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REPORT OF THE

**“Bovine Brucellosis”
TASK FORCE SUB-GROUP**

**Meeting held in
Belfast
Northern-Ireland, UK
07- 08 October 2008**

**REPORT OF THE
MEETING OF THE BOVINE BRUCELLOSIS SUB-GROUP OF THE
TASK FORCE FOR MONITORING DISEASE ERADICATION
HELD IN BELFAST, NORTHERN IRELAND, UK, 07-08 OCTOBER 2008**

PARTICIPANTS: see Annex I

AGENDA: see Annex II

LOCATION: Belfast, Northern-Ireland, UK.

DAY 1

1. Introduction and overview

Roland Harwood

The meeting was held in the Newry Divisional Veterinary Office, Glenee House, Carnbane Industrial Estate, Newry. Mr. Roland Harwood, Senior Principal Veterinary Officer (SPVO) of the Enzootics Section gave an overview on the situation on Bovine Brucellosis (BB) and the current eradication programme.

It was presented the organisation of the Department of Agriculture and Rural Development (DARD) for Northern Ireland (NI) includes the Veterinary Services which has 2 Departments, the Logistics with 3 Divisions (Enzootics, Standards and Epizootics) and the Operation with also 3 Divisions (Northern, Southern and Meat Inspection). The total staff is composed of about 650 person's, 90 Veterinary officer's (VO's) and 170 Animal Health Welfare Inspectors (AHWI's). The NI Veterinary Service structure is currently under review.

An overview on the history of Bovine Brucellosis eradication in NI and the evolution of the herd and animal incidence and prevalence during the period of the last 12 years was presented.

The recommendations and measures implemented since the last Task Force visit in May 2002 in NI were explained and discussed. Furthermore measures not or partially implemented were discussed. Also actions and initiatives of the NI scheme for Brucellosis (Br) eradication and the factors contributing to the current good situation were discussed.

The target of the NI BB eradication programme is to reduce herd incidence from 1% to 0.8% within 3 years. To achieve that purpose the key areas under consideration of the Official Services, were discussed during the meeting.

2. Epidemiology – the current picture in NI

Darrell Abernethy

The brucellosis eradication scheme commenced in NI in 1963 and resulted in near-eradication of the disease by the late 1980s. Between 1990 and 1996 the seropositive herd incidence ranged from 0.01% to 0.08% with most outbreaks considered to be false-positives (single, culture-negative reactors). Recrudescence occurred in 1997 and annual herd incidence increased to peak at 1.32% in 2002 before reducing to 0.41% by 2005. Following a large outbreak in County Armagh in late 2005 and a range of measures to improve sensitivity, the seropositive herd incidence increased over the following two years (0.56% in 2006 and 0.72% in 2007) but the incidence of confirmed outbreaks decreased (0.28% in 2006 and 0.25% in 2007).

Across the duration of the epidemic, brucellosis has been clustered and over 75% of outbreaks are located in the southern part of the country. Three spatiotemporal clusters have been identified: the first occurred in the north-west (largely in County Londonderry) and was of shortest duration (three years) while the largest cluster originated in the south of the country, lasted five years and extended across County Armagh, through western County Down and into the southern parts of County Antrim. A third cluster, also in County Fermanagh, was also five years in duration but commenced and ended a year later than that of County Armagh.

Various epidemiological studies have been undertaken to investigate the risk factors for brucellosis, explain the epidemic curve and assess the measures

taken to eradicate the disease. A descriptive study highlighted farm fragmentation, spatial clustering, latency and test sensitivity as requiring further investigation. A case control study identified herd density and size, contiguous infection, abortions and cattle movement as significant risk factors. A field study confirmed farms were highly fragmented, with land parcels up to 30 km away. The level of compensation paid for cattle, the prevalence of putative fraudulent infection and the extent of government funding were also evaluated. Four inter-related factors were identified that are likely to determine the success of the eradication programme: management of exposed contiguous herds, the level of compensation paid, bio security measures employed by farmers and the level of government investment in the programme.

Sixty percent of outbreaks occur in the immediate vicinity of outbreaks, indicating the significance of local spread and the importance of testing contact herds. Of the surveillance measures in place (routine testing, post-abortion testing, abattoir surveillance, bulk milk tank testing, pre-movement testing), routine testing detects most outbreaks but post-abortion testing was deemed to be the most important. This was due to the higher within-herd prevalence and higher percentage of confirmed outbreaks detected by this test type than by other surveillance measures. The 2001 foot and mouth epidemic did not appear to have had a significant effect on brucellosis, most likely due to the bio security measures employed by farmers at that time.

In 2003/4 a parallel field trial of six serological tests was undertaken to assess the accuracy of the serum agglutination test (SAT). Over 31 000 tests in 19 000 cattle were tested in parallel. The trial provided significant assurance of the SAT's sensitivity within the NI programme but parallel testing with both an i-ELISA and the SAT was recommended for all putatively exposed cattle.

An in-depth investigation of the 2005 outbreaks in County Armagh concluded that latent infection was the likely source of the primary breakdown. Over forty herds were subsequently infected with a small but significant proportion only identified at the third post-exposure test.

3. Northern Ireland Brucellosis Scheme

Sharon Verner

The Veterinary Service of DARD is the designated Competent Authority in NI under Council Directive 64/432/EC and the Brucellosis Control order with responsibility for the implementation of the national brucellosis eradication programme. The programme is based on a test and slaughter policy.

Testing programme

3.1 Annual blood testing of all breeding animals over 12 months in the three high incidence Divisions namely Armagh, Newry, and Enniskillen. Annual testing of all non-dairy herds and biennial herd tests of all dairy herds in the seven other Divisions, supplemented by Bulk Milk ELISA testing.

3.2 Special testing includes

- 30-day premovement test
- Post abortion testing
- Testing of restricted herds
- Testing of herds contiguous (inner and outer rings) to a disease outbreak
- Forward and backward traced animals/herds
- Parallel testing in high-risk areas
- Bulk milk ELISA carried out monthly in all dairy herds
- Abattoir surveillance of cattle over 30 month and individually restricted (BR) animals
- Screening of laboratory submissions (eg infertility investigations)
- Testing in Temporary Control Areas

The official tests are the Serum Agglutination test (SAT) EDTA (EDTA with SAT) the i-Elisa, the complement fixation test (CFT), and the milk Elisa. The serology and milk test are carried out in the laboratory of the AgriFood Biosciences Institute, which is located in Belfast. An SAT is performed on all blood samples (screening) and the CFT on samples with an SAT > 25 IU or >

1/20 in increased risk tests. Parallel testing with SAT and i-Elisa is carried out in contiguous herds in high incidence areas. Test results are electronically transmitted from the laboratory to the Divisional Veterinary Offices (DVO's).

3.3 Testing strategy

Surveillance testing is carried out for early detection and confirmation of disease outbreaks, source(s) of infection. Targeted and parallel (high risk) testing of contiguous herds for the early warning of disease spread.

Following a disease outbreak, a detailed epidemiological investigation and veterinary risk assessment is carried out. Immediate movement restrictions and extra testing on the breakdown and surrounding herds are imposed. Reactors are removed. When disease is identified the herds is normally depopulated. Depopulated herds cannot restock for 6 months. Forward and backward traces of animals movements are carried out with subsequent restriction and testing.

The compensation system is on farm valuation. The reactors are paid 75% of the average market value subject to a ceiling or 75% of the valuation and the in contacts 100% of the market value. There is no ceiling on the amount that can be paid. The salvage value of the carcasses goes to the competent authority.

4. Farm visit

After lunch a visit to a infected local dairy farm was organised in order to evaluate how dairy cattle is raised, grazed and housed in NI. The pedigree dairy herd of 260 animals with approximative 120 cows was located in the district of Newry, Bessbrook, Divernagh RD in the North west of Newry. Many other bovine dairy herds are also located in this district. Artificial insemination is carried out by the farmer, calfs are sold of an age of 4-6 month. During summer time bovines are on pastures near the farm crossing a main road.

At the herd check test in November 2007, a positive titer in a single dairy cow was found for SAT 71, CFT 264 and to the i-ELISA Test. The herd had been tested three times in the previous 1 month with negative results. Following the positive culture for *Brucella abortus* biotype 01, the entire herd was depopulated by mid January 2008. No clinical symptoms on BB were found in the herd. The herd keeper began restocking as soon as he was permitted at the moment there were 155 bovines in the herd.

At herdtest at 2 months post restocking has been carried out, results were awaited. If the test is negative, a check herd test will be allocated for 3-6 months later.

The farmer and the official veterinarian presume that the source of the infection was the contact on pastures with infected bovines from the neighbouring farm during the grazing period. Animals of both herds may have had contacts due to a broken fence in a water area separating the two farms. On the neighbouring herd BB positive bovines and abortion was found.

Before depopulation was carried out also about 40 bovines were tested positive for Bovine Tuberculosis.

5. Overview of BR in Armagh, Newry and Enniskillen DVOs

Owen Denny

This talk was presented by Owen Denny who is the Senior Principal Veterinary Officer with responsibility for implementation of Veterinary Service work in the southern half of Northern Ireland.

He began by outlining the structure at the top level in the Department of Agriculture and the geographical relationship of the 10 Divisional Veterinary Offices in Northern Ireland.

The epidemic curve for brucellosis breakdowns from January 1995 to August 2007 was then presented. This demonstrated the start of the epidemic in 1997 and the subsequent increase to the end of 2001. The number of breakdowns

steadily decreased from 2002 to the end of 2005, when a single outbreak with massive spread to more than 40 other herds, reversed the downward trend. Although the number of breakdowns has further increased from 2005 to 2008, this is largely as a result of using additional serological tests and stricter interpretation. Hence the number of confirmed breakdowns has remained essentially level in the last few years.

Mr. Denny then reviewed the strategic response by the Veterinary Service to Brucellosis. The measures employed were outlined – see table below.

Bulk Milk Elisa testing applied to all dairy herds	Compulsory 6 month restocking after a breakdown
BB Publicity Campaign – posters, press articles, etc.	HQ resource expanded to 3 DVO's dedicated to Brucellosis
Staff training and Private Vet meetings	Updated staff instructions
Abattoir surveillance for females over 30 months	Brucellosis Control Initiative – an implementation task force with dedicated staff.
Annual testing in the 3 high risk DVO's	Management database designed to facilitate local management of outbreaks
Cattle identification inspections for herds experiencing a breakdown.	Annual testing in all DVO's
Tracing and purchase of the progeny of reactors	Removal of low CFT animals
Segregation of cattle groupings on breakdown farms.	Modifications to valuation procedure to reduce potential for over-compensation
Enhanced restrictions on contiguous herds	Pre-movement testing for all eligible cattle over 12 months
i-Elisa introduced in risk herds	Slurry treatment with lime in breakdown herds to kill the bacterium
Ongoing publicity and meetings, including an emphasis on abortion publicity	Brucellosis audits of field offices by HQ staff
Temporary Control Areas with increased controls	

Several slides were then presented to review the geographical variation in incidence, i.e. 3 Divisions with higher levels of disease – Armagh, Newry and Enniskillen.

The staffing of the 10 veterinary offices was reviewed and numbers of vets and support staff outlined. Reference was made to the very comprehensive and

detailed staff instructions for Brucellosis which were available to all staff on the Department network.

The range of reports available for the management of Brucellosis was then outlined – see below.

- Are tests allocated correctly?
- Are herd keepers notified of due risk tests?
- Are tests arranged on time?
- Are restrictions applied for overdue tests correctly?
 - Are enforcement procedures in place for o/d tests?
 - Are samples processed within target time?
 - Are tests being checked in lab on time?
 - Are queries at the laboratory getting sorted out on time?
 - Are VO' s interpreting/following up tests on time?
 - Correct interpretation?
 - Are restrictions applied correctly
 - Are admin staff progressing tests?
 - Are valuations being completed on time?
 - Are valuations processed by admin within target time?
 - Are valued reactors/NIC's slaughtered within target times?
 - Brucellosis breakdown follow up tasks: VO visit, traces, mapping, Cleansing and disinfection complete, Segregation notices, CII complete, Investigation report

The detailed 6 monthly work programmes that the veterinary service uses to plan and monitor its work were demonstrated. In these, the Brucellosis tasks for Veterinary and support staff are detailed along with the associated resource which is calculated according to the predicted number of breakdowns. Approximately 10 days of veterinary officer resource is allocated to every breakdown.

The detail of the Brucellosis audit was then presented. This consisted of an Excel spreadsheet with approximately 50 questions that are asked by HQ staff during the audit of the local office.

Mr. Denny then covered details of staff training in relation to Brucellosis. This included:

- Brucellosis Induction Training for Veterinary Officers and Animal Welfare and Health Inspectors, i.e. all AWHI's and VO's are trained every few years, e.g AWHIs - June and September 2008. and VO's – planned for December 2008.
- Admin staff get training in disease basics and admin procedures
- Database, test interpretation and fraud awareness training is provided to various staff.

In addition, every year there are special training sessions and meetings organised as necessary. For example, in 2008, the following meetings have taken place so far:

- Br Workshops in Newry, Dungannon and Armagh
- Cross-border meetings – Armagh and Cookstown, in addition to regular DVO-SVI
- Br management meetings - Enniskillen and Loughry
- Br Database meeting in Newry
- Cases conferences – Armagh, Newry
- Ministers meetings
- Stakeholder Groups

After the presentation there were a number of questions and discussion points. There was optimism within Veterinary Service that with continued intensive efforts, brucellosis could be eradicated during the next few years.

6. Operation of a Field DVO and introduction to BR procedures

David Brown

The organisation of the Divisional Veterinary Office of Enniskillen and the implementation of brucellosis programme in the region were presented, as an example of DVO's organization and procedures.

The Divisional Veterinary Officer is responsible for the overall management of the programme. In particular, the DVO supervises the procedures followed, checks whether the tasks are completed on time, is responsible for decision

making, performs resource reallocation when necessary and supervises training of the personnel and DVO & DAF communications.

The region of Enniskillen is divided in 5 veterinary patches. Each patch is run by a VO, who supervises the routine tests, is in close contact with the farmers and has the local knowledge to indicate the high risk farmers. The vast majority of the farms ($\approx 80\%$) are small suckler farms with very few in-movements.

The testing scheme followed for monitoring purposes includes the following:

- Annual herd testing of all herds
- Previous outbreaks tested twice yearly for 3 years
- Inconclusive reactors retested 2-3 weeks later
- Bulk milk ELISA tests
- In case of reported abortions, immediate testing of herds and re-sampling 3 weeks later
- Abattoir samplings
- Pre-movement testing

The interpretation of laboratory results is performed as follows:

- SAT < 20 IU/ml, CFT = 0: No herd restrictions
- CFT ≥ 33 IU/ml: Positive
- CFT ≥ 20 IU/ml: Positive in risk situations
- Positive i-ELISA: Positive in risk situation

A herd is considered to be of high risk in case there was infection during the previous 3 years, the farm is contiguous to a breakdown, there is backward or forward tracing of a positive animal and multiple animals show low, however not zero, CFT titres.

After a positive result in a herd the VO visits the herd within 24h (if not possible, as soon as possible). Movement restrictions are imposed and an initial epidemiological investigation is performed, including identification inspection. Instructions are given for the reactors to be isolated immediately.

Animals should be removed within three weeks from the day the sample was taken. Backward and forward tracing is done. Movement restrictions are also imposed on contiguous herds, which are found using the GIS from payment association. Valuation of reactors is done by livestock valuation officers, within 5 days of herd keeper notification. Herd keeper has up to 3 days to accept. In case of non-acceptance an independent valuation can be done, without any obligation from the department to accept it. Full epidemiological investigation may be completed within some months.

At the herds that are considered positive a decision is taken whether to depopulate or not. There are three cases:

- Depopulation: Restocking is allowed after at least 6 months interval. Two months later, a herd test is performed.
- Non depopulation, cultures from reactors found positive: Herd tests are performed every 6 weeks, until all pregnant animals calved more than 3 weeks ago. Because animals calve throughout the year, practically this procedure requires almost 12 months period.
- Non depopulation, cultures from reactors found negative: Two herd tests are performed 30 and 90 days after the removal of reactor(s).

All samplings, laboratory results and data from epidemiological investigations are recorded in an on-line brucellosis database, which is available at all DVOs in Northern Ireland (APHIS). Through the database the time between individual steps of the procedure is recorded and the DVO can check where there are problems, if additional personal are needed somewhere, etc. Reports are produced to check how targets are met, such as if there are delays within test cycle, reactors have been valued and slaughtered on time, breakdown tasks are completed, etc. The database also provides a system of monthly letters notifying herd keepers of brucellosis herd tests due, arranged dates and times, implications of overdue tests, restrictions applied and enforcement actions.

7. Organisation of testing in a local DVO

Gerry Murphy

The Senior Animal Health & Welfare Inspector (SAHWI) is responsible, among other tasks, for monitoring work of Animal Health and Welfare Inspectors (AHWI). Brucellosis blood sampling is a key AHWI role.

The Newry Divisional Veterinary Office is divided in 8 areas with several subdivisions in these (patches). On every patch, an AHWI is the responsible for ensuring that all Brucellosis tests are arranged and completed. In addition, there are an AHWI who act like team leader and contribute to prioritise tests for an optimum use of the programme resources.

This are the priority Herd Tests in NI Brucellosis Programme:

- Reactor Herd Test (RHT)
- Lateral Check test Inner ring (LCI)
- Backward Check Test (BCT)
- Forward Check Test (FCT)
- Lateral Check Test Outer (LCO)
- Lateral Check Zone (LCZ)
- Annual Herd Test (AHT)

For Individual tests (animal tests), the priority is like following:

- Check Test Abortion (CTA)
- Reactor Inconclusive (RI)
- Check Test Clear animal (CTC)
- Check Test Tracing (CTT)

To set up the process of checking a work programme is made each Wednesday/Thursday for each AHWI available to test on the following week. Tests are selected from the APHIS test queue and a Cognos Report, that identify TB test to be carried out by Private Veterinary Practitioners who enter their work information on-line. That's aimed to join Brucellosis and Tuberculosis tests in the same herd in order to help to ensure efficient use of herd keeper's time and reduce stress to his stock. In this way, a large

percentage of BB test each week will be done alongside TB tests. Completed work programme goes to the Administration Unit, who arrange test and send appointment letter to give, at least, 3 days notice. Many of test are Brucellosis Risk tests, and some Annual Herd Tests. Tests have been performed 6 days per week when necessary.

Some problems encountered:

- Late cancellation of tests, that causes AHWI downtime
- Poor handling facilities, with danger for Health and Safety
- Numerous Out farms, that make long days with low outputs
- Not all animals be present for checks, so, return visit is required and time wasted

8. Administration organisation in a local DVO

John McKeever

The administration organization in a local DVO is under the responsibility of an Executive Officer Manager (EO), supported by administrative officers (AO) and administrative assistants (AA). The EO has 3 main areas of work: brucellosis, tuberculosis and identification restriction and movement rules.

The aim of the administrative team is to provide support and work closely with the DVO, VO and AHWI.

The brucellosis work area (administrative actions on brucellosis restricted herds) is the first priority at the administrative organisation at the local DVO.

The most important administrative actions implemented on brucellosis restricted areas are movement restriction notices, notices of intended slaughter, valuation papers and health and safety notices.

Some of the further administrative actions implemented on brucellosis restricted herds are notification of outbreaks, preparation of outbreaks herd maps, elaboration of restriction letters to the farmers.

Every outbreak has a process map for office procedures (herd inspections, inner ring mapping, changes on testing routines and herd restrictions). This process map is available to the VO, AO and AA.

In conclusion, regular staff updates (weekly briefings), staff views encouragement, well defined procedures, thorough staff training, staff rotation to enhance knowledge and managerial monitoring are considered the most important factors that help in the routine work of the office structure.

9. BR eradication programme in the Republic of Ireland- overview and progress

Garry O'Hagan

The State Veterinary Service has responsibility for the implementation of the Brucellosis Eradication Scheme. The programme is based on a test and slaughter policy. Routine testing includes an annual blood testing of all breeding animals over 12 months. Additional testing includes post abortion testing, 30 day pre/post movement tests, and targeted testing of herds of contiguous to a disease outbreak. Bulk milk i-Elisa is carried out monthly on all dairy herds.

The official test are the SAT, the i-Elisa, and the CFT and the milk Elisa. A SAT is performed on all blood samples(screening) and i-Elisa and CFT on all samples from herd with one positive SAT.

There has been a significant reduction in the prevalence of the disease between 1998 and 2008. New restrictions have decreased from 459 in June 1998 to 61 in June 2008, serological positives from 4152 to 139, and herds depopulated from 147 to zero. The last infected outbreak was in April 2006.

There were a number of new disease control measures introduced to the scheme during this period, which contributed to the success of the programme including a 30 day pre movement test, a rapid depopulation policy, treatment of infected slurry and testing of culled cows at slaughter.

Following a 12 month study (2004/2005) of non specific infection in herds with blood positives, a protocol has been developed to deal with false positive serological reactions.

A roadmap is planned for the next five years which will include applying for OBF status in April 2009 followed by a very cautious approach to a gradual reduction the serological programme, maintenance of the monthly bulk i-Elisa test and the abattoir surveillance.

DAY 2

1. Introduction to the Veterinary Sciences Division at the AgriFood Biosciences Institute (AFBI)

Sam McCullough (AFBI)

The group was first welcome by S. Kennedy, Deputy CEO and Director of the VSD. The AFBI is a non-departmental body, granted by the DARD. It now includes the Veterinary Sciences Division (VSD) that was before a full division of DARD. The VSD is divided into two branches:

- the immunodiagnostic branch that deals with *Brucella* serology;
- the bacteriology branch that deals with *Brucella* bacteriology and molecular biology.

2. *Brucella* serology

Sam McCullough (AFBI)

Since the last visit of the expert group in 2002, the number of samples tested by VSD has dramatically increased (> 1,400,000) due to the introduction of pre-movement testing in 2004 and introduction of parallel testing by both SAT and i-ELISA in 2006 (Fig.1).

In order to face this new load, additional staff was recruited, new accommodation has been made available and testing and information handling were automated.

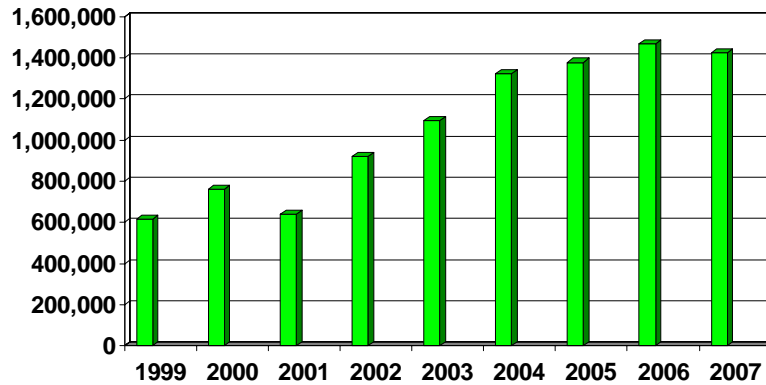


Fig.1: Serum samples tested per year at the VSD

Submission rates rise through the winter, peak in spring and fall off again in summer (Fig. 2). The lab. Deals with this by (i) ensuring lab and equipment capacity for peak demand, (ii) imposing complementary seasonality on other work programmes and (iii) transfer of staff.

The breakdown of samples types is: (i) surveillance and eradication (1,179,515 samples), (ii) pre-movement testing (235,623), (iii) Older Cattle Disposal Scheme (OCDS) (31,251) and (iv) bulk milk testing (31,280 samples).

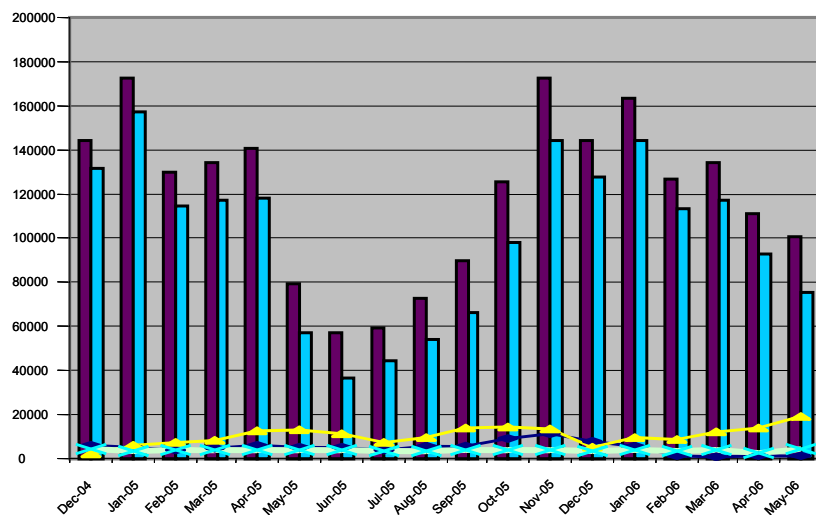


Fig.2: Seasonality of sero-testing at the VSD

The tests currently performed are microtitre serum agglutination test (MSAT), MSAT with addition of EDTA, Complement fixation test and i-ELISA. The lab has also been performing c-ELISA and Rose Bengal test for specific studies, particularly the “Parallel trial” done in 2005.

Serum samples are screened by MSAT (at 1:10, 1:20 and 1:40 dilutions) with a cut-off of 25 IU/ml.

The selection of non-negatives for further testing depends on the identified level of risk:

Low risk biennial herd test ≥ 25 I.U.

Low risk annual herd test ≥ 20 I.U.

At risk field samples ≥ 12 I.U.

If there are titres present in low risk herds which are \geq the cut-off in a low risk test, then apply "at risk criteria" to whole herd test

OTMS ≥ 20 I.U.

In all situations the cut-off taken into account is lower than the one considered in the annex C of the 64/432/ECC directive for trade (30 IU/ml)

If samples are non-negative at screening, they are titrated to end-point by MSAT, EDTA-MSAT and CFT. But, since 2006 the numbers of samples from risk tests which have been screened by both MSAT and i-ELISA in parallel have been increased by the lab. (118,000 i-ELISAs in 2007; 171,500 already in Sept. 2008).

Information management and sample tracking of serum samples are as follows:

Each submission is accompanied by form BT20a where details of animals sampled are given and that also functions as lab work-sheet. Samples and details are checked and discrepancies are looked for according to a procedure. The negative results on screening are entered on BT20a and validated by lab manager. The non-negative sample details are transferred to lab work-list for further tests and final results are entered on BT20a and validated by lab manager. The results are then entered on APHIS and validated by work area manager. The hardcopy original BT20a is completed, and work-sheets and work-lists are archived.

Information management and sample tracking of milk samples are as follows:

Samples are received from dairies, bar-coded by producer number. The results are currently reported manually but an electronic transfer system is nearing completion.

The tests are carried out according to requirements laid out in Technical Annex of Directive 64/432/ECC. For MSAT and CFT, there is a daily control using national standard serum, checked against OIE standard from VLA Weybridge (UK). Antigens and i-ELISAs kits are commercial products. Tests are completed within 3 working days for screening and 5 working days when further testing is needed. Information about the samples process is accessible to private vet. Practitioners on the lab. Website.

While not yet accredited according to ISO 17025 standard, the lab. Has implemented a quality assurance system. This includes: 1% of samples chosen at random for retesting, cross checks of test plate reading skills, training period and assessment prior to carrying out tests, random checks on work-lists, validation of all results prior to reporting, and monitoring for errors, with action taken and monitored. The lab. Has also participated in UK and ROI ring-trials, in 2000, 2002, 2003 and 2005 previous European ring-trials (organised by VLA, UK) and to the first proficiency ring-trial organised by the newly established EU-CRL in 2007-2008. All results were within acceptable ranges.

Finally, the four following recommendations of the previous Task Force visit were implemented:

- The NI authorities are greatly encouraged to assess other tests (as RBT for instance) comparatively to the MSAT currently in use in order to check whether MSAT (SAT+ EDTA) could not be at least partially responsible of this low sensitivity of the surveillance system.
- Parallel screening of cattle in the higher risk areas, would be done using what would be considered as the most adequate test and CFT could be used for confirmation of outbreaks.
- The laboratories adaptation should be considered if the number and especially the kind of tests carried out are due to change.
- According to Directive 64/432/ECC, results of the SAT should be expressed in units and the CFT results in EEC Units.

3. *Brucella* culture, characterisation and typing– D. Graham & Stanley McDowell (AFBI)

From its previous visit in 2002, the Task Force subgroup had noted that due to laboratory constraints (i) isolation of *Brucella* was only performed on lymph nodes and vaginal swabs, (ii) the culture of foetuses, abortion material and milk, was not currently performed with a standard incubation period of 6 days. The group then recommended (i) the extension of the lab and improvement of the equipment, (ii) the culturing of other more heavily infected samples (especially the stomach content or lung of the aborted foetus) that could help in a more rapid confirmation of outbreaks and (iii) an incubation for 10 days at least or using enrichment procedures (broth sub-cultures).

The laboratory Facilities were rebuilt in 2008 (granted by DARD) with a new modern building including containment level 3 well equipped bacteriology laboratories, one of them being dedicated to *Brucella*. Then, the VSD received the Specified Animal Pathogens Order (SAPO) licence.

Samples submitted to *Brucella* culture are collected at slaughter (since 2007, parotid, retro-pharyngeal, sub-mandibular (head), supra-mammary in female [or superficial inguinal in males] and iliac lymph nodes), vaginal swabs from aborted animals in high risk herds and foetuses and aborted material submitted by private veterinary practitioners for diagnostic service.

Up to three animals sampled per herd - typically high titre & / or freshly aborted animals.

For foetal submissions, foetal stomach content is cultured (if not available: pool of foetal organs (liver, lung and kidney).• Placenta is cultured the same way if available.

Samples are not released to other labs until cleared for *B. abortus*.

The procedure used for culture is according to the OIE Manual and to the UK National Reference Method (NRM004).

For primary isolation from tissues, all 5 lymph nodes are cultured individually selective Farrell's medium (2 plates per lymph node), and incubated at 37°C in 10% CO² for 14 days (with a reading every 3-4 days).

As regards identification of species and biovars, the OIE manual recommendations are followed except for small details (the pararosaniline

sensitivity test is not recommended by OIE; the biovar 7 of *B. abortus* was deleted from the *Brucella* classification in 1986 ; there is a biovar 9 for *B. abortus* that is not considered by VSD).

Most isolates in Northern Ireland are typical *B. abortus* biovar 1 strains. From 2001 to now only 10 *B. abortus* biovar 2 strains were also isolated.

Figure 3 shows the number of animals sampled and the number of samples submitted to *Brucella* culture at VSD in comparison with numbers of reactors at NI level(1995-2008).

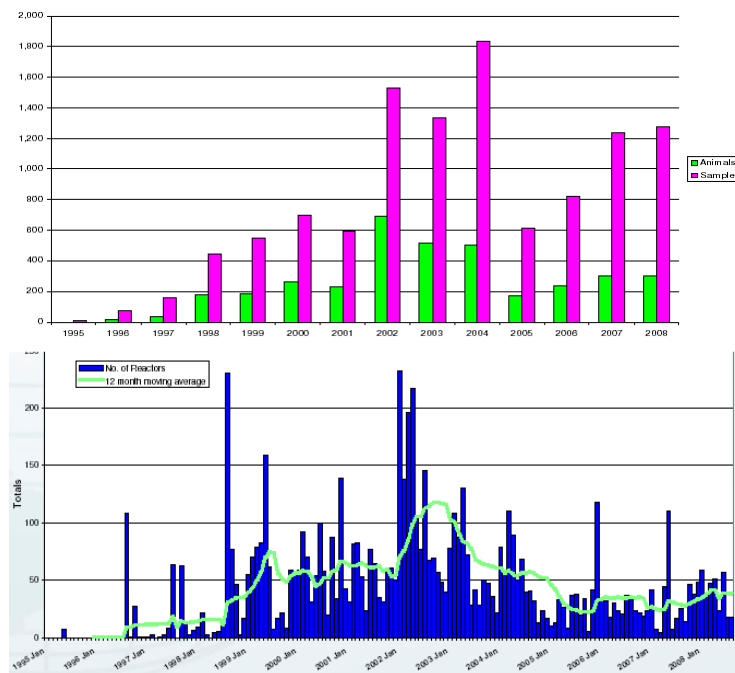


Fig. 3:Numbers of animals sampled and samples submitted to *Brucella* culture at VSD in comparison with numbers of reactors at NI level(1995-2008)

Tables 1- 3 gives details on respective isolation rates according to the kind of lymph node cultured. Results are in accordance with those reported elsewhere showing that the supra-mammary LN gives the highest rate of isolation and that the culture of at least 4 different LN optimises the global isolation rate.

Table 1: Isolation rates for individual lymph nodes

Lymph Node	Parotid	Retro-pharngeal	Sub-mandibular	Supra-mammary	Iliac
Number tested	1662	1678	1653	1602	230
+ve	695 (42%)	787 (47%)	719 (43%)	<u>810 (51%)</u>	68 (30%)
-ve	967 (58%)	891 (53%)	934 (57%)	792 (49%)	162 (70%)

Table 2: Numbers positive only in 1 lymph node

Based on culture positive animals received between 2002-2007 with all 4 LN (n=940)

Lymph Node	Parotid	Retro-pharngeal	Sub-mandibular	<u>Supra-mammary</u>
Number	15	26	16	57
Percentage	1.6%	2.8%	1.7%	6.1%

Based on culture positive animals received in 2007 with all 5 LN (n=90)

Lymph Node	Parotid	Retro-pharngeal	Sub-mandibular	<u>Supra-mammary</u>	Iliac
N	0	4	0	9	0
%	0.0%	4.4%	0.0%	10.0%	0.0%

Table 3: Numbers positive in head compared to supra-mammary LNs

Based on culture positive animals received between 2002-07 with all 4 LNs (n=940)

Lymph Node	Positive in Head LN's only	Positive in Both	Positive in Supramammary only
Number	131	752	57
Percentage	13.9%	80.0%	6.1%

Fig. 4 and 5 give the numbers of samples submitted for culture and the isolation rates for respectively the fetuses (400-800; 0.0% to 1.2%) and the vaginal swabs (23-163/year, 12-25% positive). Overall animal confirmation rate is 49% (based on samples from 2434 animals received between 2002 and 2007). The herd confirmation rate is 51% (based on 1206 herds represented by samples received between 2002 and 2007). However it is not uniform according to years (Fig. 6).

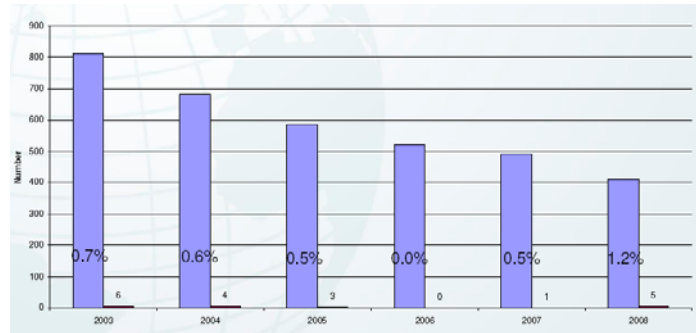


Fig. 4: Foetal submissions with isolation rates(2003-2008)

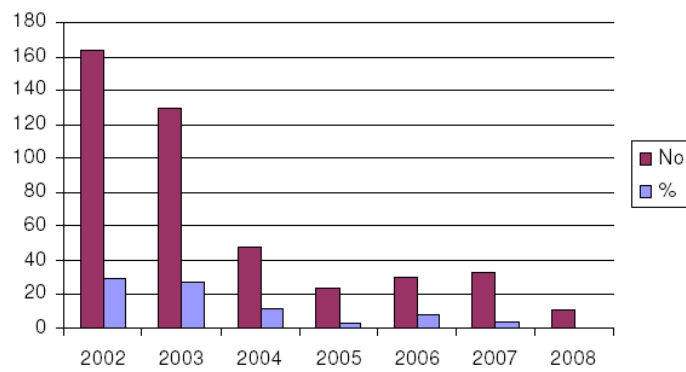


Fig. 5: Foetal submissions with isolation rates(2003-2008)

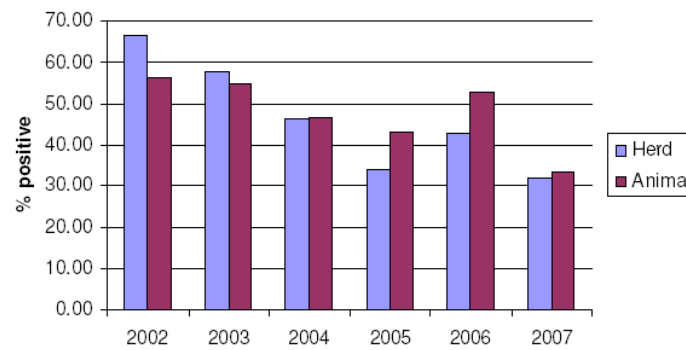


Fig. 6: Results overview Isolation rates in herds and animals (2003-2008)

4. Molecular typing of *Brucella abortus*: NI pilot study

R. Skuce, T. Mallon, C. McCormick, S. McDowell (AFBI) and A. Whatmore (VLA, Weybridge, UK)

The objective of this pilot study was to investigate the potential interest of new molecular tools, particularly the MLVA (VNTR analysis) in giving additional epidemiological information as regards the relationship between breakdowns at historical and geographical levels.

In this study VLA tested VNTR profiling on 26 different VNTR loci (8-marker Hoof-Prints assay from USA, 13 VLA VNTRs and 5 BRUCE VNTRs [Université Paris-Sud, France]).

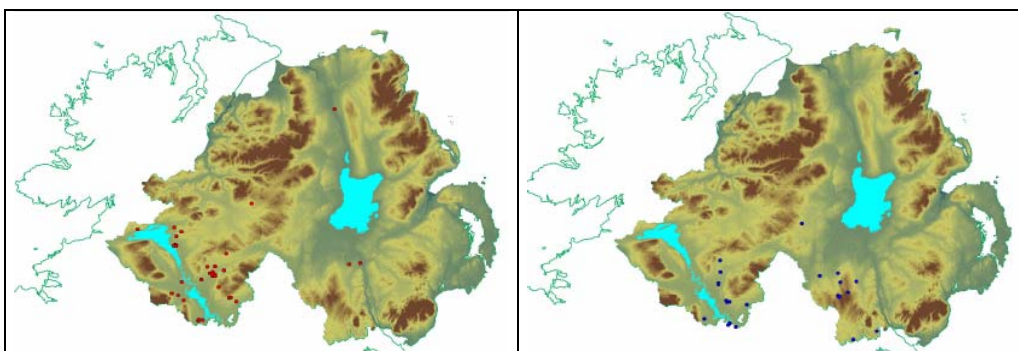
513 *B. abortus* isolates from 269 herds (2000 and 2004-2007) were tested. The analysis of results was performed at AFBI using the Hunter-Gaston diversity index (D) per marker or marker string.

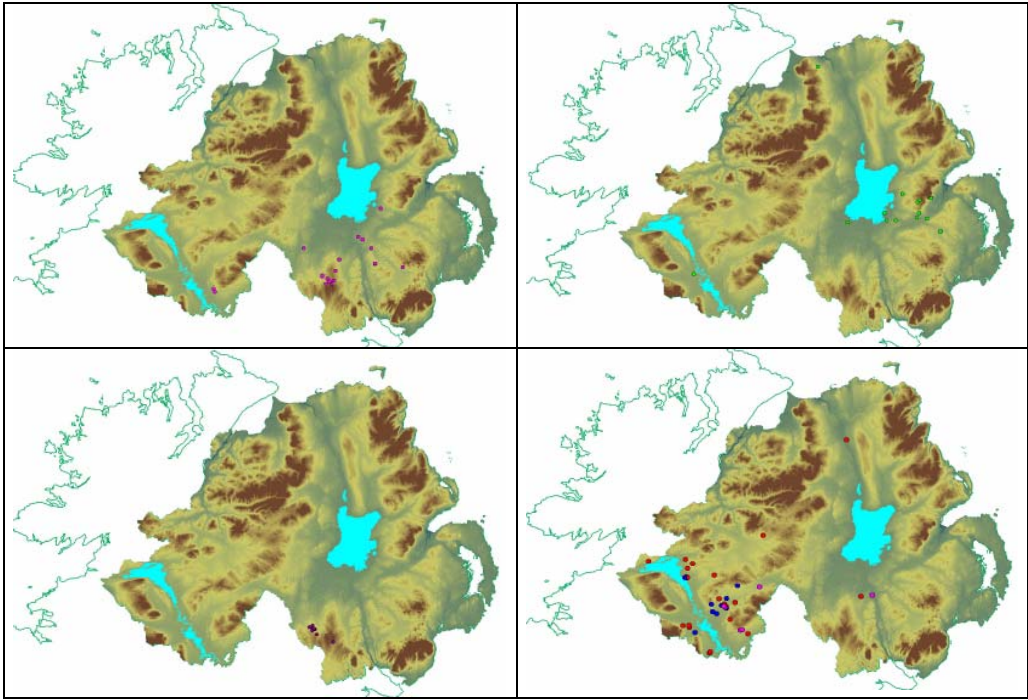
The results disclosed within-herd multiple isolates (88 herds with 2-13 isolates) and within-animal multiple isolates (46 animals with 2-4 isolates) and showed that some markers do not discriminate this sample at all. Some markers showed unacceptable instability. Multi-locus assay selects markers which better balance discrimination and stability.

In the 513 *B. abortus* isolates, 63 profiles (10 VNTRs, D=0.94) were identified with a marked degree of geographic localisation for profiles. This was not evident with 26VNTRs or Hoof-Prints alone. However results could be consistent with epidemiologically-linked outbreaks?

Therefore VNTR 'genetics' allows relationships between strains to be inferred. As a conclusion of this pilot study, the markers and assay merit further investigation.

The following figures show the different VNTRs clusters identified.





CONCLUSIONS

The Bovine Brucellosis Task Force Subgroup acknowledges the important efforts made by the NI CA in order to improve the situation of the disease in NI. The Task Force was pleased to see efforts being made both in the programme and in the laboratory. In particular, the main recommendations made by the subgroup in 2002 have been fully implemented.

However while the situation improved clearly up to 2005, the incidence of disease increased again regularly up to now.

But the outbreaks are generally clustered in limited numbers of areas. This shows the efficiency of the detection strategy implemented. However, the initial source of infection remains in some cases unidentified, which highlights the difficulty in identifying sources of infection and emphasises the need for continuing intensive efforts.

The experts identified potential reasons for the ongoing epidemic:

- insufficient depopulation of confirmed outbreaks which could favour latent carrier animals remaining in the field
- the group is concerned that the compensation scheme could encourage fraud and does not encourage farmers to implement biosecurity measures.

In order to limit the spread of the disease from outbreaks and to prevent new outbreaks, awareness of breeders in terms of biosecurity is essential. This could be improved through reinforcement of awareness campaigns already in place and greater involvement of farmers and their associations.

While sheep and goats are not very sensitive to B. Abortus, with usually no abortion, this species is receptive and could constitute a reservoir of infection. The potential existence of such a reservoir should be investigated.

RECOMMENDATIONS

- Continue with, and intensify the measures being employed.
- All infected herd should be depopulated. In truly exceptional circumstances a herd may not be depopulated, if subject to a thorough epidemiological investigation and the decision agreed at a senior level.
- Review of compensation scheme should be completed as soon as possible. Upper limit (cap) should be set. The compensation levels in other MS should be considered.
- Brucellin Skin Testing should actively considered.
- Further involvement of the technical and producers associations in the programme is strongly encouraged in particular to improve biosecurity measures.
- All sheep & goats on infected farms should be tested.
- Lab accreditation should be finalised and obtained

Meeting of the Bovine brucellosis sub-group of
the Task Force for monitoring disease eradication
held in BELFAST, Northern Ireland, UK, 07-08 October 2008

PARTICIPANTS

Task Force Sub-Group - members

- | | |
|----------------------------|-----------------------|
| ▪ Ernst STIFTER, Chairman, | Italy |
| ▪ Georgia BARGOULI | Cyprus |
| ▪ Bruno GARIN-BASTUJI | France |
| ▪ Garry O'HAGAN | Ireland |
| ▪ Antonio PINA-FONSECA | Portugal |
| ▪ José-María PARAMIO | Spain |
| ▪ Darrell ABERNETHY | UK (Northern-Ireland) |
| ▪ Valentina PIAZZA | Commission |
| ▪ James MOYNAGH | Commission |

Northern-Ireland Representatives (main list)

A. Veterinary Services of Northern Ireland

▪ Roland Harwood	Senior Principal Veterinary Officer – Enzootics Section (SPVO)
▪ Owen Denny	SPVO –Implementation, Southern Region
▪ David Brown	DVO – Enniskillen office
▪ Sharon Verner	DVO Brucellosis, Enzootics Section
▪ Gerry Murpy	SAHAWI – Newry office
▪ John Mckeever	Office manager, DARD, district Newry
▪ Judith Graham	VO – Brucellosis section
▪ Barbara Geddis	VO – Brucellosis section
▪ Michael Bready	DVO – Newry Office
▪ Gerald Henderson	Deputy Principal Officer, TBIBR Policy Branch, Animal Health and Welfare Division, Central Policy Group
▪ Darrell Abernety	Epidemiology DVO and Task Force member

B. Agri-Food and Biosciences Institute (Veterinary sciences Division)

- Seamus KENNEDY, Deputy CEO and Director of the VSD
- Sam Mc CULLOUGH AFBI, VSD, Immunodiagnostic Branch
- David GRAHAM, AFBI, VSD, Bacteriology Branch
- Robin SKUCE, AFBI, VSD, Bacteriology Branch

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**MEETING OF THE BOVINE BRUCELLOSIS SUB-GROUP OF
THE TASK FORCE FOR MONITORING DISEASE ERADICATION
HELD IN BELFAST, NORTHERN IRELAND, UK, 07-08 OCTOBER 2008**

AGENDA

Timing	Item	Presenters/ Rapporteurs
DAY 1	NEWRY Divisional Veterinary Office (DVO)	
09.30	1. Introduction and Overview	Roland Harwood
10.00	2. Epidemiology – the current picture in NI	Darrell Abernethy
11.00	<i>Tea / coffee</i>	
11.15	3. Northern Ireland Brucellosis Scheme	Sharon Verner
12.00	4. Questions and discussion	Roland Harwood
12.30	<i>Lunch</i>	
13.00	5. Farm visit	
14.45	<i>Tea / coffee (on return to the DVO)</i>	
15.00	6. Overview of BR in Armagh, Newry and Enniskillen DVOs	Owen Denny
16.00	7. Operation of a Field DVO and introduction to BR procedures	David Brown
16.30	8. Organisation of testing in a local DVO	Gerry Murphy
16.45	9. Administration organisation in a local DVO	John McKeever
17.00	10. BR eradication programme in ROI- Overview and progress	Garry OHagan
17.20	11. Questions and Discussion	
18.00	close	
19.00	<i>Visit Dinner – Canal Court Hotel, Newry</i>	
DAY 2	AGRI-FOOD AND BIOSCIENCES INSTITUTE (AFBI) and VETERINARY SERVICE (VS) HQ	
09.30	1. Introduction to Veterinary Sciences Division (AFBI)	Sam McCullough
	2. <i>Brucella</i> serology	Sam McCullough
	3. <i>Brucella</i> culture, characterisation and typing	David Graham
	4. Molecular typing of <i>Brucella abortus</i> : NI pilot study –	Robin Skuce
	5. Tour of laboratories	
11.30	<i>Tea/ coffee (on return to VS HQ, Room 1033)</i>	
12.00	6. EU Task Force BR subgroup meeting (Room 1033)	
13.00	<i>Lunch (Room 601)</i>	
14.00	7. Final conclusions and recommendations Final opportunity for questions (Room 601)	All Speakers including any HQ staff TF Subgroup
15.00	close	
	<i>Tea/ coffee</i>	