Chapter 1

Introduction

I have chosen to write my thesis on Bovine brucellosis Eradication. I chose this topic as I have a personal interest in the eradication schemes in place in Ireland. Ireland is currently officially brucellosis free, a status reached in 2009. Currently testing for brucellosis is a daily requirement for veterinary surgeons in Ireland. Although the country has reached brucellosis free status we still need to remain vigilant to the prospect of reoccurrence. Brucellosis infection in a herd can cause major production and economic losses not to mention the emotional trauma experienced by herd owners as a result of a rapid depopulation programme.

The brucellosis Eradication programme in Ireland commenced in 1965 and is governed by European legislation. The main legislation with regard to eradication is council directive 64/432/EEC, council directive 78/52/EEC and Irish legislation S.I no.114 of 1991 all of which will be discussed in greater detail in the context of this thesis. Although at the beginning of the eradication programme several problems were encountered, namely and possibly the most important lesson learnt was the relaxation of the programme in the 1980's which resulted in the resurgence of the disease. However from 1998 onwards progress was made, several changes were made to the eradication scheme which were instrumental in reaching officially brucellosis free status in 2009.

Chapter 2

Brucella an Introduction

Brucella the causative agent of brucellosis is a gram negative bacteria named after David Bruce who discovered it in 1884. They are small, non-motile, non-spore forming, intracellular, facultative, coccobacilli that belong to the genus α2-proteobacteriacea (Neta C et al 2009).

Ten species of *brucella* have been identified which have approximately 94% homology, *Brucella* species have a strong affiliation for a specific natural host; the classification is based mainly on differences in pathogenicity and host preference (Oreno E. Et al 2002). The species and hosts are listed in table 1. Distinction between species is based on phenotypic characterization of lipopolysaccharide (LPS) antigens, phage typing, dye sensitivity, requirement for CO_2, H_2S production and metabolic properties (Whatmore A.M. 2009). These factors are used as important diagnostic parameters.

Table 1: *Brucella* species and Host's.

Species	**Host**
B.abortus	Cattle
B.melitensis	Goats and sheep
B.Canis	Dogs
B.suis	Pigs
B.Ovis	Sheep
B.pinnipedialis	Seal
B.ceti	Dolphin, tortoise, whale
B.microti	Common vole

2.1. A synopsis of *Brucella* species: The following is a brief synopsis of the most important species causing disease in an agricultural environment. As brucellosis is an important zoonotic disease causing human infection will also be discussed briefly.

2.1.1 *Brucella abortus.*

Is the major cause of abortion in cattle. It is caused by biovars 1, 2, 3, 4, 5, 6, and 9. It is known to cause zoonosis, undulant fever in humans. Transmission is through uterine

discharges or congenital infection. The most susceptible animals are sexually mature cattle, usually in first calf heifers and older cows. Clinical signs include abortion in last trimester of pregnancy, still born or weak calves, a drop in milk production and orchitis and epididymitis in bulls. Clinical pathology findings usually include necrotizing placentitis and inflammatory changes in the foetus. (Neta C. Et al 2010)

2.1.2 *Brucella Melintensis*

Is the causative agent of disease in goats, sheep, humans and occasionally cattle. It is caused by biovars 1, 2, 3.Transmission is through congenital, and ingestion or contact with infected placenta or vaginal discharges of infected animals. It can also be transmitted through milk. Clinical signs include abortion in the last trimester and weak newborns. Clinical pathology findings usually include placentitis (Godfroid et al 2010).

2.1.3 *Brucella Suis*

Is the causative agent in pigs. It is caused by biovars 1, 2, 3, 4 and 5. Biovars 1-4 can cause disease in cattle however this is rare. The infection in pigs is transmitted by contact, ingestion and venerally. Clinical signs include infertility, small litters and abortion and stillborns. In boars it can cause orchitis and lameness. Clinical pathology findings reveal placentitis (Godfroid et al 2010)

2.1.4 *Brucella ovis*

Is the causative agent in sheep. The infection is transmitted by sexually mature rams by direct contact or venereal. Clinical signs include infertility in rams due to epididymitis, abortion in the last trimester in ewes and still born lambs (Godfroid et al 2010)

2.1.5 Humans brucellosis

Brucellosis in humans is mainly caused by B.*melitensis*. It is transmitted by the consumption of contaminated foods, most commonly through unpasteurised dairy products and by direct contact, as a result it is usually an occupational disease. Clinical signs include joint and muscular pain, sweating, miscarriage headaches and depression. Blood tests usually reveal leukopenia and anemia. The disease can last from a few days, weeks, months or even years (Godfroid et al 2010)

Chapter 3

Epidemiology of brucellosis.

Brucellosis recognised since the 20th century is a worldwide highly contagious zoonotic disease. It is caused by members of the genus *Brucella*, which are small, non-motile, aerobic gram-negative, facultative intracellular bacteria (Radostits 2007). Unless controlled it has the potential to cause serious economic losses directly through abortion and decreased milk production and indirectly through trade limitations. In humans it's associated with the consumption of unpasteurised dairy products, direct or indirect contact with animal products, or inhalation. It is mainly considered an occupational disease affecting slaughter house workers, butchers and veterinarians (Corbel M.J 1997). Recently in France a human case of brucellosis was diagnosed, the source was raw milk cheese (Mailles A. et al 2012). Common reservoirs that may infect humans include cattle, dogs, sheep, goats and pigs. Cats are resistant to *Brucella* infection (Neta A et al 2010) There are six species known to cause human disease, *B.melitensis* in goats and sheep, *B.abotus* in cattle and buffalo, *B. Suis* in pigs, *B canis* in dogs *and B. Ceti and B. Pinnipedialis* in marine animals. *B. Microti and B. Neotomae* occurs in wild rodents, although they have not been implicated in human infection (Corbel MJ 1997).

B.abortus the main causative agent of bovine brucellosis (B.melintensis can also cause infection in cattle), has eight biovars, biovar 1 is the most frequently identified in cattle and is an important determining factor when tracing outbreaks. Brucella abortus has been reported in virtually every country.

Although reported cases of the disease is on the decrease bovine brucellosis caused by B.abortus is the most widespread form and is a serious disease problem in the Mediterranean region, Western Asia, and parts of Asia and Latin America (Gul S.T et al 2007). As mentioned previously *B. abortus* has 8 biovars of which 1, 2, 3, 4 and 9 are the most prevalent. Ruminants are most susceptible to *B.Abortus*, this is of particular importance in areas where eradication programmes are currently been undertaken. Infection occurs in cattle of all ages but is most common in sexually mature animals, particularly dairy cattle (Neta C. 2010).

Infection of herds with Bovine brucellosis is associated with abortion in the last trimester, weak calves and infertility, reduced milk production and an increase in somatic cell count.

Bulls show clinical signs of orchitis, epididymitis and seminal vesiculitis (Neta c et al 2010). Although abortion is the main clinical signs, animals may not have subsequent abortions but may have a normal parturition thereafter. Infact 80% of infected cows abort only once, however they continue to shed the bacteria following subsequent parturitions and milk products. Asymptomatic infections are central to maintaining the infection in a herd, although infection depends on the number of organisms excreted, survival of the organism in the environment and level of exposure of susceptible animals.

The main factor for the spread of brucellosis is abortion. In particular aborted foetuses, foetal membranes and uterine secretions are the most important source of infection. Cows infected with *Brucella* are 3 times more likely to abort that cows that are unexposed. Infection can also be spread to calves through contaminated milk as infected cows shed *Brucella* in their milk. Congenital spread may also occur; the animal may remain serologically negative until its first parturition, when the animal will begin to spread the organism. The use of semen for artificial insemination is also a risk factor.

Infection can be transmitted by ingestion, penetration of the intact skin and conjunctiva and contamination of the udder during milking. The main route of infection in cattle is oral or ingestion, through the gastrointestinal tract, following uptake of food or water contaminated with secretions from infected animals. In pasture it has been reported that *Brucella* can remain viable for long periods, it does not multiply in the environment but can survive for up to 12 months. From the gastrointestinal tract the infection spreads to the lymphatic system leading to systemic infection, the most favoured sites been the pregnant uterus, male genital organs and mammary glands. The strong attraction to the uterus is taught to be due to the high concentrations of erythritol. Erythrophagocytic trophoblastic cells are considered the primary site of invasion of foetal placental tissues, resulting in insufficient foetal-maternal exchange, ultimately resulting in abortion (Neta A et al 2010).

Animals become infected with *B.abortus* when animals which have not yet been diagnosed with the infection are introduced into susceptible non infected herd. Of particular risk are animals that have been taken to fairs or shows where they come into contact with positive animals and are then reintroduced into their herd of origin (Samartino L.E. et al 1993). However in recent years this has become a less likely scenario as animals participating in fairs and shows must come from a brucellosis free herd and have passed a pre-movement test. In countries like Ireland which gained brucellosis free status in 2009, 20 % of the herds are

tested annually with all breeding animals over 24 months been tested. Importing of breeding cattle from a country that is not brucellosis free to a country that is brucellosis free is another important risk factor. To deal with this problem countries such as Great Britain have introduced post-calving testing for imported cattle. Regular testing of animals is paramount to any eradication programme as rate of spread depends on the level of surveillance testing.

Brucellosis occurs in many wild species including Elk, bison, deer, wild boar, fox, and other wild ruminants (Godfroid J 2002). Bison and Elk are potential reservoirs for cattle as the infection can remain latent for several years. Sheep can become infected if exposed to cattle with infection; this is a very important factor in eradication programmes. Dogs can also be important in eradication as they can acquire *B.abortus* from infected cattle. The re-introduction of infection from wildlife is a major concern in member states which are officially brucellosis free. In an outbreak of brucellosis in cattle in the USA it's believed the infection originated from elk. (Beja-Pereira A. et al 2009). In France an outbreak of *B.suis* in domestic pigs originated in wild boar (Garin-Bastuji et al 2000).

Chapter 4

Vaccination

As brucellosis infection can result in serious economic losses and because of the risk of zoonosis in humans, efforts have been made to prevent infection of animals by using vaccines. Often the first step in development of an eradication programme is vaccination; however on its own it's not sufficient to eradicate disease. The major factor inducing an antibody mediated response in animals has been identified as Lipopolysaccharide (Moriyon I. et al 2004). Induction of an immune response can also be associated with phenotype, *Brucella* can have either a rough or smooth morphology. *Brucella* devoid of the O-LPS are termed rough or "R", or smooth of those carrying S-LPS. However it is possible that they may revert to either type and this change could be associated with a decline in virulence (Moriyon I. et al 2004). Most diagnostic tests are based on the detection of antibodies to the O-LPS rough type antibodies. The main vaccines used in prevention of brucellosis are, B.*abortus* strain 19 (S19), B. *abortus* strain RB51, B. *Melitensis* Rev.1, and in the past B.*abortus* strain 45/20 (Schurig G et al 2002)

4.1 B.*abortus* strain 19

This vaccine is the most widely used for prevention of brucellosis in cattle (Nicoletti, 1990). It induces the production of antibodies to O -LPS. It is a live vaccine; its effectiveness is based on route of administration, age of the animal and amount of vaccine given. It is given to female calves between 3-6 months of age as a single subcutaneous dose. A reduced dose can be given to adult cattle, however in a pregnant animal this may cause abortion and excretion in milk, adult animals can be vaccinated via a conjunctival route to reduce this risk (OIE 2009). Following vaccination antibodies can be detected with serological assays and this can cause a major problem in distinguishing vaccinated from infected animals. As animals age they are at an increased risk of developing persistent antibody titres to O LPS antigens. In a study of serological tests carried out pre and post vaccination, tests which had negative results pre-vaccination showed positive results post-vaccination with S19, within a 2-10 weeks period (Stevens M et al 1994).

4.2 B.*abortus* strain RB51

This vaccine has become the official vaccine in use in many countries for prevention of brucellosis. It is usually the vaccine of choice because it does not interfere with serological diagnosis. In the USA the vaccine is administered between 4-12 months of age subcutaneously. Vaccination of animals over 12 months of age is only given following authorisation. In other countries a second vaccination is given as a booster following the first vaccination as calves. The vaccination can induce abortion in pregnant animals and may be excreted in milk. If the dose of the vaccine is reduced abortion in adult animals can be avoided, however administration of a reduced dose to calves with not protect against infection (OIE 2009). In a study of serological tests carried out on animal's pre and post vaccinations with RB51 results were negative both pre and post vaccination. These results indicate that vaccination with RB51 does not produce antibodies that can be detected with serological tests used for the detection of brucellosis (Stevens M et al 1994). RB51 vaccine has low levels of O LPS antigen and this may be the reason that it is not detected. It has also been reported that animals vaccinated as calves with S19 and then vaccinated with RB51 as adults will not have a positive result on serological tests for brucellosis (Olsen S et al 1996).

4.3 B. *melitensis* strain Rev.1 vaccine

In countries with a high prevalence of B.*melitensis* in small ruminants this vaccine can be used in cattle as there is controversy on the protection S19 gives against this strain (OIE 2009).

4.4 B.*abortus* strain 45/20

This strain of the vaccine has been shown to protect cattle from infection. However the strain has been reported to revert to smooth virulent form when injected into cattle. As genetic defects in this strain are unknown, and it can cause unpredictable serological results, the vaccine is no longer on the market. (Moriyón I et al 2004)

Chapter 5

Diagnosis

Diagnosis of Brucellosis is based upon the isolation and identification of the bacteria directly or with the use of serological testing methods. Any abortions on a holding in late gestation should be treated as a potential brucellosis infection and should be investigated immediately.

5.1-Serology

In order to support diagnosis of brucellosis the European commission has set up a Community Referenence Laboratory (CRL) in France which communicate with the National References laboratories (NRL) of each member state.

No serological test for brucellosis is 100% sensitive or 100 % specific. In animals vaccinated with *brucella* false positives are common due to cross-reactions of antibodies with wild strains (Nielsen K 2002). Serological tests measure antibody response to the lipopolysaccharide (LPS) O antigen of wild type strains of B.*abortus* (Moriyon I et al 2003). As no test can identify all positives or all negatives it is imperative that confirmatory tests are used to identify true positives especially in areas that are free of brucellosis. The interpretation of results from samples should consider, the percentage of positives, disease prevalence and incidence, presence or absence of clinical signs, vaccination protocol, herd, and area and country status.

In use tests since 1987 include:

5.1.1 *Agglutination tests*

Serum Agglutination test (SAT). The SAT test first described in 1987 by Wright and Smith, is subject to high false positives and its use is no longer recommended by the OIE (Nielsen K.2002).

Rose Bengal test (RBT). The RBT is a simple spot agglutination test using *B.abortus* S99 or S1119.3 whole cells stained with Rose Bengal. Any visible reaction is considered positive (Nielsen K.2002).

Buffered plate agglutination test (BPAT). Uses B.abortus S1119.3 whole cells stained with crystal violet and brilliant green. Any visible reaction is considered to be a positive (Nielsen K. 2002).

With both RBT and BPAT false positives can occur and it is recommended by the OIE that other tests be used for confirmation.

5.1.2. *Complement Fixation Test (CFT).*

This test is widely used and is acceptable as a confirmatory test. The principle of this test is that if antibody is present in serum, it will bind antigen and complement will be activated, an indicator system is added which consists of sheep erythrocytes sensitized with rabbit antibody. Sera giving a titre of 20 ICFTU/ml or more are considered to be positive (OIE 2009).

5.1.3. *Primary binding assays- radioimmunoassay*:

Indirect enzyme immunoassay (I-ELISA). I-ELISA was developed by Caelsson in 1976. Numerous variations of the test now exist, with commercial kits been widely available. I-ELISA uses antigen, antiglobulin-enzyme conjugate and substrate/chromogen. Although the tests are highly sensitive for detection of anti-*brucella* antibodies, they cannot differentiate from antibodies resulting from S19 vaccination (Nielsen k. 2002, OIE 2009).

Competitive enzyme immunoassay (C-ELISA) . This test works on the principle that a competing antibody can be selected to inhibit binding of vaccination antibodies but not wild type induced antibody. It uses monoclonal antibodies specific for one of the epitopes of Brucella species. The C-ELISA is commercially available and the sensitivity is comparable with BPAT and I-ELISA. C-ELISA is a prescribed test for international cattle trade prescribed by the OIE.

Fluorescence polarisation assay (FPA). The FPA is a rapid simple test which can be performed in the field. The mechanism of the test is based on the fact that a small molecule will rotate faster than a large molecule. If a fluorochrome attached to an antigen is added to a test solution that contains antibody, the antibody will bind and thus its rotational speed will be slower. It's this rotational speed which can be measured (OIE 2009).

5.2- The Bucklin skin test

The use of skin delayed-type hypersensitivity (SDTH) or the brucellin skin test is another method used to diagnose unvaccinated animals (Z.Bercovich 2000). This test has a high specificity and may be used to confirm results obtained from serological tests. However not all positive animals are detected and hence it is not recommended as a test for international trade (OIE 2009). During the test procedure 0.1ml brucelin is injected intradermally and the test is read 48-72 hours later. Before injection a skin thickness measurement is taken and compared to a skin measurement taken 48-72 hours later. If the skin thickness increase is greater than 1.5-2mm it is considered a positive reaction (OIE 2009). A study in 1999 comparing the use of SDTH with SAT and CFT in an outbreak of brucellosis found that 95% of infected cattle were detected with SDTH compared to 61% when SAT and CFT were used (Bercovich Z. 2000).

5.3 MILK TESTS

The screening of dairy herds by taking milk bulk tank samples is common place as a diagnostic tool in the eradication of brucellosis in many countries. However dry cows do not participate in this test (OIE 2009). This is a cheap and more readily available test than taking bloods samples from animals. In some countries the milk ring test has been replaced with the more sensitive and reliable I-ELISA test (Sheahan.M et al 2006)

The milk ring test is an adaptation of the agglutination test using hematoxylin stained whole cell antigen added to milk. However it can produce false positives caused by mastitis, colostrums and milk at the end of the lactation period. In small herd where these factors can have a greater impact on results it is not recommended to use this test (Nielsen K 2002).

As was discussed with the serum I-ELISA various commercial variations are available. Milk samples are tested at lower dilution than with serum samples. The I-ELISA can also be used with whey samples. (OIE 2009)

5.4 Isolation and Identification

Samples taken from organs of fallen animals or biological fluids are tested for the presence of *Brucella* species by the Stamp Ziehl-Neelsen method (positive samples stain red against a blue backround), based on colony morphology, urease, oxidase and catalase tests and slide agglutination test with anti-*brucella* polyclonal serum. However it should be noted that other

organisms causing abortions such as *chlamydophila arbortus* and *Coxiella* species are difficult to differentiate from *Brucella*. (OIE 2009)

Brucella species can also be detected by direct isolation and culturing allowing *Brucella* to be identified clearly. The most usable samples for culturing include stomach contents, spleen and lung of aborted foetuses, foetal membranes, vaginal secretions, milk and semen. From deceased animal, samples are taken from the lymph nodes of the head, mammary and genitals along with the spleen, uterus and udder. They can be grown on both basal and selective solid media; liquid media can also be used. After 2-3 days *Brucella* colonies are visible, however cultures are not deemed negative until 8-10 days have passed. Colonies are round, smooth, pale honey colour, 1-2mm in diameter (OIE 2009).

For further identification of *Brucella* species nucleic acid recognition methods can be used including, Polymerase chain reaction (PCR), real-time PCR, PCR restriction fragment length polymorphism and southern blots (Bricker B.J 2002). The use of nucleic acid methods in the identification of *Brucella* can be extremely useful in tracing outbreaks from an epidemiologically point of view.

Chapter 6

Legislation on bovine brucellosis

6.1 European Legislation

The following is a summary of the legislation governing the eradication of bovine brucellosis; EU legislation is used for eradication programmes with the objective of gaining brucellosis free status as soon as possible.

Within the European Union the European parliament has to approve EU legislation together with the council. EU law confers rights and obligations on the authorities in each member country. The Authorities are responsible for implementing EU legislation in the national law and enforcing it.

6.1.1 Legislation referring to official controls in the veterinary field.

Regulation (EC) no.882/2004 of the European parliament and of the council on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules

This regulation aims at:

- Preventing, eliminating and reducing the risk to humans and animals either directly or through the environment.
- Fair practises in feed and food trade.
- That information deemed necessary is available on labels.

European Council directive 64/432/EEC of the 26th June 1964 on animal health problems affecting intra-community trade in bovine animals and swine.

It lays down the requirements for trade of bovine animal. The procedures necessary for obtaining, maintaining, suspension and withdrawal of officially brucellosis free status of a member state.

This directive states that:

- The country exporting the animal is required to ensure that the animal is not a source of contagious or infectious disease
- Each animal must be accompanied by a health certificate
- The Member States has the right to refuse entry of any animal if they are suffering or are suspected of suffering from a contagious or infectious disease.
- Are subjected to an identity check.
- Are subjected to a health check within 24 hours of departure.
- Must be from a holding free of restrictions
- Animals must not have come into contact with other animals of the same species other than those with a similar health status
- Must be transported as meets requirements of directive 91/628/EEC.
- Bovine animals for breeding and production must, in the case of uncastrated animals greater than 12 months of age, have passed a pre-movement test for bovine brucellosis in the past 30 days.
- Animals for slaughter must be slaughtered within 72 hours of arrival,
- If moved to an approved assembly centre, they must be slaughtered within three working days of arrival at the assembly centre.

6.1.2 Legislation regarding the Eradication of bovine Brucellosis

6.1.2.1 The Council directive 64/432/EEC is outlined above.

6.1.2.2 Council Directive 77/391/EEC of the 17th may 1977 introducing community measures for the eradication of brucellosis, tuberculosis and leucosis in cattle.

The purpose of this directive is to improve the state of health of cattle by accelerating or intensifying the eradication of brucellosis and tuberculosis and to eradicate leucosis.

Plans must be drawn up by member state, which gives the details for accelerating the eradication of bovine brucellosis in their country. Upon their completion herds are classed as "officially brucellosis free". These plans must give

- The percentage and number of herds subject to control measures
- The number of herds with confirmed bovine brucellosis,

- The number of animals with suspected brucellosis or considered to be infected. The number of infected animals and of animals slaughtered.
- The format of the initial eradication programmes and of the accelerated plans.
- Member states with bovine free brucellosis cattle population shall inform the commission of all the measures taken to prevent reoccurrence of the disease in their country.

6.1.2.3 Council Directive 78/52/EEC of 13 December 1977 establishing the Community criteria for national plans for the accelerated eradication of brucellosis, tuberculosis and enzootic leukosis in cattle.

In order to qualify for financial contributions as outlined in directive 77/391/EEC herds must satisfy the criteria in this directive.

For the purposes of this Directive, the following definitions shall apply:

- *" in the case of brucellosis in cattle: (a) type B1 bovine herds: herds in whose case the previous clinical history and vaccination and serological status are unknown;*

  (b) type B2 bovine herds : herds in whose case the previous clinical history and vaccination and serological status are known and in which routine monitoring tests are carried out in accordance with the national rules for bringing these herds up to type B3 or type B4 status;

  (c) type B3 bovine herds : brucellosis-free herds within the meaning of Council Directive 64/432/EEC of 26 June 1964 on animal health problems affecting intra-Community trade in bovine animals and swine, as last amended by Directive 77/98/EEC (3);

  (d) type B4 bovine herds: officially brucellosis-free herds within the meaning of Directive 64/432/EEC"

Member States shall ensure that under a plan for the eradication of brucellosis:

- The presence and suspected presence of brucellosis is immediately notifiable
- Any therapeutic treatment of brucellosis is prohibited;

- Vaccination is only carried out under supervision and must be ceased to gain officially brucellosis free status

- A herd that is suspected of containing a brucellosis positive animal is investigated by the authorities as soon as possible

Following the outcome of the investigation

- The herd can be placed under official surveillance,
- All movement into and out of the herd is forbidden unless authorized for slaughter without delay.
- Movement of the castrated cattle on the farm may be authorized after the isolation of the suspect animals, provided that the castrated animals are moved to fattening herds, and hence to the slaughterhouse.
- Isolation within the herd of the suspect animals.

If brucellosis is officially confirmed in a herd, the following precautions must be implemented:

- All movement into or out of the herd in is forbidden, unless the animal is to be slaughtered without delay ;
- Castrated males can be moved only to fattening herds and then to slaughter
- Animals which may have been infected are isolated
- Animals which have positive results or are considered to be infected must be isolated until slaughter
- Milk from infected cows may only be fed to animals on the same farm following heat treatment.
- Milk from cows from an infected herd, cannot be delivered to a dairy, except to undergo suitable heat treatment,

- *"Carcases, half-carcases, quarters, pieces and offal from infected animals intended for use as feed for animals are treated in such a way as to avoid contamination*"

- "*foetuses, still-born calves, calves which have died from brucellosis after birth or placentae are carefully disposed of and destroyed immediately, unless they are to be examined*"

- *“Straw, litter or any other matter and substance which has come into contact with the infected cow or calf or with the placenta is destroyed immediately, burnt or buried after soaking in disinfectant”*
- *“Manure from sheds or other quarters used by the animals is stored in a place inaccessible to farm animals, treated with a suitable disinfectant and stored for at least three weeks. Use of disinfectant is not required if the manure is covered with a layer of uninfected manure or earth. Liquid waste from sheds or other quarters used by the animals must be disinfected if it is not collected at the same time as the manure”*.

- Animals that have tested positive and are considered to be positive must be slaughtered no less that 30 days after the person in charge of the herd has been notified.
- After the slaughter of animals and before restocking, the premises including all containers, equipment and other tools used for animals are cleaned and disinfected, in accordance with the instructions given by the official veterinarian. Pastures which have contained these animals cannot be used for 60 days after their removal except in the case of castrated animals where permission must be granted, these animals can only move to a fattening herd or for slaughter.
- “*All means of transport, containers and equipment are cleaned and disinfected after the transport of animals from an infected herd, or of materials from such animals, or of materials or substances which have been in contact with such animals. Loading areas for such animals must be cleaned and disinfected after use*”
- “*The disinfectant to be used and its concentrations are officially authorized by the competent authority of the Member State concerned*”.
- Brucellosis tests are carried out to confirm that the disease has been eliminated.

- Animals remaining on the holding over 12 months of age must pass a serological test for brucellosis before the herd can be restocked.
- All female animals and all bulls from type B1 herds destined for type B2 herds: - if greater than 12 months must pass 30 day pre-movement test and be accompanied by a certificate from the official veterinarian. These animals must be isolated for 60 days and pass another serological test before movement into the type B2 herd
- Do not come into contact with animals of a lower health status

- Control measures are implemented to prevent re-infection
- all movements of cattle is subject to official monitoring,

6.2 Irish Legislation

With regard to Irish legislation the following abbreviations are used in the text

- "Minster" refers to minister of agriculture and Food
- "Authorised officer "refers to an inspector appointed by the minister.
- "Animal refers to a bovine animal.
- "District Veterinary Office (DVO), refers to an office of the Department of Agriculture and Food.

6.2.1. Disease of Animals Act, 1966.

This is the main law in Ireland concerning Animal health. It is the principle act in Ireland making disease notifiable and concerning control and eradication. Since 1966 several amendments have been made to this act to comply with European legislation.

6.2.2. S.I No. 114 of 1991 Brucellosis in cattle (General provisions) Order 1991.

This order provides that:

- no animal shall be vaccinated with anti-abortion vaccine unless authorised by the minister,
- If authorisation is given the animal must be marked by means of an ear-tag or otherwise.

6.2.2.1 Sample taking

- A test carried out on an animal shall be by taking a sample and this sample can only be tested at an approved laboratory.
- The taking of a sample shall only be carried out by a registered veterinary surgeon or by an officer of the minister.
- The owner occupier or person in charge of the animal must inform the person taking the sample of any circumstances that may affect the outcome of the test.
- A record shall be made of the animal from which the sample is taken.

- If an animal concerned shall give a positive reaction, identity cards shall be surrendered to the District Veterinary office.
- If none of the animals tested have given a positive or inconclusive reaction to the test, the person shall endorse each identity card given to him with an indication that the animal has passed the test and shall return the cards to the person in charge of the herd.
- If a test is returned as inconclusive the person shall return the cards to the District Veterinary office
- No animal from which a sample has been taken can be removed from the holding until such time as the tests have been completed and the person in charge of the animals have been informed of the results of the test.
- A test shall not be carried out without the permission of a veterinary officer.
- No person shall administer to an animal any substance, or in any way interfere with a sample, for the purpose of affecting the accuracy of any test of the animal.

6.2.2.2. Reactor identification and restriction

If a reactor is identified on a holding or a veterinary inspector has reasonable grounds for suspecting that brucellosis may be present, the veterinary inspector involved shall by notice in writing declare the holding to be a restricted. The keeper who is notified shall surrender all identity cards of animals on the holding.

Once a holding has been declared restricted the following applies to that holding

- No bovine animal may be moved into or out of the holding except under the terms of a movement permit
- The veterinary inspector may by notice require that an animal be housed or confined to a designated part of the holding for a specified period.
- Upon notice from a veterinary inspector the holding shall cease to be a restricted holding.

Once a reactor is disclosed the following provisions shall apply

A mark consisting of a diamond shape hole of 1.5cm on each side and punched in the left ear may be applied to the reactor and a tag bearing a yellow disc may be attached to the same ear of the reactor, this shall only be done by a veterinary inspector or an officer of the minister

- The reactor shall be immediately isolated pending it been removed for slaughter.
- Where a reactor is taken possession of under this order it may be disposed of as the veterinary inspector or authorised officer thinks fit.
- An inspector or authorised office may by notice require the person in charge of the holding to cleanse and disinfect the holding or any vehicles used in transporting of the reactor, within a defined period of time. In this article "holding " includes any pond, stream or water source and land otherwise covered with water.

6.2.2.3. Notification of Disease and Abortion

If a person has reason to believe that or suspects the existence of brucellosis in any herd or in any carcass on any land or that any animal has aborted, he shall notify the Department of Agriculture. If an animal aborts the person in charge shall isolate the animal together with any infective material and shall notify the department of agriculture or arrange for a specimen of the abortive material or a blood sample to be taken from the animal which has aborted by a registered veterinary surgeon who shall submit the specimen or sample to an approved laboratory.

6.2.2.4. Use of milk or milk products**.**

Unpasteurised milk or any unpasteurised dairy by-products shall not be moved onto land or premises for feeding to bovine or other animals unless such milk or milk by-products came from an officially brucellosis free herd or has been converted to powder form.

Heat treatment of milk shall be carried out from any reactor, before it is fed to any animal.

6.2.2.5. Movement of Animals

A person in charge of an animal must have a valid cattle identity card in order to move an animal into or out of a holding. Females aged 18th months or more or males aged 24 months or more may not be moved into or out of a holding unless for slaughter unless the animal has passed a blood test within a 60 day period prior to movement.

A person in charge must keep records of births, deaths, acquisitions and disposals of animals which have been kept on their premises.

If an animal is sold or bought they must have records concerning the name/address of the seller/buyer, and the sale date.

Chapter 7.

Brucellosis prevalence in the European Union.

Control and eradication programmes of brucellosis in the EU member states have more or less been implemented successfully. Many member states mainly countries of northern Europe have been declared “officially bovine brucellosis free” on the basis of directive 64/432/EEC and decision 99/466/EC. The official bovine brucellosis status of the European Union at the end of 2012 is depicted in Figure 1 and Table 2. The disease is concentrated in southern member states. The disease is present in Greece, the southern regions of Italy, in regions of Spain, in Portugal and Cyprus.

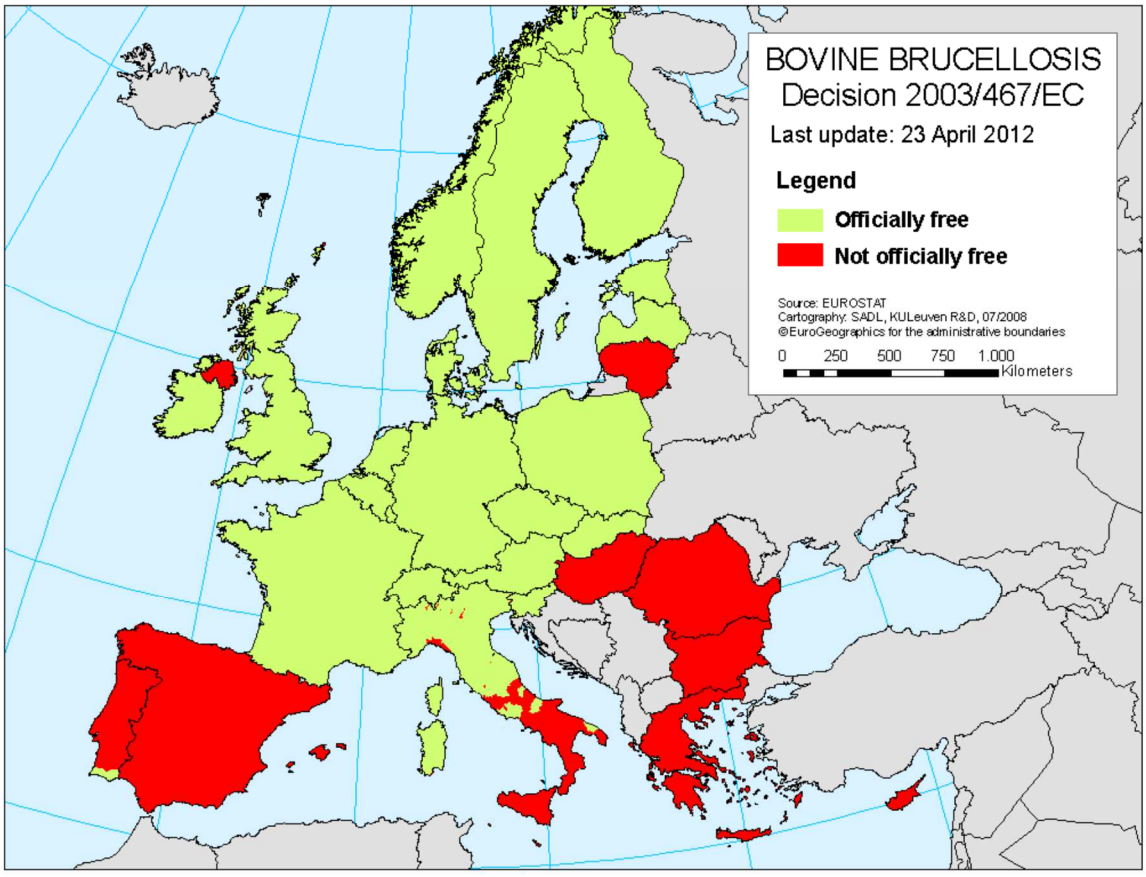

Figure 1 – bovine brucellosis status of the European Union

Source: European commission annual report 2011 on bovine and swine disease.

Table 2 – countries officially free of bovine Brucellosis.

Countries officially free	
Austria	Latvia
Belgium	Luxemburg
Czech Republic	Netherlands
Denmark	Norway
Estonia	Poland
Finland	Slovakia
France	Slovenia
Germany	Sweden
Ireland	Switzerland

Although France has been declared officially brucellosis free since 2005, there has been a case of brucellosis in a dairy cow in a herd in April 2012. In order to prevent re-emergence of the disease animals are screened annually using the Ross Bengale Test and CFT. Abortion is notifiable and investigation of abortion includes examination for brucellosis. All animals on the infected farm were slaughtered and their lymph nodes sampled. All except 1 were seronegative. Veterinary investigations are still ongoing to determine the origin of single breakdown (Mailles A et al 2012).

From data provided by the European commission in 2012 France reported 99.97 of its herds as been officially free herds. Number of suspended herds was 77, of which 116 were serologically positive, however none were B.S.T positive.

The bovine brucellosis eradication programmes implemented in Portugal and Italy has met with some obstacles in performance at regional level as reported by the European commission in 2011. In Italy's southern regions the prevalence of the disease is still high in bovines and buffaloes. In Portugal difficulties are also been experienced with regard to geographical locations, incidence been higher in the regions of Alentejo and Tars-os-Montes. However since 2005 bovine brucellosis in cattle has decreased or remained at a low level in most countries. In order to advise member states on the design and improvements of brucellosis programmes a brucellosis sub-group was set up in 2000. This subgroup provides the member state with conclusions and recommendations in order to improve the

effectiveness of their eradication programme. The following gives further details on the progression of the eradication programmes in aforementioned countries.

7.1 Brucellosis prevalence in Cyprus –

As of 2013 Cyprus has applied for official brucellosis free status. The bovine brucellosis eradication programme started in 2001, after reappearance of the disease in 1998. Vaccination has been prohibited since 1978 and treatment of brucellosis in farm animals is prohibited by law. It has been a notifiable disease since 1978. Prevalence of the disease between 2003 and 2005 showed significant progress Figure 2. Following a peak of the disease between 2001 and 2003 intensification of the eradication programme was implemented. Animals greater than six months of age were tested by serology, positive animals were slaughtered in no less than 30 days. The infected herd was placed under quarantine and suspected animals were tested one month later. When an outbreak was confirmed testing and culling continues until a herd has two consecutive negative tests. The last reported case of the disease was a single outbreak in 2008. Currently the total number of animals is 39462, total bovine herds stands at 309, with herds tested at 99.02%. To maintain free status the following programmes are currently in place: farms with greater than ten animals must have three negative bulk milk samples at 3 month interval and All animals greater than twelve months of age must have a negative Ross Bengal test. In herds with less than 10 animals, animals must have two negative Ross Bengal tests yearly. Animals are required to have a pre-movement test (UECBV 2013).

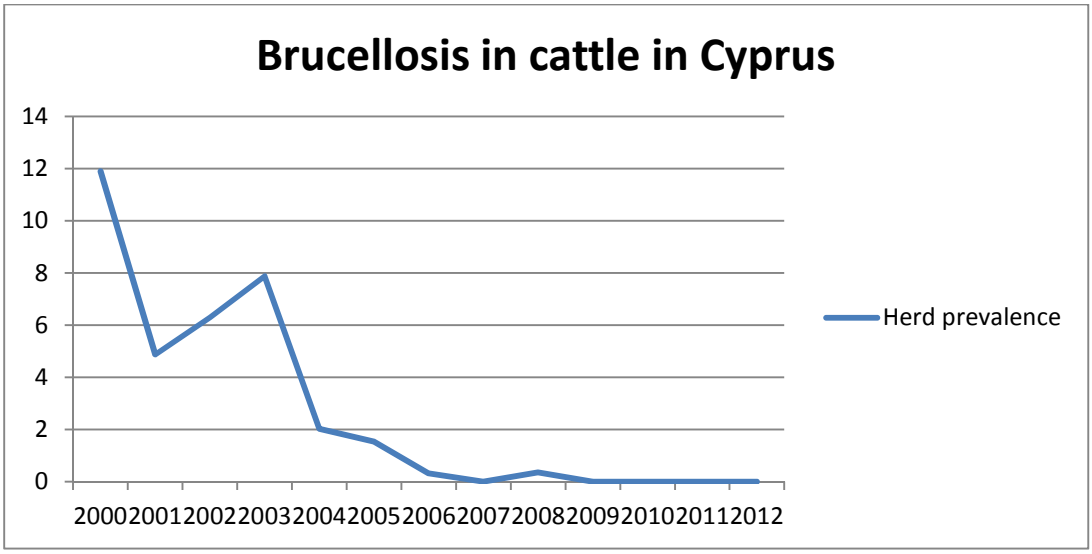

Figure 2 – Herd prevalence of brucellosis in cattle in Cyprus.

7.2 Brucellosis prevalence in Portugal

In Portugal a national eradication programme has been implemented in 4 regions on the mainland including Montalegre and Vieira do Minho, Ribeira de Pena, Alentejo region and Cuba/Alvito (FCEC 2011). From 1999- 2012 the programme has seen a decrease in herd prevalence from 917 to 72 see Figure 3. General implementations include serological testing of blood and milk bulk tank samples and slaughter of animals along with a RB51 vaccination program. As part of the vaccination programme a total of 607 herds are involved on the mainland, with Autonomous regions of the Azores also involved (UECBV 2013). All adult and young females are vaccinated. This vaccination programme has seen a progressive drop in disease incidence especially in the Montalgre and Vieira do Minho region. Stamping out of herds in Portugal only occurs under certain condition, including no improvement in a 12 month period, where the epidemiological conditions have worsened, prophylactic measures are not possible and *Brucella* species have been isolated.

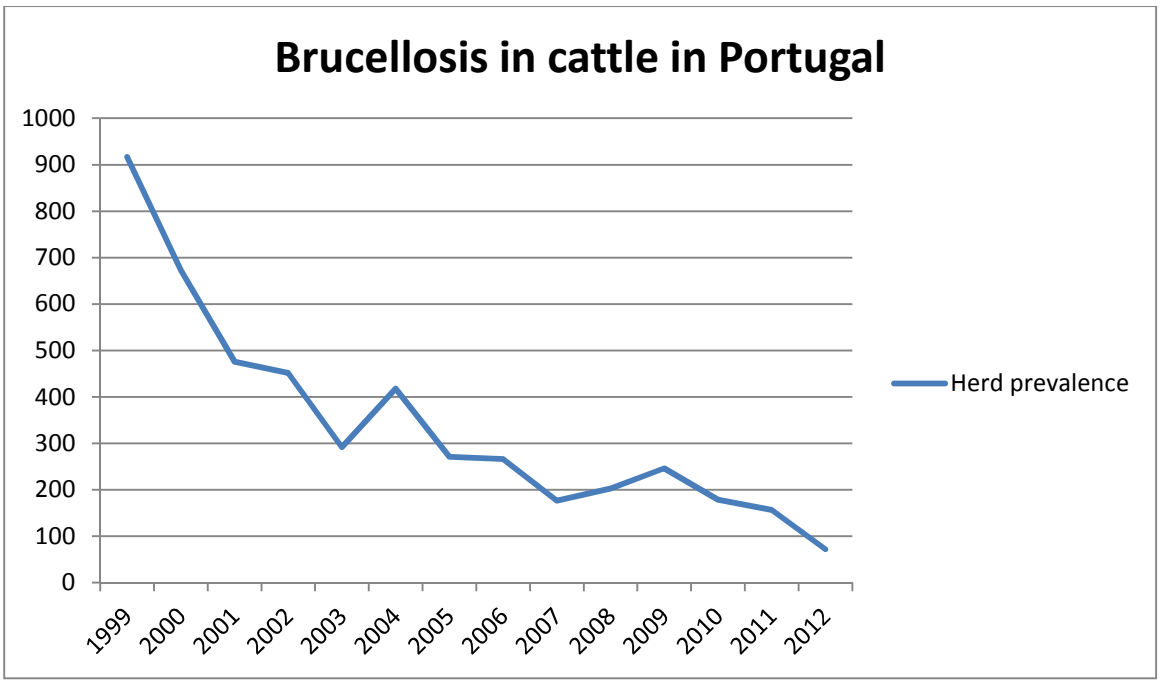

Figure 3. The herd prevalence of brucellosis in Portugal.

7.3 Brucellosis prevalence in Spain

In Spain a national eradication programme has been in place since 1965, the programme at this time was based on the vaccination of females between 3 and 6 months of age in milk production herds and was focused on those regions where the disease was endemic. A change of strategy was implemented in the 1990's which prohibited the use of vaccination in order to facilitate intra-EU trade in live animals. The period 1986-2012 has seen a progressive decline in the incidence of disease see Figure 4. The herd prevalence of brucellosis has reduced from 6.59% in 1986 to 0.08% in 2012. From 2006 there has been a reduction from 1,167 herds infected to 83 herds infected in 2012, without any new hotspots. However this trend has not been uniform throughout the country with brucellosis still persistent in certain area due partly to special epidemiologically condition referred to as special incidence areas (SIA). To combat this problem a comparative study was carried out where special control measures were implemented in these areas. Including, stamping out and/or vaccination with RB51 and S19. The results indicate that both stamping out and vaccination may be an effective strategy to deal with brucellosis infections in these regions (Saez JL et al 2011).

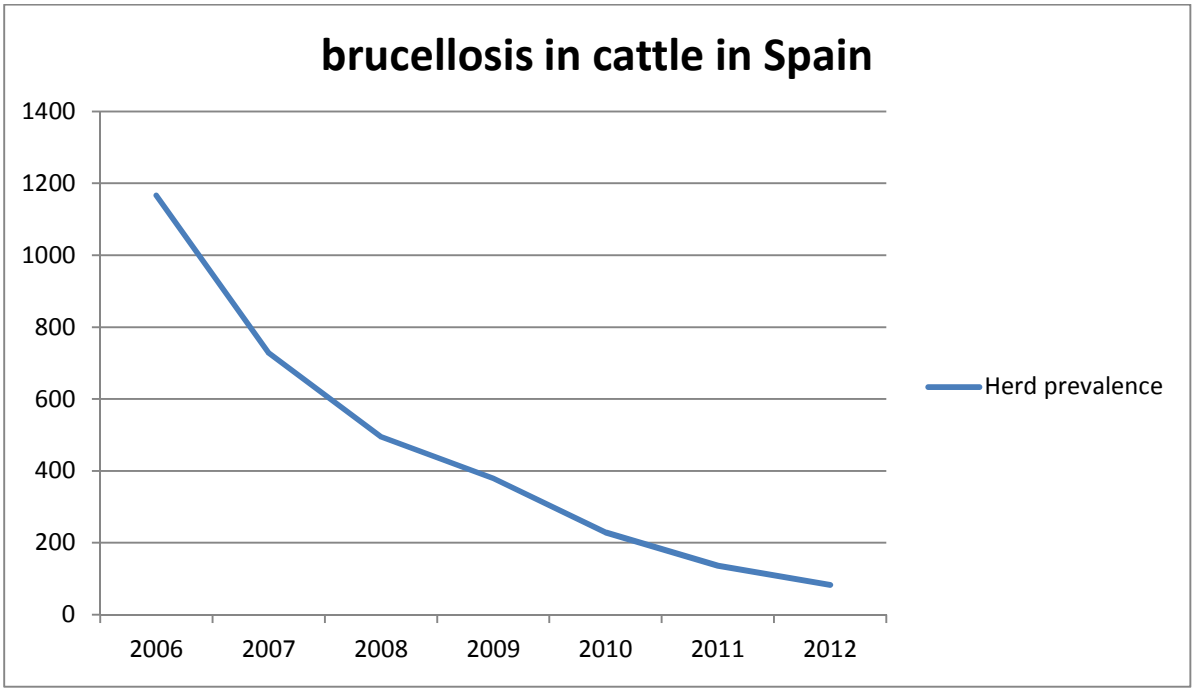

Figure 4 – herd prevalence of brucellosis in cattle in Spain.

7.4 Brucellosis prevalence in Italy

Almost all regions of central and northern Italy are officially brucellosis free; however Italy's southern regions still have a high incidence of disease, with Sicily having the highest prevalence accounting for more than 60% of the disease. A recent study of the possible risk factors contributing to the prevalence in this area was carried out. Results showed that two main clusters of infection were shown to be in the Messina and Siracusa provinces. The infections are associated to the largest herds and beef and mixed herds (Calistri P et al 2013).

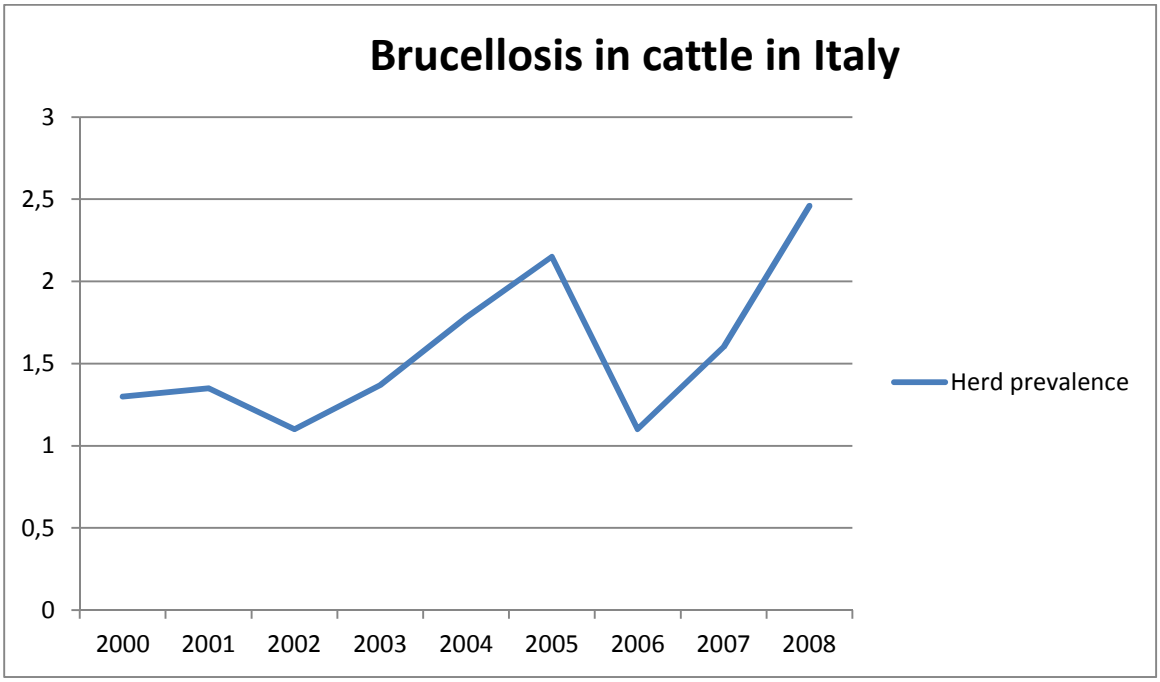

Figure 5. Herd prevalence of brucellosis in Italy.

7.5 Brucellosis prevalence in The United kingdom- Northern Ireland.

In Northern Ireland the eradication programme started in 1963, resulting in the disease almost been eradicated in the 1980's. In Northern Ireland a test and slaughter policy is implemented. Routine testing of animals is carried out annually on all female animals over 12 months. Vaccination of animals is prohibited. Individual identification of animals, movement control and a computer recording system are also essential elements been implemented. A brucellosis programme management team has also been set up their functions include monitoring and management of the programme, and provision of veterinary advice. Intensive control

measures from 2007 has ensured significant improvements in disease levels as seen in figure 6 , despite an increase in 2010 which is taught to have resulted from deliberate infection of a herd. Outbreaks in Northern Ireland were for the most part confined to an area in Co. Armagh, where cattle and herd density is the highest in the country figure 6 (Abernethy D.A et al. 2010)

Figure 6. Map of Northern Ireland illustrating outbreaks in Co. Armagh (Source Abernethy D.A et al 2010)

The total number of cattle tested in 2012 was 879,831 with a confirmed herd incidence of 0.005% a reduction from 0.25% in 2007 see figure 7.Currently the percentage of herd that are officially brucellosis free is 99.92%, the last confirmed outbreak was in February 2012. Possible eradication is foreseen in 2015 (EUCBV 2013).

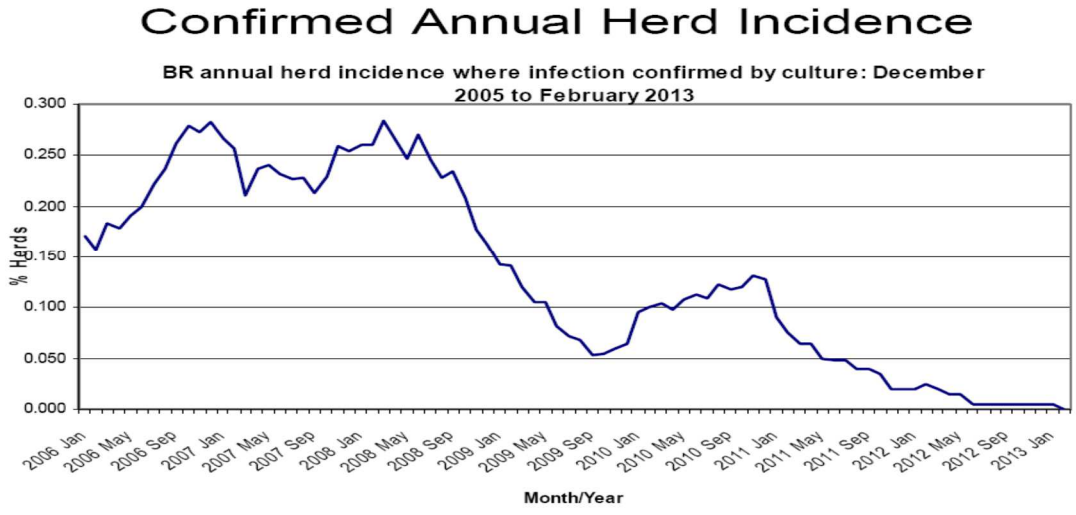

Figure 7. Herd prevalence of brucellosis in Northern Ireland

Chapter 8

Brucellosis in Ireland

8.1 History

A programme to eradicate brucellosis in Ireland began in 1966. A milk ring test survey at this time indicated that between 12% and 15% of 105,000 bovine animals were infected with *Brucella abortus* a further 3% were inconclusive. The highest disease incidence was recorded in the south, an area which contained the highest proportion of dairy herds (Hynes M.G 1973). Following the implementation of a vaccination programme using a killed 45-20 adjuvant vaccine as a diagnostic agent , a test and slaughter policy , considerable progress was made , brucellosis almost been eradicated in the 1980's. With the number of herds restricted at between 300 and 500. However disaster struck, the programme was relaxed too soon, Vaccination was stopped in 1984, together with the stopping of annual testing and pre-movement testing in 1986 and 1988 respectively. In 1986 the herd incidence had fallen to 0.2% and restriction was limited to the south of the country. By the late 1980's brucellosis had re-emerged as major a problem, and the early 90's showed an increase in prevalence in areas where brucellosis had not been detected for a number of years. During the early 1990's a few setbacks were encountered originating from a dispute with veterinary practitioners which saw the programme curtailed, and the introduction of a suckler cow scheme for farmers which saw farmers receiving EU funding for each suckler animal. This scheme led to farmers moving and buying large numbers of cows throughout the country, and perhaps older animals been kept for an extended period in order to meet quotas following reform of CAP. From this period onwards the prevalence of brucellosis in Ireland began to increase see figure 8 with a peak in 1998 of 0.74% herd prevalence.

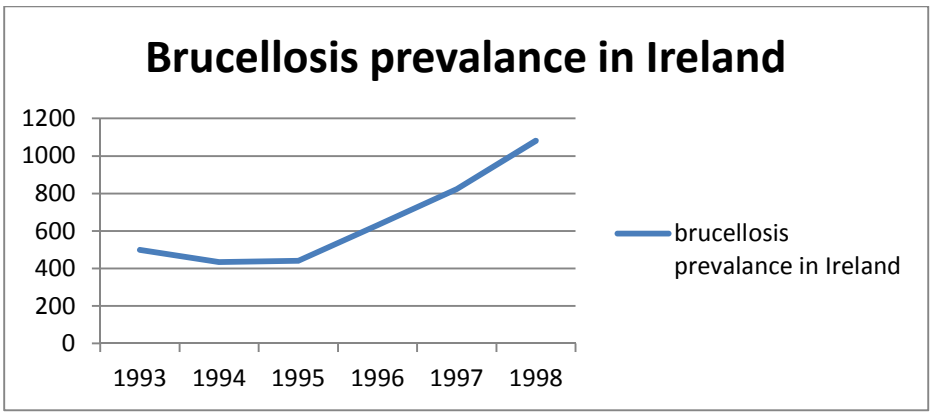

Figure 8. Herd prevalence of brucellosis in Ireland.

8.2 Movement towards eradication

In 1998 the government revamped the programme, reintroducing full round serological testing of all female animals and bulls over 12 months of age, compulsory testing of contiguous herds , a 30 day pre-movement test and in 1999 the introduction of blood sampling from cows at slaughter (Sheahan M. et al 2006) .In 1997 epidemiological investigations revealed that in 29% of outbreaks the source could be traced back to animals that were bought into a holding emphasising the importance of pre-movement testing. By 2001 this statistic had been reduced to 12.5% possibly as a result of the enforced pre-movement testing of animals. From the beginning of the implementation of the brucellosis eradication programme in 1966 bulk milk samples were taken and tested using the milk ring test (MRT) which is an adaptation agglutination test with milk. However it was discovered that this test can result in a high number of false positives caused by various milk conditions such as mastitis, colostrums and milk at the end of the lactating cycle. As a result in 2000 the use of a more sensitive test the whey ELISA was implemented (Nielsen K 2002).

Following various epidemiological studies and learning from past experiences Ireland adopted a Rapid depopulation policy. In a short a time as is possible infected herds were removed for slaughter to prevent contiguous spread. This has proved to be a very important strategy in the eradication of brucellosis in Ireland.

Diagnosis of Brucellosis in Ireland was essentially based on the use of serological tests. As no test is 100% sensitive or specific a number of different tests have been used throughout the eradication programme. The microtitre serum agglutination test (MSAT) is the standard test used in Ireland with the complement fixation test used for confirmation. Other tests available include indirect ELISA (EIA), the competitive ELISA (cEIA) and the fluorescence polarisation assay (FPA). Following a study of cattle herds with inconclusive serological evidence of bovine brucellosis the testing policy was changed to include re-sampling of all animals with a CFT reading greater than 20 IU. As a result of this study there was a reduction in the number of herds restricted and restriction duration (Hayes M. Et al 2009)

Another control measure implemented from 1998 was the compulsory treatment of slurry on infected farms. *B.abortus* is able to survive for up to 12 months in slurry and is isolated

without difficulty on many farms. Treatment of slurry with hydrated lime in liquid form to raise the pH of slurry to 12 became part of the eradication programme from 2001onwards.

In 2005 dramatic improvements were seen in brucellosis levels. In particular laboratory cases were down by 66%, there were 144 new herd restrictions in 2005, representing a fall of almost 50%on 2004. These figures represented a fall of 96% for blood positives and a fall of 91% in depopulations compared to peak figures in 1998.

In 2006 further progress was made as efforts were intensified to eradicate the disease. Incidence of the disease had fallen by over 60% compared to 2004; in the first 3 months of 2006 numbers had fallen by a further 25% (Department of Agricultural fisheries and food). During 2006 no herd was depopulated an indicator of the progress been made, the goal of eradication was a realistic prospect. In 2007 and 2008 no herd depopulations took place and in 2009 Ireland was granted officially brucellosis- free status.

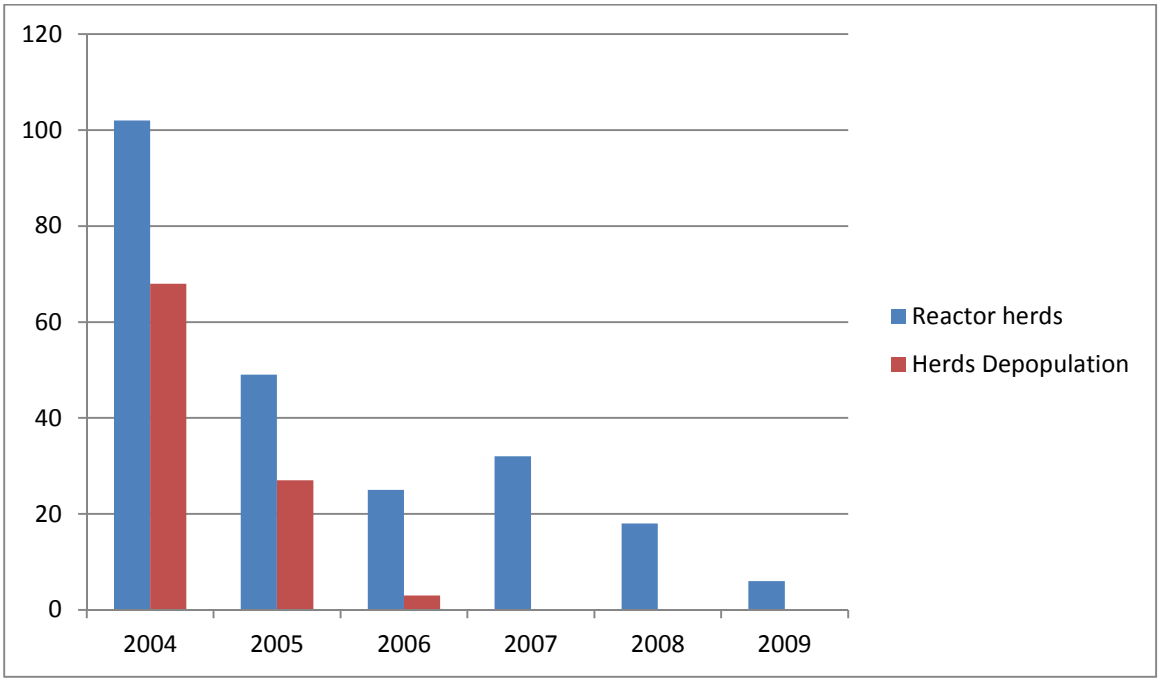

Figure 9. Number of reactor herds and depopulated herds between 2004 and 2009.

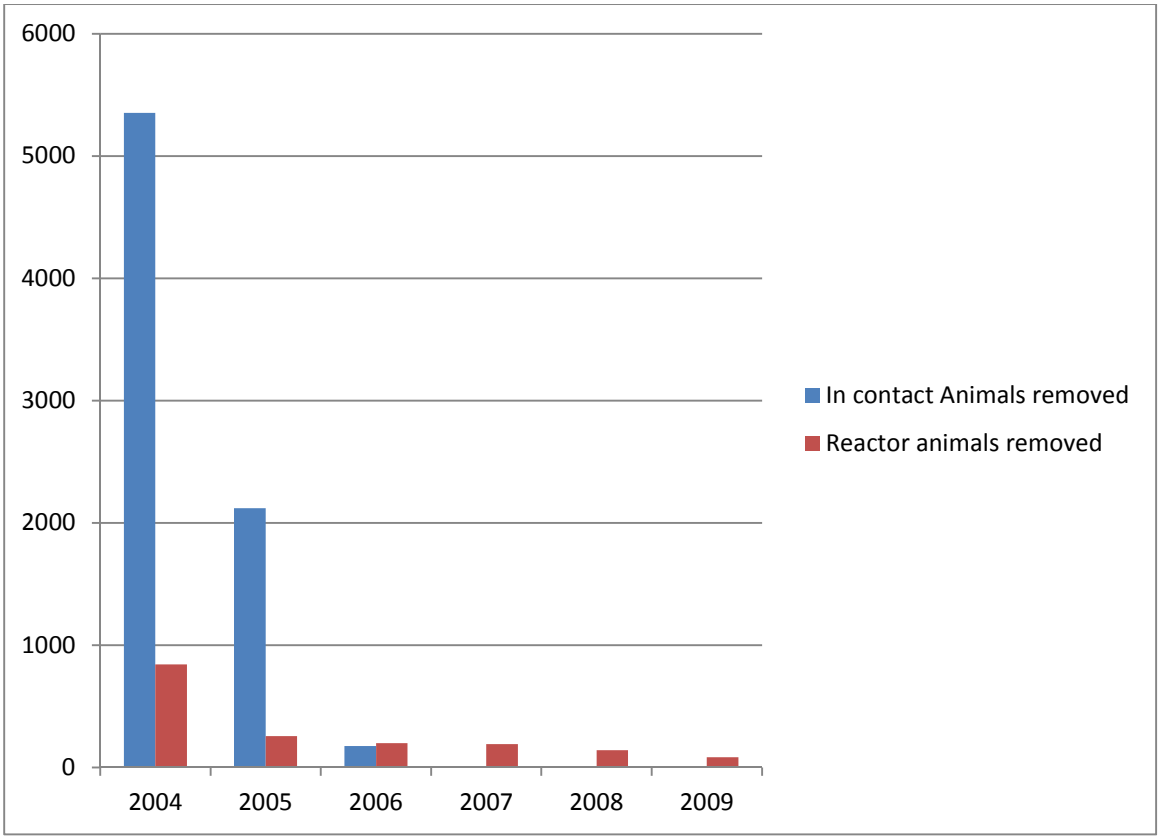

Figure 10. Number of reactor animals and in contact animals removed between 2004 and 2009.

In September 2009 changes were made to the eradication programme. The age threshold for the annual round test of bulls and females was increased from 12 to 24 months. A pre-movement test was only required for bulls greater than 24 months and females greater than 18 months. The pre-movement test was increased from 30 days to 60 days. In 2010 it was announced that herd would only be tested every second year. 2011 saw this change to all herds every second year. In 2012 testing was relaxed further with dairy herds been tested every fifth year, removing 2.5million animals from the testing regime. In 2013 all herds are tested every five years.

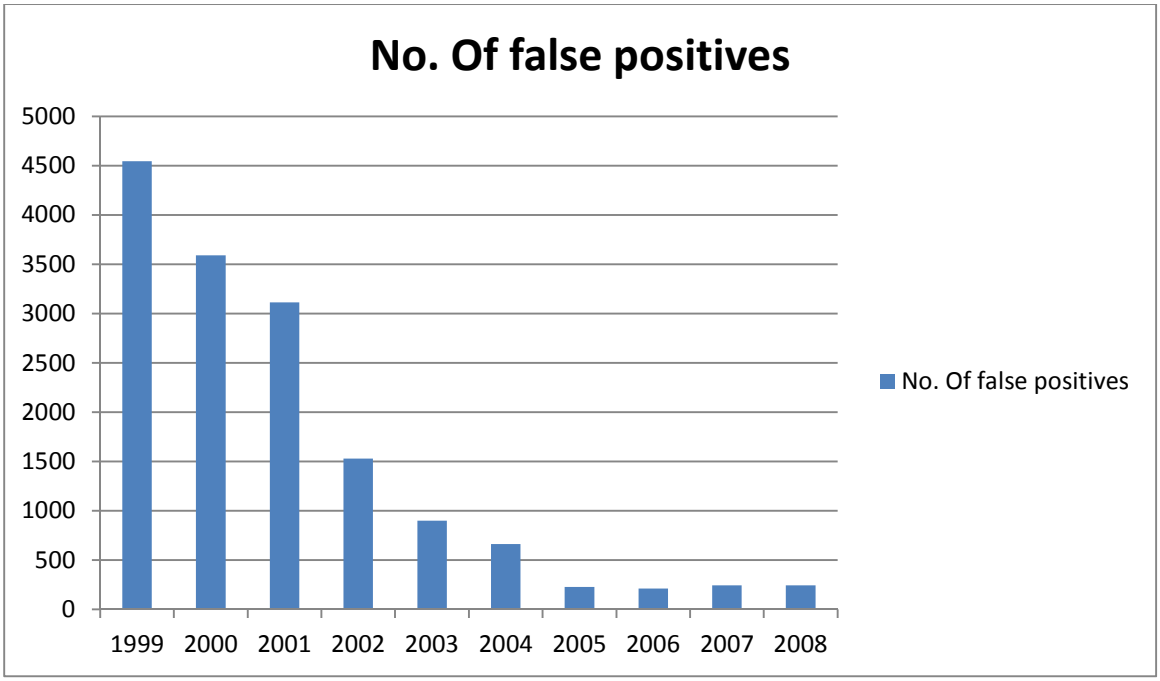

Figure 11. No. of false positives detected in Ireland 1999-2008.

8.3 **Details of provisions given for brucellosis testing in Ireland 2013.**

Each year the private veterinary practitioners PVP are issued with a copy of ERAD document ER 4 which they must sign and return to the department. In 2013 it contained the following details: with regard to brucellosis testing to be carried out in Ireland.

- Veterinary surgeons must be a registered practitioner in Ireland
- Be authorised by the minister
- Be approved
- Must follow instructions as laid down in ER4
- Failure to comply with rules set out in ER4 can lead to prosecution.

Instructions for sampling.

The level of testing required in Ireland has been scaled down since brucellosis free status was granted in 2009. Animals are only tested every fifth year, and only females and bulls over 2 years are required to be tested. Pre-movement tests are only required for animals greater than 2 years of age, the validity of the test is 60 days, as of the 1st January 2013 the one movement per test rule has been abolished.

Each animal presented for testing must be correctly identified according to tag number, sex, stage of pregnancy, age and abortion history.

Equipment:

- Bold testing kit
- Official list of animals to be tested.
- Tagging equipment and tags
- Protective clothing
- Disinfectant effective against brucellosis

Bleeding: A separate needle must be used for each animal. Each tube must be at least 2/3 full. A pre-coded label must be attached to each bottle, this must correlate with the identification of the animal. The person taking the samples is responsible to ensure that samples are correlated properly. Sample tubes are placed in correct order in a sample box in sequential order left to right. An ER16 form is completed and enclosed in the sample box. The blood samples are then forwarded to the Department of Agriculture testing laboratory.

Chapter 9

Conclusion

The implementation of an Eradication Programme for brucellosis in Ireland has been a success. Since the scheme began, Ireland has introduced a series of successful strategies enabling the country to be declared offically brucellosis free. The success of the programme in Ireland gives other member states a template to follow to allow them to reach free status.

The importance of annual testing and pre-movement testing cannot be over emphasised. The rate of spread and detection depends on the level of surveillance testing. In Ireland the stopping of annual testing and pre-movement testing in the late 1980's meant that there was a resurgence of the disease in the early 1990's a lesson about the importance of testing dearly learnt. The type of testing, and the combinations of tests used is also important. The risk of false negative results due to latency or false positives resulting from vaccination programmes needs to be addressed especially in those countries nearing official free status. Paramount to this process is the co-operation of veterinary services, they must be organised properly, with adequate financial, administrative and personnel support. Adequate education of farmers on the importance of eradication is also important.

Many animals cross the border from Northern to Southern Ireland each year, as Northern Ireland is not brucellosis free there is a risk of infection crossing the border. Recent outbreaks of brucellosis in Northern Ireland have been confined to border counties. To prevent resurgence of the disease in the South a strict pre and post movement testing regime must be maintained.

At peak prevalence of brucellosis infection in Ireland a slaughter /stamping out regime was enforced, with many herds been depopulated. The major success of the eradication programme could also be attributed to this efficient process.

The last confirmed case of brucellosis in Ireland was in 2006, as the country heads into 2014, eight years since the last outbreak, we must remember the importance of continuing the surveillance for brucellosis. Currently the programme has been relaxed, with 20% of the national herd been tested annually in 2013. A decrease from 50% of the national herd in 2012, however if a case of brucellosis is identified the programme must revert to more stringent measures.

Chapter 10

Summary

Brucellosis is a worldwide disease caused by the *brucella* genus of bacteria. The main clinical signs observed in animals include abortion, infertility, and weak offspring and reduced milk production. It causes disease in many species including Cattle (B.*abortus*), goats and sheep (B.*melitensis*), pigs (B.*suis*), Dogs (B.*canis),* and in marine animals (B.*ceti*, B.pinnipedialis), all of which are capable of causing a zoonosis in humans. The zoonotic potential of this infection emphasises the importance of implementing eradication programmes in areas affected by the disease. Most eradication programmes are based on a test and slaughter policy. The main diagnostic methods used include isolation and identification of the bacteria, or serological testing of either blood or milk samples at an approved laboratory. Currently the most widely in-use tests include different variations of agglutination tests, ELISA and complement fixation. Many countries employ a vaccination policy as part of the eradication programme. The two most common vaccines used are B.*abortus* strain 19 and B.abortus strain RB51, care must be taken with vaccination programmes as the vaccination of animals can cause false positive serology results.

Brucellosis is a notifiable disease. Legislation exists within the European Union and each member state on its notification, control and eradication. The main legislation regarding brucellosis in the EU is council directive 64/432/EEC, council directive 77/391/EEC and council directive 78/52/EEC. In Ireland the main legislation regarding brucellosis eradication is the Disease of animal's act 1966 and S.I No.114 of 1991.

Within the European Union brucellosis has been successfully eradicated in many member states. However some countries such as Cyprus, Portugal, Spain, Italy and Northern Ireland are still experiences difficulties. Ireland is a success story, as of 2009 they have been declared officially brucellosis free. The success of the eradication plan, owes its origin to a not so successful story of eradication in the 1980's. Relaxations of the programme too soon lead to a resurgence of the disease in the 1990's. However the re- introduction of procedures such as annual testing, pre-movement tests, and depopulation lead to Ireland been declared brucellosis free.

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