

COMMISSION
OF THE
EUROPEAN COMMUNITIES

SANCO/401/2002

Directorate General for
Health and Consumer Protection

SANCO/E.2

REPORT ON THE

**TASK FORCE MEETING
OF THE
“BOVINE BRUCELLOSI“
AND
“SHEEP&GOAT BRUCELLOSI”
SUB-GROUPS**

SPAIN, CORDOBA

9-11 JULY 2001

REPORT OF THE
“BOVINE BRUCELLOSIS” AND “SHEEP & GOATS BRUCELLOSIS” TASK FORCE
SUBGROUPS
MEETING IN CORDOBA, SPAIN, ON 9-11 JULY 2001

Participants: see Annex 1

Agenda: see Annex 2

Introduction

The meeting was held in Cordoba in the headquarters of the Sub-delegation of the Government in Córdoba. The Spanish Authorities provided one person that provided the Spanish-English simultaneous translation service.

The first day was dedicated to the sheep and goats brucellosis national eradication programme and several examples of local implementation. Bruno Garin-Bastuji chaired this first part of the meeting.

The second day included in the morning a visit to two ruminant flocks (one sheep/goat and one bovine), and a visit to the Regional Laboratory of Animal Health in Andalucía. In the afternoon a paper on the "Compensation rules for the sanitary slaughter in brucellosis of ruminants" in the MS (Member States) was presented and discussed among the members of the subgroups. This was followed by a meeting with the Spanish authorities for clarification of matters and discussion of the sheep and goat brucellosis programmes.

The third day was devoted to discussion on the Spanish national bovine brucellosis eradication programme and local implementation. The Sub-secretary of Agriculture of the Ministry of Agriculture Fisheries and Food and the Sub-delegate of the Government in Andalucía welcomed the subgroups of the Task Force during this meeting and confirmed the need for Spain in pursuing the control of this important zoonosis.

DAY 1

THE NATIONAL ERADICATION PROGRAMME FOR OVINE AND CAPRINE BRUCELLOSIS IN SPAIN - *Ignacio Sánchez Esteban* - *Chief Veterinary Officer* - *Subdirección General de Sanidad Veterinaria (SGSV)*

General aspects on the sheep and goat and bovine brucellosis eradication programmes in Spain

Spain is made up of 17 Autonomous Communities (AC) of which 2 are the islands of Balears and Canaries. The AC have the responsibility for the implementation of the eradication programmes. The Central Veterinary Services of the Ministry of Agriculture (SGSV) are responsible for planning, programming and co-ordination. This co-ordination, and follow up of the eradication programmes is done through the Committee of National Co-operation and Implementation of Eradication Programmes (CNCS), which includes representatives of the Ministry of Agriculture and of the AC.

As a general rule, the Official Veterinary Services of ACs are directly responsible for the implementation of most of the tasks regarding the programme. But depending on the AC, tasks for vaccinating (part), tagging and sampling could be developed under two different systems:

- A first system, mainly implemented in the territories where there is an adequate structure of farmers associations devoted to animal health tasks (Agrupaciones de Defensa Sanitaria -Sanitary Defence Groups-ADS). Activities in the holdings are conducted by ADS Veterinarians under the co-ordination of the local units of the Official Veterinary Services of the AC.
- A second system, where the activities in the holdings are implemented by Veterinarians of contracted enterprises (by the AC) under the supervision of the local offices of the Official Veterinary Services of the AC.

The main legislation that governs the programmes includes two National Royal Decrees, two Council Directives and three Council Decisions from the EU.

Financing of the National Eradication Programmes is shared between the Ministry of Agriculture and the AC that together provide the compensation payments to farmers for slaughter of animals and the cost for equipment and consumables. The AC bears the entire costs of the field teams. Brucellosis represents 58% of the budget dedicated to EU supported eradication programmes.

Sheep and goat brucellosis eradication programmes in Spain

Considerable efforts have been made by the Spanish Authorities in the last 10 years to control brucellosis in the small ruminants. The numbers of both flocks and animals tested each year has increased steadily from 41,000 flocks and 2.1 million animals in 1990, to 136,290 flocks and 18.8 million animals in 2000. A set of tables that recorded the number of animals and flocks tested and the number of these that were positive, with percentages, was presented.

The prevalence of sheep and goat flocks recorded as positive for Brucellosis in the year 2000 ranges from none in the Canary Islands to 42% in Cataluña (National mean rate of free flocks = 88.65%). Over the country as a whole, the percentage of flocks reported positive has fallen steadily from 29% in 1991 to 11% in 2000. Over the same period there has been little change in the number of animals reported positive, which varied between 1% and 3% of those tested each year. The AC with the lowest rate of free flocks were Andalucía (73.5%), Valencia (65.5%), Aragón (63%) and Cataluña (58%)

The national programme recognises 5 areas of disease status determined by the percentage of positive cases and whether vaccination is practised. These are as follows:

- Area 1- Zones qualified as officially free or free. The serological control is done by random sampling.
- Areas 2 and 3- Prevalence equal or less than 2% without or with vaccination. Serological survey and slaughter. (This is the status of most of Spain)
- Area 4 – Prevalence greater or equal to 2% with vaccination. Serological survey and slaughter.
- Area 5 – High disease incidence. Implementation of emergency vaccination and restrictions to animal movement.

Vaccination is done with Rev.1 vaccine and is administered to animals aged 3-6 months. No specific regulation regarding the route of administration is provided in the National legislation.

The programme also recognises five levels of disease status based in the regulations laid down in the Royal Decree 2611/1996, that is a transposition of the EU Directive 91/68. Regarding brucellosis the flocks are classified as follows:

- M1: flocks in which clinical history and situation with regards vaccination and annually serological controls are unknown.
- M2: flocks in which clinical history and situation as regards vaccination and serological controls are known and in which routine control tests are carried out to upgrade the said flock to M3 or M4.
 - M2 negative: Brucellosis free, vaccination practised, clear flock test (negative in the last annual survey).
 - M2 positive: Brucellosis free, vaccination, seropositive animals at last flock test
- M3: *Brucella melitensis* free flock (BMF), as laid down in Council Directive 91/68/EC.
- M4: *Brucella melitensis* officially free flock (BMOF), as laid down in Council Directive 91/68/EC.

Live animal movement control depends on the qualification of the flocks:

- M4 flocks can move animals without restrictions to flocks and fairs and markets.
- M3 flocks can move animals to other flocks with the same or lower status
- M2 flocks cannot move to M3/M4 and the movement to other M2 flocks is conditioned by a 30 day pre-movement test. Can receive animals from M4 after a risk assessment done by the official services.
- M1 flocks can move animals to M2 flocks if 2 tests, with negative results and with the minimum interval of 6 weeks, are performed.

Transhumance

In Spain, all transhuming flocks should be compulsorily vaccinated. Movements for transhumance should be approved by the Official Veterinary Services. For M2 flocks at least a negative test up to 30 days prior to the departure is required.

In Cataluña the summer pastures are classified M3 or M4 and the transit documents allow the transit for pastures according to this prior defined status.

Vaccination: no figure was put to the vaccine coverage at the national level.

Diagnostic tests: The official testing methods used in the Programme are the Rose Bengal and the Complement Fixation Tests, as indicated in Annex C of Directive

91/68/EC, transposed to the Royal Decree 2121/93, and in annex C of Directive 64/432/EC.

According to these provisions, the Rose Bengal Test is used as a screening test followed by the Complement Fixation Test for confirmation of individual positive results. When more than 5% of animals react positively in a flock, all samples are tested by CFT and all positive results (titres of 20 IUs or more) are considered as positive animals (even if negative to Rose Bengal).

In conclusion, Spain stated that the procedures laid down by the EU were being followed, all areas were improving, although some faster than others. Then the programmes for Andalucía, Extremadura and Cataluña were presented.

OVINE AND CAPRINE BRUCELLOSIS IN THE AUTONOMOUS COMMUNITY OF ANDALUCÍA - Miguel Angel González - Head of the Regional Veterinary Services

This is the Southern most AC of Spain. It includes 8 Provinces. The small ruminant stock is 2.5 million sheep and 850,000 goats. In this AC there are 59 Veterinary Services in the Comarcas, 212 veterinarians working for the official services and 90 veterinarians working under contract.

The qualification of flocks implies two sequential negative tests. A positive result implies the isolation, and slaughter of the animal followed by disinfection.

In Andalucía the phases for the control of brucellosis were:

1st phase: 1978-1987 – No control

2nd phase – 1987-1994 – Beginning of control. Veterinarians were recruited for serological surveys and vaccination campaigns.

3rd phase – 1994 –1997 – Transition from the Official Services to Private firms with yearly contracts

4th phase – Since 1997 – 100% of the flocks are controlled and there is the implementation of the ADS, which presently include 90% of the flocks in the area.

The number of animals tested has increased steadily over the last 10 years in Andalucía. The number of positive tests has fluctuated but has shown a steady decrease since 1996. There has been an increase of the number and percentage of positive animals from 1997 to 2000 (70,804 [3.02%] to 103,026 [3.52%] while the number of positive flocks infected decreased from 7,890 in 1997 to 4,900 in 1998 and since then it has been relatively stable (in 2000; 27.5% of flocks were recorded as

‘positive’). In the last year about 43% of flocks tested have improved their disease status while 50% have remained the same.

The epidemiological investigations performed in 2,531 positive flocks in 2000 showed a mean prevalence of 8.7%. The presence of the following risk factors was established:

- presence of dogs (94%). The representatives of Extremadura and Cataluña stated that epidemiological investigations indicate that dogs play an important role in maintaining infection in the flocks. No legal provisions are laid down for this issue.
- illegal re-stocking (93%)
- access to other animals’ foetus (70%)
- common grazing pastures (60%)
- no quarantine (40%)

In 30% of these flocks there were cases of human brucellosis. This has been occurring with 10-12 years peaks and it has been decreasing in the last years. Human Brucellosis has a rate higher than the national average but it has been decreasing (from 15 cases per 100,000 persons in 1985 to 2 cases per 100,000 persons in 2000).

The problems detected by the sanitary authorities are that:

- 50% of the flocks have only 1 positive animal.
- 40% of the positive flocks are related to insufficient vaccination. The region has a policy of compulsory vaccination, however individual farmers can apply for exclusions and this is decided on a case by case basis. The producers after the first negative serological survey and attaining the M4 status, apply for permission to stop vaccination as there is the feeling that with this approach false-positive reactions due to Rev-1 vaccination can be avoided. This happens generally in all the AC of Spain and after being proposed by the producer can be agreed by the official services after an area evaluation and is only allowed for isolated and non-transhuming flocks. It was reported that in only 1% of the cases was there a new occurrence of the disease in these flocks.

- Contact among flocks
- Difficulty in eliminating the reactor animals from the flock (97.4%)
- The frequency of sanitary controls is 1.2 per year per flock and according to the authorities they should be increased to a semester control, which is being achieved with the implementation of the ADS system.

- The effect of the premium system on sanitary measures

According to the authorities improvement could be achieved with the following measures:

- Assuring the continuity of the program and increasing the frequency of controls in the positive flocks.
- Increasing the vaccination coverage. If the financing of the vaccine would include the cost of the application and not only the product cost as it is presently.
- Development of new combinations between the use of vaccines and diagnostic tests.
- A training programme for agents responsible for the implementation of the programme.

OVINE AND CAPRINE BRUCELLOSIS ERADICATION CAMPAIGNS. PERSPECTIVES FROM THE LOCAL VETERINARY SERVICES - *Juan Borrero - Oficina Comarcal Agraria (OCA) de Linares (Jaén-Andalucía)*

A Veterinary Officer from Andalucía, responsible for the eradication programmes in this Comarca that includes 8 municipalities, presented his perspective of disease control of sheep and goats brucellosis. This OCA controls and inspects the activities of survey and vaccination developed by the local ADS services. The approach to eradication is to vaccinate, maintain sero-surveillance and slaughter seropositive animals. All animals are identified and if vaccination targets are not reached a farm visit is carried out to investigate why. All data are maintained electronically on a database. The results of the serological diagnostic are sent to the OCA that organizes the slaughter of the positive animals within a period of 30 days after the results are received. Whenever possible the notification of a positive result to a farmer is made in person by the veterinary services. There is a 10% payment incentive for positive animals to be slaughtered within 2 weeks of notification. If the farmer does not accept the validity of the result the farm is retested. The official services organize every 14 days an expedition for the killing of positive animals followed by the transport of animals for sample collection for bacteriological isolation and for incineration. If the positive animal is not found all the animals without a tag are surveyed and the producer pays the costs. Cleansing and disinfection is often difficult, as there are earth floors.

Only 10 to 15% of the reproductive stock is vaccinated as young. If the animals are vaccinated the first serological control is done after 18 month of age. The speaker remarked that this can give the producer a feeling of false security as these non vaccinated animals will only be controlled at 18 months of age and subsequently with the chance of aborting and spreading the *Brucella*.

OVINE AND CAPRINE BRUCELLOSIS ERADICATION CAMPAIGNS. PERSPECTIVES FROM THE ANIMAL HEALTH ASSOCIATIONS (ADS) –Agustín González - President of the Andalusian Federation of ADS

The Animal Health Association (ADS) Representative gave a short presentation of the history of the ADS implementation in Spain. In 1985 it was implemented for swine and in 1994 was implemented for ruminants. Minimum requirements for the constitution of an ADS in a given territory are: the association should include at least a 30% of the holdings in the area and 50% of the animal population. Membership of ADS is voluntary. With the extinction in 1997 of the private enterprises that provided this service to the State and did not charge the producer, their area of action was largely increased and e.g. in Andalucía covers 90% of census and 70% of the flocks and 34% of M3/M4 flocks. The producer is charged per head up to attaining the qualification and is then paid back the entire amount spent. They provide in addition lower cost veterinary products to the producers. They support the programme, however not all farmers support it. The ADS provides free sanitary services for flocks, which achieve M3 and M4 status.

OVINE AND CAPRINE BRUCELLOSIS IN THE AUTONOMOUS COMMUNITY OF EXTREMADURA - Antonio Cabezas - Head of the Regional Veterinary Services

This region, in the West of Spain has 2 Provinces and an area of 41,602 km². It has the biggest agricultural sector and the lowest income *per capita* of Spain and the agricultural sector accounts for 16% of its productivity and within this the animal production accounts for 48%.

The veterinary services have 15 local “Comarca” Services, a Regional Laboratory in Badajoz and, with limited activity, 2 laboratory Sections in Caceres and Zafra.

The small ruminant census for 2000 includes almost 12,000 ovine flocks with 4,400,000 animals and almost 6,000 caprine flocks with 314,000 animals. In this region there is a legal basis to promote the co-operation of farmers with the ADS,

which has enabled the AC veterinary service to concentrate on seropositive flocks. Therefore the 198 ADS that exist in the Extremadura control 1.5 million sheep and 92,000 goats in negative flocks. The remaining activities of tagging, vaccination and sampling in the field are implemented by an enterprise, selected by tender, that is in charge of contracting private veterinarians. All activities conducted by this enterprise are directly supervised by the Official Veterinary Services. The individual registration of animals and flocks, the vaccination activities, results on test and slaughtering actions, etc, are recorded in a database system with territorial implantation. This database system has been implemented for small ruminants and cattle movement control.

The coverage of eradication programme was greatly increased from 1.7 million animals and 10,869 flocks surveyed in 1995 up to almost 4 million animals and 20,000 flocks in 1999-2000.

The rate of positive animals dropped within the same period from 1.47% to 0.66% and 0.54% in 1999 and 2000. All flocks are controlled at least once a year and 85% of the flocks are surveyed twice a year. The number of brucellosis free flocks increased from 85.65% in 1997 to 91.26% in 2000. Rev.1 vaccination is performed by subcutaneous route and more than 50% of the flocks are vaccinated (12,000 in the year 2000). As reported on the previous presentation as soon as they reach the M3 status the producers apply to stop the vaccination programme and press sanitary authorities to withdrawal the vaccine.

From 1995 to 2000 for paying 149,230 animals slaughtered the compensatory payments reached 1,062,823,856 pesetas [6,387,700 €] (which gives an average of 7,122 [42.8 €] per animal) of which only 50% is supported by the AC. It was estimated that the total cost of the programme is of 1,712,109,098 pesetas [10,300,000 €].

OVINE AND CAPRINE BRUCELLOSIS IN THE AUTONOMOUS COMMUNITY OF CATALUÑA - *José Gou Torrent - Regional Veterinary Services*

The ovine and caprine sector in Catalonia has 4,000 flocks and 660,000 animals and only accounts for 0.74% of the livestock in this AC. What predominate are the fattening and finishing flocks with animals imported from other CA and from the EU.

There are 41 local (“comarca”) veterinary services, 153 official veterinarians, and 8 animal health laboratories. The field activities are done by 41 veterinarians that have contract with 5 private entities selected by tender (there are no ADS).

In 2000 there was 100% coverage of the flocks and 1,667 flocks (42%) were positive during the first survey, and half of those (due to the lack of time) were investigated on a second round with 447 positives (60%). This refers to 656,844 animals on a first survey and of which 30,488 (4.6%) were positive and of 185,211 animals repeated 7,726 (4.2%) were positive.

In the campaign of 2001 only 2000 flocks were surveyed up to the date, as there has been a greater input of the veterinary services into the control of the Classical Swine Fever epidemic in this Region. 38.04% of the flocks done have seropositive animals and this happens especially in vaccinated flocks that have 1 to 3 reactor animals. 80-90% of the replacement animals are systematically vaccinated. Rev.1 applied by the conjunctival route has also been used in emergency in 2 or 3 highly infected holdings.

SUMMARY OF DAY 1

In the opinion of the subgroup, some aspects of the programme should be carefully reviewed and new or reinforced measures introduced to successfully achieve the goal of final eradication of the disease:

Vaccination:

- It would be desirable to have more precise data on vaccination coverage of eligible animals in each area. Those areas should be defined based on epidemiological criteria.
- The findings of positive reactors, which might have been induced by the vaccination could be overcome with the practice of vaccination by conjunctival route.

Movements control:

- Controls on animal movements should be reinforced, especially in those areas with transhuming practices.

Diagnostic tests

- *False negative reactors:* Great concerns of AC Official Veterinary Services were focused on the possibility of false negative results, due to the limited sensitivity of the official test.
- *False positive reactors:* False positive reactions (FPR) to a serological test could compromise the results of the programme in some particular flocks or areas in Spain. It was clear that in many cases the laboratory test is used as the sole mechanism to define the flock status, thus the findings of low number of positive animals within a flock must be carefully evaluated by additional investigations, based on complementary serological test, bacteriological studies or epidemiological inquires. It therefore seems likely that in some areas of Spain the disease situation is better than is presented.

DAY 2

The morning was spent visiting sheep and cattle farms that were representative for the management and production (but perhaps of a higher standard than the Spanish mean holdings) and the regional laboratory of Andalucía.

In the afternoon, the representatives of Andalucía, Cataluña and Extremadura were requested to continue the meeting in order to make clearer some aspects of their respective programmes and to explain in detail questions that were raised by the experts during the day. The meeting with the Spanish colleagues went on for one and a half-hours.

In the afternoon the Chairman of the bovine brucellosis sub-group presented a description of the compensation rules, which apply for animals slaughtered due to Brucellosis in each of the Member States. The compensation rules vary widely between Member States and there is concern that in some cases they reward the presence of disease or fail to encourage reporting. It is not possible for the sub-group to assess the effect of compensation on the likelihood of disease reporting and efficiency of control. This should simply be flagged as a possible cause of failures.

Day 2 ended with a too brief group discussion about the Sheep and Goat Brucellosis programme. This started at about 7.30pm and continued until 9.15pm. Several proposals were put forward and the Commission will consider them. These included suggesting that the sub-group should be presented as 'available expertise' and the Member States should be encouraged to design the programme for the visit to address the problems that they themselves perceive. Several experts suggested that key data should be sent to members of the sub-group before the visit so that they could be prepared. It was also suggested that there should be key measures identified for assessing the progress of eradication (*e.g.* vaccination coverage, numbers of flocks in each disease status category in each region, and incidence of human disease in each region). These should be supplied, with an overview of the policy and an explanation as to why some areas have higher levels of disease than others.

The information given by the Spanish authorities during the meeting was based on the provisions of different Decisions of the EU Commission. But the sub-groups feel that it is necessary to show a progression in order to identify those indicators able to reflect in detail how different aspects of the programme are going on the spot. Indicators that would make possible an evaluation of cost/benefit relations of actions

and activities, and thereby determine which of them must be suppressed and which reinforced. Anyway the subgroups consider it necessary to undertake a more in depth and co-ordinated data analysis of the programme's outcome both at regional (AC) and at central level. This will make possible the identification of short and medium term targets.

DAY 3

The bovine meeting was chaired by Fernando Boinas and occupied the 3rd day of the visit. Members of both subgroups and various Spanish representatives attended it.

THE NATIONAL ERADICATION PROGRAMME FOR BOVINE BRUCELLOSIS IN SPAIN

J.L. Paramio - *Subdirección General de Sanidad Veterinaria (SGSV)*

The disease eradication programmes in Spain are governed by Royal Decree 2611/1996 as well as Community Directives 64/432 and Decisions 90/424 and 93/368. Of the financial assistance provided by the European Union for disease eradication, 29% is devoted to bovine brucellosis. A similar proportion is dedicated to small ruminant brucellosis (*B. melitensis*) while tuberculosis receives 17% and leucosis 7%.

Official diagnostic tests include the Rose Bengal Test (RBT), Complement Fixation Test (CFT), Serum Agglutination Test (SAT), Milk Ring Test (MRT), and milk and serological ELISA tests.

For bovine herds to qualify as officially free and to maintain their official freestatus they must satisfy the requirements laid down on Directive 64/432.

The brucellosis free status of a herd is suspended when there is failure to comply with the necessary requirements, or following laboratory, epidemiological or clinical suspicion of disease. If the latter, the animal(s) are isolated or slaughtered.

Requirements for lifting the suspension are as provided in Directive 64/432. The brucellosis free status is withdrawn following confirmation of disease in a herd. In this case the affected cattle are slaughtered and 2 negative tests (30 days and 60 days post-slaughter) are required to re-achieve the free status. Pregnant cattle require a negative test 21 days post-partum.

The proportion of brucellosis free herds in Spain has progressively increased from 96% in 1990 to 98.61% in 2000, while the animal prevalence has decreased from 1.08% to 0.26% over the same time. A slight decrease in the percentage of free herds from 1999 to 2000 (98.72% and 98.61% resp.) was attributed to a decrease in the number of herds, due to herds combining together, leading to fewer but larger herds.

In 2000, the number of cattle herds varies between AC, from 116 in Murcia to 29773 in Asturias. The range in the proportion of Free herds in 2000 varied from 93.86% to 100%, while the animal prevalence was between 0.92% and 0.00%. The animal prevalence exceeded 0.26% in 4 AC: Cantabria (0.92%), Castilla y León (0.38%), Cataluña (0.32%) and Andalucía (0.27%). In the remaining 13 Communities it ranged from 0.18% to 0.00%. It is considered that the best results of the bovine eradication programmes in Spain were obtained in the AC with a higher proportion of milk herds,

BOVINE BRUCELLOSIS IN THE AUTONOMOUS COMMUNITY OF GALICIA– *David Fernández- Director of the Laboratory of Animal Health and Production of Lugo*

Galicia lies in the extreme North-West of Spain, comprises an area of 29,575 km². It contains 865,464 cattle, approx. 16% of the national herd, which are distributed among 72,801 herds. It is an important dairy area, containing 40% of the national dairy herds.

In 2000 the Official Veterinary Services comprised 147 staff. In addition there are 150 vets that implement the eradication programme in the field. The 7 central units and 65 peripheral offices of the Service are linked through a common animal health database that is updated daily. The cattle industry is largely regulated through 56 agrarian cooperatives that oversee various sanitary programmes which are changing into sanitary defence groups (ADS) as in other Regions, aiming at an increased collaboration in the eradication campaigns. The number of vets in each cooperative is in proportion to the cattle population.

Brucellosis eradication began in 1988 when an area of eradication was defined within Galicia. In 1990, eradication became compulsory across all of the AC with intensification of control measures, and vaccination ceased at that time. The herd prevalence has progressively decreased from 1.08% in 1993 to 0.36% in 2000, while the animal prevalence decreased from 0.38% to 0.18% over the same period. This corresponds to 72,201 herds and 771,152 bovine qualified.

All cattle older than 12 months are subjected to an annual serological screening with a modified Rose Bengal microagglutination test; the positives are confirmed by CFT (threshold of positivity: 20 UCEE/ml). Test-positive animals are slaughtered and the test repeated at a minimum interval of 2 months up to 2 consecutive negative results

for the sanitary accreditation of the herd. This refers to officially bovine free herds as vaccination is not done in this area. Apart from routine testing, additional measures include biotyping, set up of serum banks and evaluation of a Competitive ELISA in collaboration with the National Reference Laboratory.

BOVINE BRUCELLOSIS IN THE AUTONOMOUS COMMUNITY OF CASTILLA-LA MANCHA –*Sagrario Munoz. AC Veterinary Services*

Castilla La Mancha lies in the centre of Spain, comprises 79 000 km² and consists of 5 provinces. The Department of Agriculture and Environment of the AC of Castilla La Mancha have a Livestock Service, with Sections of Animal Health, Animal Production and Laboratory. The Laboratory Services is made up of one laboratory in each province and a central, regional laboratory. There are 79 ADS that implement the sanitary programmes in 85.5% of the bovine herds.

In 2000, there were 2,703 herds in the AC. The proportion of brucellosis free herds increased progressively from 1996 (93.68%) to 2000 (97.61%) with a slight decrease in 1999 (95.75%). The animal prevalence decreased from 0.19% in 1998 to 0.10% in 2000 with a rise to 0.25% in 1999.

It was reported some problematic areas such as some mountainous areas in the province of Guadalajara that have communal pastures and in which a high herd prevalence occurred in 1999.

THE LABORATORY DIAGNOSIS OF BRUCELLOSIS, IN THE CONTEXT OF THE IMPLEMENTATION OF NATIONAL ERADICATION PROGRAMMES.

Organization and activities of the National Reference Laboratory for Animal Brucellosis – Dr Fulgencio Garrido, Director

The National Reference Laboratory for animal brucellosis is situated in the Central Veterinary Laboratory in Santa Fe, Granada and has direct dependence from the CVO of Spain (SGSV)

Quality Performance is considered as an important aspect of the laboratory's work and it has connections with some international reference laboratories. Domestically, it is responsible for the harmonisation of the official diagnostic tests performed by the 50 regional and local laboratories, including the QC of all antigens, vaccines and test kits. It is also responsible for confirmatory diagnosis and typing of *Brucella* organisms isolated. The task of periodic inter-laboratory trials was initiated a decade ago, and nowadays it was well established. The Laboratory provides training for staff from the regional and local Official Veterinary Laboratories and organises workshops.

The incidence of human brucellosis cases in Spain has decreased consistently from 15.7 cases per 100,000 inhabitants in 1985 to 2.85 cases per 100,000 in 2000.

In 2000, the National Reference Laboratory isolated 60 *B. abortus* and 38 *B. melitensis* strains. *B. ovis* is isolated periodically, the last time in 1999. Within the species *B. abortus*, biovar 3 is the most common, comprising 53 of the 60 strains, while biovar 1 accounted for the remainder. Of the *B. melitensis* strains, 29 were biovar three in 2000, the remainder being biovar 1. No vaccine strains were isolated in 2000 although two S19 and three Rev.1 strains had been identified in 1999 and one S19 strain in 2001 isolated together with the field *Brucella*. In each of these situations an epidemiological investigation was done.

From 1991 to July 2001, 270 strains of *B. melitensis* were identified by the laboratory: 85 biovar 1, 22 biovar 2 and 163 biovar 3. Over the same period 316 *B. abortus* strains were identified: 75 biovar 1, 241 biovar 3.

In cattle, six *B. melitensis* strains were identified in 2000, two of biovar 1, four of biovar 3.

With regards the inter-laboratory trials, detailed information on the last trial was explained . In a Coefficient of Variability (CV) study for the CFT, the CV exceeded 0.10 in 5 laboratories (10%), while it was below this threshold in the remaining 45 laboratories. All the laboratories out of the threshold of acceptability were included in a programme of training, at the end of which they succeeded in a new restricted ring trial specifically organised for them.

Preliminary clinical studies in isolated flocks are being done using the vaccine RB51 under the competent authority supervision.

Activities of the Regional Laboratory of Cordoba for Animal Brucellosis –
Antonio Gasca, Director

This presentation informed about the procedures done with the samples sent to the laboratory for serological diagnosis of bovine brucellosis and the quality controls made including ring trials co-ordinated by the National Reference laboratory. In the year 2000 114,787 samples were analysed.

In 2000, a total of 1,113,601 serum samples were processed of which 813,201 were ovine and 300,401 were caprine sera.

During the year 2000, 40 samples were received for bacteriological isolation and 14 isolations were made. It was considered that most of the samples were in bad condition.

Competitive ELISA (Svanova) is a test that is in the process of being licensed in Spain to be used as a routine. This is considered to have a lower sensitivity than the indirect ELISA.

Microplate method is used in the testing protocol for RBT. This was considered by the members of the TF as not recommended as it is necessary to use a standardized technique. The National Laboratory reported that the data to validate this technique was already available and efforts are being made to publish it in a scientific journal.

Also included in the presentation was the protocol used for serology of small ruminants as laid down in Directive 91/68/EEC.

The Laboratory of Cordoba also performs the control of REV 1 vaccine, in order to control the physical and the biological properties.

**RECOMMENDATIONS OF THE BRUCELLOSIS SUBGROUPS –
GENERAL RECOMMENDATIONS TO THE TASK FORCE**

1. As in previous meetings, large amounts of information were delivered in the presentations during this meeting. It was agreed among the members of the subgroups that it would be more convenient to obtain the data before the meeting so that a more fruitful discussion and more accurate questions could be addressed for clarification.
2. To speed up the writing of the minutes of the meetings the Commission suggested that the members of the sub-groups that are native English speakers could elaborate the draft minutes and the chairmen revise it before sending to the other members for comments.

RECOMMENDATIONS TO SPAIN

1. The subgroups recognized the competence's of the ACs and the restricted role of the Central Veterinary Authority in implementing the programme. Nevertheless, the latter was responsible to the Commission for the design, implementation and monitoring of the programme. Consequently, the group recommends that the role of monitoring, assessment and coordination of the programme by the Central Authority should be reinforced and recognized.
2. The brucellosis subgroups complimented the veterinary representatives of the AC of Andalucía, Extremadura Castilla-La Mancha, Galicia and Cataluña on their considerable efforts in the brucellosis eradication programme. The results of their hard work were a significant increase in the number of herds tested and a reduction in disease incidence over several years. The subgroups also acknowledged the considerable amount of data collected by the veterinary authorities. However, a more adequate and deeper analysis would help both the groups and the Spanish authorities to properly assess the strengths and weaknesses of the programme or the extent of progress achieved. Comprehensive analysis would not only benefit the subgroup but also the AC in that they could monitor their programmes more effectively, identify possible weaknesses, establish meaningful targets and better allocate resources. The sub-groups therefore recommend that all collected data should be subjected to proper epidemiological analysis. This might require the collection of additional data and/or changes in the way data is captured.

3. A more structured approach is required for the upgrading of flocks from M3 to M4 status. This should include a formal assessment of the disease and vaccination status of flocks in the area, and the extent of transhumance. Consideration should also be given to slowing the transition from M3 to M4, possibly by insisting on a minimum period of vaccination within the M3 status.
4. A special concern on proper vaccine coverage should be given to the transhuming flocks.
5. Animal movement control should be re-enforced.
6. Herds with small numbers of seropositive animals should be thoroughly investigated to assess the possibility of false positive reactions. This requires a clearly defined structure in the use and interpretation of complementary tests, epidemiological investigation of the herds and an assessment of infection in the area.
7. More effort should be made to attempt isolation of *Brucella* in the serologically positive herds.
8. The upgrading of the status of a herd should not be impeded by false positives and greater use of the “suspended” status as stated in the Directive is recommended.
9. Consideration should be given to using Rev.1 conjunctival vaccination in sheep and goat herds if cross-reaction to subcutaneous administration is suspected of causing false positive reactions. There is the need for an information campaign to the producers and field veterinarians.
10. The preliminary field testing that is presently taking place in some areas of Spain using the RB51 vaccine in bovine should be thoroughly reported to the Commission.
11. The Rose Bengal test methodology should comply with prescribed, standardized methods unless it has been adequately validated and approved by the procedure referred in Article 17 of Directive 64/432/EEC. The criteria establishing the threshold of 5% positivity for further testing should be revised.
12. The subgroup is concerned about the limitations of the available official tests for the detection of *B. melitensis* infection of sheep and goat in some epidemiological conditions (SCAHAW, Doc SANCO C.2/AH/R23/2001), and any alternative or complementary methodology should be properly standardised and evaluated.