with the respective Provincial Services.

Human resources at provincial level comprise 82 veterinarians and 20 livestock controllers. At local level are 103 UVL where309 "Official vets" are working , 26 "Livestock controllers", and 210 contracted veterinarians. The implementation of sheep and goats Brucellosis Eradication Program is also supported by the ADS (Health Defence Groups), in 58 % of farms. Nine provincial laboratories work for animal health, involving 205 people (analysts, technicians, auxiliary personnel and other). These labs are coordinated by a "Animal Health Laboratories Network Coordinator".

The CyL AC is undergoing an important economic effort to strengthen its veterinary services.

Brucellosis

The prevalence of small ruminants (SR) brucellosis at farm level has been decreasing steadily over the past years. Flock prevalence decreased from 17.73 % in 2000 to 1.44 % in 2008 (see next figure). In 2008, animal prevalence was 0.05 % and 215,714 animals were slaughtered with a compensation always under market value.



The decrease in the prevalence of SR brucellosis is followed by a decrease in human reported cases, 0.86 per 100,000 inhabitants (above the national figure of 0.35 per 100,000 inhabitants).

Measures adopted for SR brucellosis eradication

Between 1997 and 2001 the main investments were on individual animal identification, registration of all farms and the development of a computer-based information system (SAGA) to manage the ruminant's diseases eradication programmes. This information system has the advantage of integrating the health history of all farms and its classification, allowing an rapid and efficient data transmission and the traceability of individual animals. This permits a better control of the time intervals between activities (testing, slaughter, follow-up) required for the correct implementation of the programme, the identification of problems and the control of animal movements connecting farms.

Nine steps are fundamental for the field data entering in the SAGA: 1. Data capture of target herds at the UVL; 2. Data loading to the laptop; 3. Automatic reading of animal identification code in the farm; 4. Blood sampling and registration; 5. Printing of herd registration sheet on the farm; 6. Deliver samples and registration sheet to the Lab; 7. Data loading into the Central Database System; 8. Data transmission to the Lab for the issuing of results; 9. Data transmission from the Lab to the Central Database System.

In 2002, due to the slow progress of the programme, a decision was made to increase the sensitivity of diagnosis and the laboratory procedure was changed to use not only RBT but also CFT in all samples. The use of tests in parallel allowed an increase in 11.6 % of the diagnosis sensitivity with a loss of only 2.5 % in specificity.

In 2004, a new boost was made to the SR brucellosis eradication programme with the issuing of new regulations, the "Order AYG/162/2004, 31st January", which differentiates measures according to the classification of municipalities regarding the disease.

One of the most important and difficult to apply measure was the pasture classification and the interdiction of use of common pastures to flocks with lower classification.

Other measures were those directed to protect free flocks from infection and disease spread, namely: *i*) Shorten the period from test results to slaughter to 15 days; *ii*) Mark and slaughter the positive animals at the farm (reducing errors and frauds and speeding the process); *iii*) Increase the re-testing of positive flocks, with time intervals of 2 months between tests up to 2 negative consecutive results, what means that each positive flock is checked at least 3 times a year.

In 2004, a surveillance plan of brucellosis in wild animals was also implemented checking from 226 to 445 samples annually from different animal species. Only in 2005 positive results (7 in wild pigs and 1 in wild ruminants) were obtained.

In 2006, an important investment was carried out in the electronic identification of SR with ruminal bolus; 1,403,640 heads were identified (39 % of CyL population, above the national level, 26 %).

In 2006, another problem was identified in the implementation of the programme: a high number of farms appeared with only one positive animal, without bacteriological confirmation of brucellosis. The false-positive serological reactions have been increasing in the last years. In 2008, 460 farms were analysed (687 samples) and only 1 was truly positive (*B. melitensis* biovar 1).

As the slaughter is carried out in the farm, sampling for bacteriology is not systematic, because of biosafety. Some samples are collected at the rendering plant where carcasses are sent for destruction.

The vaccination programme in 2006 and 2007 allowed the exclusion of vaccination when the positive flocks were dispersed in a region, which is the case of CyL. Furthermore the prevalence is decreasing, allowing for 43 UVL to be considered M3 (BmF) or M4 (BmOF), and false positive serological reactions have been of more importance since 2006. Therefore the vaccination programme is being reduced.

Depopulation of infected flocks is carried out only if the UVL has 0 % prevalence and *Brucella* is isolated, regardless of the size of flock. This decision is centralised at the Regional level.

The 2008 classification of the 11,495 farms is the following: 9,272 BmOF (80.7 %); 1690 BmF (14.7 %); 97 suspended (0.8 %) and the remaining 436 (3.8 %) infected or not qualified. The distribution of qualified local units is presented in the following map.

In January 2008, a protocol for the integrated official control of food chain was established. This protocol aims at providing feedback from problems of identification at the slaughterhouse, in order to implement a more efficient control of each disease, with integrated measures and a good flow of information. The coordination committee includes the chief of Animal Health Service (Servicio de Sanidad Animal), the chief of the Official Sanitary Control and Surveillance (Servicio de Vigilancia y Control Sanitario Oficial), a representative of Animal Health Service of the Autonomous Region (SSA de la Consejería de Agricultura y Ganadería) and a representative of the Health Protection and Food Safety Agency (SVCSO de la Agencia de Protección de la Salud y Seguridad Alimentaria).

As conclusion remarks, it was referred that the eradication programme was progressing well in CyL AC with a steady decrease of prevalence. A closer epidemiological follow-up is required as well as the adaptation of measures to the program results, with special relevance to diagnostic methods. The vaccination policy of young females with Rev.1, needs to be revised, excluding most of the Region.

Future plans include the increase of sampling at the rendering plants and the increase of bacteriological diagnosis and the development of the information system (SAGA) to include an abattoir module to incorporate sampling and sanitary data collected at slaughter.

- **The eradication programme of Sheep and Goats brucellosis in the autonomous Community of Castilla La Mancha. Evaluation of the epidemiological situation and implementing measures.**
  Dr. Pilar García Jané. Head of Animal Health Service. Autonomous Community of Castilla la Mancha.

The Region

The Autonomous Community of Castilla la Mancha (C-M AC) is composed by 5 provinces, with an area of 79,463 km2, an average altitude of 600 m and over 98 % of its territory classified as rural.

Temperature have wide variation over the year (reaching 50 °C) and rain is irregular (400 ml/year). This AC has 1,932,261 inhabitants (low density) and a problem of ageing population.

The Veterinary Services

The Veterinary Services are within the General Directorate of Agriculture and Livestock Production of the AC (responsible for planning at Regional level), which includes the services related to agriculture products and 3 Services related to animal production, namely "Animal Health", "Production and Animal Welfare" and "Livestock Production" each with 2 technical services. There are 5 Provincial Delegations (responsible for the programmes coordination and management) with their "Comarcas" (52 OCA) (responsible for implementation and control of the programmes). At field level, veterinary services are constituted by the ADS (230 for sheep and goats) and a Public Company.

There are 4 laboratories at provincial level coordinated by the provincial laboratory.

The veterinary services quality is supported by several mechanisms. There is a system of internal auditing, directly under the supervision of the "Consejeria" of Agriculture and Rural Development. The C-M AC also has a Regional Commission for Food Quality and Safety (Decree 97/2001) which provides uniform criteria for food safety requirements and present a working group on zoonosis. Another Autonomous Organism of Natural Environment is also collaborating with the official veterinary services as well as Game Resources Institute and National Reference Laboratory for Brucellosis (Santa Fé).

The official veterinary service also collaborates with the national diseases alert and reporting systems, in education programmes and in abattoirs control.

A protocol for inspection of field teams was also implemented. In 2008 there were 72 inspections to authorised veterinarians and 81 inspections to ADS.

Animal Population

The SR population of C-M is almost 3,439,900 animals distributed by 7,580 farms, with an average of 414 animals per farm.

Brucellosis

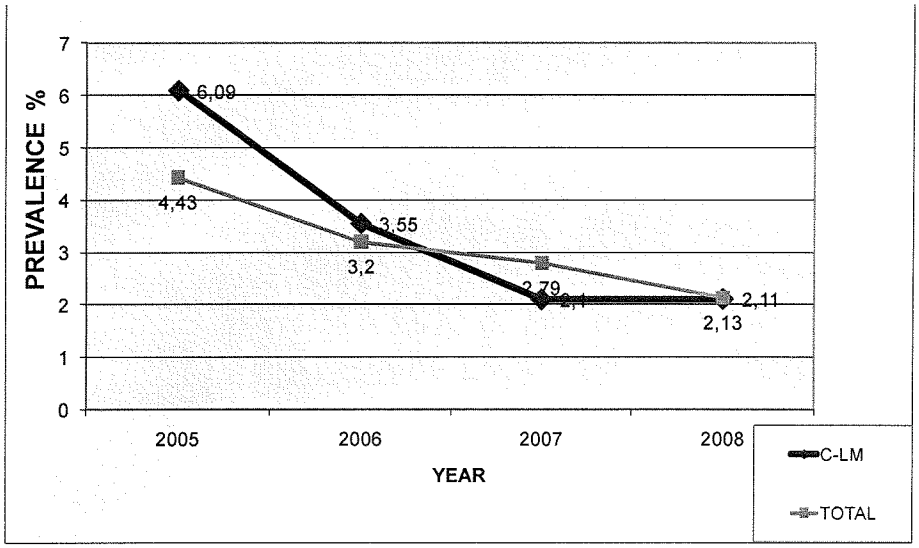
The C-M AC is classified as a "low prevalence" (below 2.5%) region at national level, therefore implementing the programme dedicated to this type of region.

In 2008, animal prevalence was 0.12%, (3,341 positive animals) and 9,968 animals were slaughtered.

Flock prevalence decreased from 6.09% in 2005 to 2.11% (150 positive flocks) in 2008 (see following figure). In this year 18 flocks were depopulated.

Human reported cases decreased from 48 in 2005 to 14 in 2008 (0.69 per 100,00 inhabitants), above national level (0.35 per 100,000 inhabitants).

The next graphic shows the evolution of prevalence im C-M AC and at national level:



Brucellosis eradication programme

The programme follows national and regional legislation.

In terms of responsibilities, the Regional Services are responsible for the approval of the programme, the elaboration of norms and instructions, general planning, authorization of depopulation of flocks, compensation payment, sanitary information, management of the information system and financial management.

The 5 Provincial Delegations have the responsibility of planning and organizing activities and controlling the programme, accreditation of the veterinarians working for the programme, planning of farm checks, provision of materials, communication with producers, formulate proposals for depopulation of farms, proposals for compensation payment and production of sanitary information.

The OCA (Comarca) have to implement the programme and coordinate activities, communicate results, mark and control the slaughter positive animals, elaborate the processes for compensation, control the authorised veterinarians working for the programme, elaborate the certification of flocks, control animal movements, produce sanitary information and respond in case of sanitary alert.

The authorised veterinarians from ADS have to implement the programme in M3 (BmF) and M4 (BmOF) farms and the Public Company in non-qualified flocks. This includes identification of animals, collection and shipment of samples to the laboratory, vaccination of replacement animals in non-qualified flocks, marking positive animals, control of cleaning and disinfection, certification of the accomplishment of the programme and elaboration of sanitary information.

All the animal population over 6 months (or 18 months for vaccinated animals) is submitted to annual screening. Positive flocks are checked every 3 months by personnel from the official veterinary services.

Brucellosis diagnosis is based on serology (RBT and CFT) and bacteriology. In 2008, 2,853,883 RBT tests were carried out as well as 49,112 CFT.

Positive results lead to notification and slaughter at the farm within 15 days and without previous warning. Carcasses are removed and destroyed as Category 1 sub-products without costs to the farmer. This approach has

lead to a decrease of the number of days the positive animals stay at the farm and a faster clearance of infected flocks.

Positive results also lead to a rapid transmission of information from the laboratory to the OCA and to farmers, for the adjustment of sanitary classification and restriction of movement of animals and products. Dairy companies are also informed in case of milking flocks.

In positive farms there is sample collection from positive animals for bacteriological examination, biosecurity is re-enforced and cleaning and disinfection is carried out.

Flocks within 1 km radius around the positive farm are also checked to confirm/avoid the spread of disease.

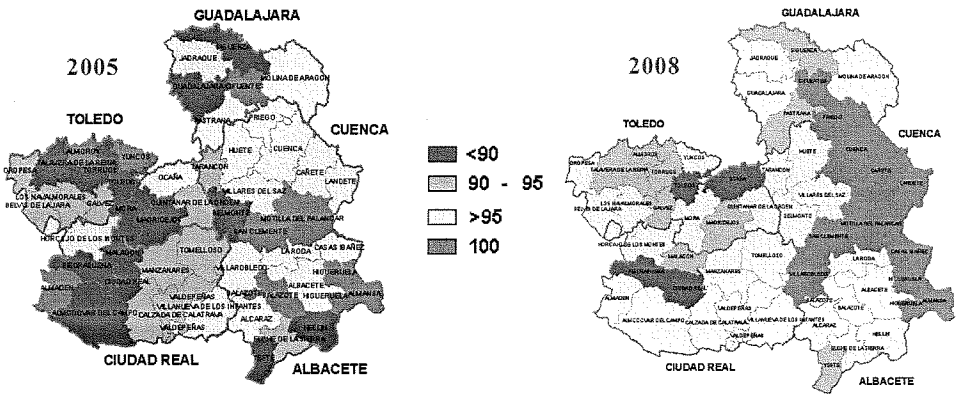
Pre-movement check is another measure implemented, at least 30 days before the departure to communal pastures or transhumance in order to avoid the spread of brucellosis. In 2008, 384 farms were checked and 2 were positive (60,316 animals, 3 positive).

Young replacements vaccination programme includes non-qualified flocks. It was observed in 2008 that several farms did not maintain animals for replacement which difficult the calculation of animals to be vaccinated. Epidemiological studies are being carried out to evaluate the advantages of vaccinating replacement animals within 1 km of a positive farm regardless of its classification.

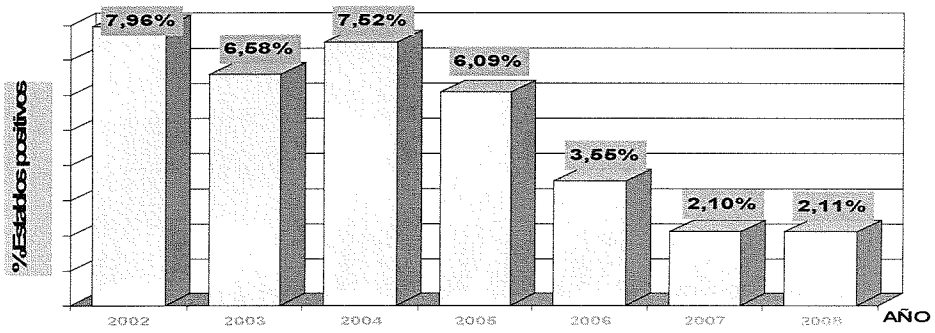
Additional measures included in the programme are the application of RB and CFT to all animals of positive flocks (parallel testing), the development of individual epidemiological studies and the consideration of adequacy of depopulation (farms with positive bacteriology, 3 consecutive positive checks and within farm prevalence over 5 % of animals).

Maps and charts were finally presented that gave details of the SR brucellosis situation in C-M, including an analysis on the progress of the time intervals between sampling and laboratory results and from laboratory results to the slaughter of positive animals.

The following maps and figure give an idea of the important progress made during the last 4 and 6 years respectively.



S&G Brucellosis in Castilla La Mancha: % of BmF and BmOF flocks



Evolution of the flock prevalence of S&G Brucellosis in Castilla La Mancha (2002-2008)

5 SECOND DAY

- **The Bacteriological Diagnosis of Brucellosis in Spain – Results.**

  Dr. D. Fulgencio Garrido Abellán, Head of the NRL, Granada, Santa Fé, MARM.

The NRL has implemented techniques for isolation and biotyping of *Brucella* according or close to Internationally standardized procedures, with training of the regional laboratories by the NRL for *Brucella* isolation. The consequence is an apparent increasing number of strains isolated from small ruminants in the past years with a better knowledge of the species and biovars in cause. *B. melitensis* biovar 3 is the most frequently isolated strain form small ruminants in Spain. The following table shows the details of the strains isolated in Spain and identified by the NRL in 2008. However, despite in high numbers, the strains received in the NRL come from a limited number of communities (most are from Andalucía where the NRL is situated), some communities with a remaining prevalence rate of brucellosis having not or rarely isolated and/or sent *Brucella* strains to the NRL for typing.

CC.AA.	SHEEP					GOATS					UNKNOWN		TOTAL	Flock Prevalence 2008
	B. melitensis					*B. melitensis*					*melitensis*			
	bv. 1	bv. 2	bv. 3	Rev. 1	unknown	bv. 1	bv. 2	bv. 3	Rev-1	unknown	bv. 1	bv. 2		
ANDALUCIA	23	2	187	4	5	74	3	46	16	5	2	3	370	8,55
ARAGON													0	0,70
ASTURIAS													0	0,00
BALEARES													0	0,00
CANARIAS													0	0,00
CANTABRIA													0	0,78
C-LA MANCHA	2		15					1	2				20	2,11
C-LEON													0	1,51
CATALUÑA			7	14				2					23	3,14
EXTREMADURA													0	0,95
GALICIA													0	0,00
LA RIOJA													0	0,70
MADRID	3			4									7	4,01
MURCIA	12		25	4					2				43	7,00
NAVARRA				1									1	0,00
PAIS BASCO													0	0,15
VALENCIA	13		23					2					38	7,58
	53	2	257	27	5	74	3	51	20	5	2	3	502	
	Sheep: 344 strains					Goats: 153 strains								
	497										5			

- **Immuno-diagnosis of sheep and goats brucellosis in the context of eradication campaigns – Inter-laboratory ring-trials.**

  Dr. Manuel Durán Ferrer, NRL, Granada, Santa Fé, MARM.

The NRL is a service of the Central Veterinary Services of the MARM and is participating to the veterinary laboratories network that includes also regional (n=17) and local (comarca/provincial; n= 33) vet. laboratories. The NRL organizes training sessions (once a year), workshops and meetings and gives continuous technical advices to improve the personnel skills and the quality assurance. The NRL is also is in charge of the following tasks:

- Control of diagnostic reagents
- Establishment of national standard sera and working sera
- Edition of SOPs
- Organisation of Proficiency ring-trials (50 participants, all with good results)
- Confirmation diagnosis (investigation of singleton reactors, large outbreaks)

The NRL has regularly participated to collaborative trials organized at European (VLA, UK then CRL, AFSSA, France) and international level (OIE). It gave satisfactory results to the last EU CRL proficiency ring-trial (2007-2008).

The NRL has also recently implemented some molecular typing methods (MLVA and Bruce-Ladder) and participated in the development and validation of a commercial kit of Bruce-Ladder as well as collaborative ring-trials organized in Spain with a limited number of laboratories as regards Bruce-ladder and MLVA (organized by FLI, Jena, G.). Finally, the NRL has started a study project on quantitative real-time PCR.

- **Descriptive epidemiology of Brucellosis in Wild Ruminants in Spain.**

  Dr. Mariana Boadella, Instituto de Investigación en Recursos Cinegéticos (Wildlife Research Institute), University of Castilla La Mancha, Ciudad Real.

A joint research project aiming at describing brucellosis epidemiology of Brucellosis in wild ruminants in Spain was presented. Wild ruminants present in Spain are:

- Cervidae
  - Sub-familly Cervinae
    - *Cervus elaphus* (Ciervo) – Red deer
    - *Dama dama* (Gamo) – Fallow deer
  - Sub-familly Odocoileinae
    - *Capreolus capreolus* (Corzo) – Roe deer
- Bovidae
    - *Capra pyrenaica* (Cabra montés) – Iberian ibex
    - *Rupicapra pyrenaica* (Rebeco/Sarrio) – Southern chamois
    - *Ovis aries* (Muflón) – Mouflon
    - *Ammotragus lervia* (Arrui) – Barbary sheep

In Spain, limited contacts could occur between wildlife and domestic ruminants in some areas, especially when fencing is in place and/or artificial feeding is practiced, increasing population density.

Wild ruminant populations (2579 bovidae and 6448 cervidae) were investigated (iELISA) for brucellosis in several Spanish regions with the collaboration of laboratories of CITA (Aragon), Neiker (Bask country) and SERIDA (Asturias).

The global sero-prevalence was estimated 0.3-0.6 % what is compatible with the specificity limits of the test. There was no spatial aggregation and highest prevalence rates were observed in chamois (1.4 %) and red deer (1.9 %). When samples were available (93 animals), bacteriological examination was performed and resulted in isolation of one *B. melitensis* (Iberian ibex with clinical signs) and one *B. abortus* (red deer) strain.

Nombre común	Nombre científico	Prevalencia	IC 95%	Muestras de tejidos enviadas para cultivo	Aislamientos obtenidos
Arrui	*Ammotragus lervia*	0	0-36	0	
Rebeco[1]	*Rupicapra pyrenaica*	0.8	0.4-1.4	11	0
Cabra montés[2]	*Capra pyrenaica*	0.1	0-0.6	1	1 *B. melitensis*
Muflón	*Ovis aries*	0	0-5	0	
Corzo	*Capreolus capreolus*	0	0-1	0	
Ciervo	*Cervus elaphus*	0.4	0.3- 0.7	81	1 *B. abortus*
Gamo	*Dama dama*	0	0-1	0	
TOTAL		0.4	0.3-0.6	93	2

As a conclusion of this study:

- The geographical distribution of prevalence rates in wild ruminants is specially due to sampling pressure rather tan real sero-prevalence differences
- Wild ruminants did not appear to be a reservoir of the disease but could serve as sentinels
- The clinical case observed demonstrates the importance of passive surveillance of wildlife.

6 GENERAL DISCUSSION – CONCLUSIONS & RECOMMENDATIONS OF THE SUB-GROUP

General conclusions

The sub-group acknowledged that:

- Significant progress has been made in the eradication of S&G brucellosis at national level;
- The level of control that the central veterinary services have over the progress of the programme has duly increased;
- The level of investment in human resources and information management has been improved;
- There is added value in the external auditing of the veterinary services and in the inspection of the field teams;
- A well-advanced system of electronic identification of individual animals is being put in place;
- The level of understanding and monitoring of the progress at both national and regional levels has also increased. However, the analysis of the situation based on sero-positive results could be biased and result in overestimation of the real prevalence and incidence in some almost free areas;
- Although in some previously highly infected regions good progress has been made, there still remains localised areas of high prevalence and incidence which require special attention;
- In some regions the flock prevalence and incidence rates are similar indicating either difficulties in preventing the spread of the disease or interference of false positive serological reactions (FPSR);
- In most regions, and particularly in infected areas, there is insufficient vaccination coverage with targets that are far from being reached. The sub-group recalls that vaccination of replacement animals remains the most effective tool in preventing the spread of the disease and should not be stopped prematurely;
- There are regional initiatives that have proved effective in:
    - accelerating the decrease of prevalence, as for instance parallel testing and enforcement of stamping out policy in Castilla La Mancha;
    - preventing the spread of infection between flocks by a pre-movement test including for transhumance and the qualification of common pastures;
    - monitoring the efficiency of the programme (for instance the evaluation of the time needed from detection to slaughter);

- Regarding laboratory diagnosis:
  - As regards direct diagnosis:
    - The use of culture for confirmation of infection which is really useful especially in the latter stages of an eradication programme, has significantly increased as demonstrated by the increasing number of *Brucella* strains biotyped by the NRL in the recent years;
    - A significant effort has been made by the NRL in implementing the most recently developed molecular methods aiming at detecting and identifying *Brucella* species, biovars and strains;
  - As regards serology:
    - Important efforts have been made by the NRL in order to improve the quality of serological testing and the standardisation of diagnostic tools at National level with the involvement of the NRL in several international collaborative studies;
    - Some regions use RBT as a screening test and CFT only as a confirmatory test, which could result in a lower sensitivity (compared to RB & CFT parallel testing) in detecting infected animals in flocks where infection has been confirmed.
- Finally, important efforts have been taken to verify the status of brucellosis in Spanish wildlife, with no indication that there is a significant risk of the establishment of a wild reservoir at this time.

Recommendations:

1. The prevalence and incidence should be based on real infection rates which would allow better targeting of additional disease eradication measures (stamping out, additional testing, etc.) provided that the definition of infected flocks and infected animals is solidly based (bacteriological investigation and epidemiological assessment, follow-up testing, etc.);
2. As soon as outbreaks are identified, epidemiological investigation should be extended to all epidemiologically-linked flocks rather than based on a limited radius from the farm;
3. In areas (*e.g.* local veterinary units, provinces, etc.) with a low prevalence, it is advisable to intensify measures aiming at achieving final eradication (*e.g.* systematic stamping out of any confirmed breakdown) in the shortest possible time;
4. Guidelines should be drafted at national level on:
   a. Criteria for the definition of infected animals and infected flocks;
   b. Criteria for the identification and clarification of FPSR;
   c. Recommendation of systematic RBT and CFT parallel testing for cleansing infected flocks;
   d. Criteria for bacteriological investigation sampling and culture (type and number of samples, media etc.);

e. Criteria for systematic application of mandatory stamping out in infected areas (that are not already foreseen in the programme);

f. The qualification of common pastures and pre-movement testing of flocks before transhumance.

5. Vaccination of young females should be maintained and reinforced when necessary to decrease the incidence of infection as much as possible, preferably up to the point that *Brucella* is no longer circulating in the respective region. The cessation of vaccination in order to access to the officially free (M4) status should be discouraged in free flocks that remain at-risk.

ANNEX 1

PARTICIPANTS
OF THE "SHEEP & GOATS BRUCELLOSIS" TASK FORCE SUBGROUP MEETING
HELD IN VALLADOLID, SPAIN, ON 23-24 JUNE 2009

- **Task Force Sub-Group**
  - Dr. Bruno GARIN-BASTUJI — Chairman, France
  - Dr. Manuel DURAN FERRER — Spain
  - Dr. Anastasios MINAS — Greece
  - Pr. Dr. Yolanda VAZ — Portugal
  - Dr. Fernando CRESPO-LEON — Spain
  - Dr. Valentina PIAZZA — Commission
  - Dr. James MOYNAGH — Commission

- **Spanish Representatives (main list)**
  - **Ministry of Agriculture [National Veterinary Services]**
    - Dr. Lucio I. Carbajo Goñi
    - Dr. José Luís Sáez Llorente
    - Dr. José Luis Paramio Lucas
    - Dr César Fernandez Salinas
  - **National Reference Laboratory**
    - Dr. D. Fulgencio Garrido Abellán
    - Dr. Manuel Durán Ferrer
  - **Community of Castilla y León (Veterinary services)**
    - Dr. Baudilio Fernandez-Mardomingo Barriuso
    - Dr. Olga Mínguez González
  - **Community of Castilla La Mancha (Veterinary services)**
    - Dr. Agapito Portillo Sánchez
    - Dr. Pilar García Jané
  - **National Wildlife Research Institute (IREC / CSIC-UCLM-JCCM)**
    - Dr. Mariana Boadella

ANNEX 2

AGENDA
OF THE "SHEEP & GOATS BRUCELLOSIS" TASK FORCE SUBGROUP HELD IN VALLADOLID, SPAIN, ON 23-24 JUNE 2009

Tuesday, 23 June

9.30-10.00	Welcome & introduction. Presentation of the Expert Sub-group & Introduction.
10.00-10.30	Structure and organization of the Official Veterinary Services in Spain. Other veterinary services in the implementation of the sheep and goats brucellosis programme.
10.30-11.30	Evolution of the National Eradication Programme on sheep and goats brucellosis in Spain. Epidemiological situation. The National Eradication Programme 2008-2010.
11.30-11.45	Coffee break.
11.45-12.15	Discussion.
12.15-13.00	The eradication programme of Sheep and Goats brucellosis in the autonomous Community of Castilla y León. Evaluation of the epidemiological situation and implementing measures.
13.30-15.30	Lunch.
15.30-16.45	The eradication programme of Sheep and Goats brucellosis in the autonomous Community of Castilla La Mancha. Evaluation of the epidemiological situation and implementing measures.
16.45-17.00	Coffee break.
17.00-18.30	General Discussion.

Wednesday, 24 June

9.00-9.30	The Diagnosis of Brucellosis in Spain – Results.
9.30-10.15	Immuno-diagnosis of sheep and goats brucellosis in the context of eradication campaigns – Inter-laboratory ring-trials.
10.15-10.45	Descriptive epidemiology of Brucellosis in Wild Ruminants in Spain.
10.45-11.00	Discussion.
11.00-11.30	Coffee break.
11.30-13.00	Meeting of the Sub-Group
13.00-14.00	Final meeting: conclusions and recommendations.
14.00-15.00	Lunch