Прилози, Одд. биол. мед. науки, МАНУ, XXXI, 1, с. 145–165 (2010)
Contributions, Sec. Biol. Med. Sci., MASA, XXXI, 1, p. 145–165 (2010)
ISSN 0351–3254
УДК: 636.3.09:615.371]:616.98:579.841.93

CONTROL AND ERADICATION STRATEGIES FOR *BRUCELLA MELITENSIS* INFECTION IN SHEEP AND GOATS

Blasco JM.

Animal Health Unit
CITA (Unidad de Sanidad Animal, Gobierno de Aragón)
Zaragoza, Spain

A b s t r a c t: *Aim:* To describe the various strategies for the control and eradication of *B. melitensis* infection in sheep and goats.

Methods: The advantages and drawbacks of these strategies are discussed on the basis of the author's personal experience and a revision of the relevant literature.

Results: Vaccination programmes in various combinations can be applied either to decrease the prevalence of infection in the animal population or, when combined with adequate complementary eradication measures, to achieve a brucellosis-free status.

Conclusion: Controlling the disease should be the primary goal of the veterinary services involved. However, eradication should be the final objective of any control programme implemented. The selection of an eradication or control strategy is of paramount relevance, and a frequent cause of controversy among decision-makers. The final strategy should be established according to the quality of the veterinary services organisation, the economic resources available and the extent and prevalence of disease. Cooperation with farmers is essential to succeed with the application of even the most elementary control programme. When brucellosis is highly prevalent, mass (whole-flock) vaccination is the choice to control the disease, independently of the socioeconomic situation. Once effective control of the disease has been accomplished, its eradication is feasible. For successful eradication, the adequate quality and organisation of veterinary services, the strict control of animal movements and the provision of adequate economic compensation to affected farmers are compulsory. When the disease is fully eradicated, a surveillance strategy has to be implemented for the early detection of eventual new outbreaks or disease reintroduction.

Key words: brucellosis, *Brucella melitensis*, zoonosis, vaccination, control/eradication strategies.

Introduction

Brucellae are Gram-negative, facultative, intracellular bacteria showing a wide range of species-specificity and causing important diseases in both humans and animals. At present, eight species are recognised: *B. abortus* (affecting mainly cattle), *B. melitensis* (sheep and goats), *B. suis* (swine), *B. neotomae* (desert rats), *B. ovis* (sheep), *B. canis* (dog), *B. ceti* (cetaceans) and *B. pinnipedialis* (pinnipeds). A couple of new species: *B. microti* (isolated from common voles) and *B. inopinata* (isolated from a human patient) have been proposed but not yet accepted by the Taxonomy Subcommittee on *Brucella*. With the exception of *B. ovis* and *B. neotomae*, all the accepted species are pathogenic for humans. In human beings, brucellosis is one of the most important and universally distributed zoonotic diseases. Swine brucellosis (due to *B. suis*) is an important disease in many parts of the world (essentially America, Asia and Oceania), but brucellosis of cattle, sheep and goats are the most relevant from the socio-economic point of view, and are generally submitted to official control campaigns all over the world. Brucellosis in cattle (caused by *Brucella abortus* infection) is a disease eradicated or practically eradicated in many developed countries, but remains highly prevalent in many parts of the developing world. *Brucella melitensis* is the main species responsible of brucellosis in sheep and goats (although in extensive breeding systems it affects frequently also cows, yaks, camels and buffaloes), and is a disease highly prevalent in many countries, most having great difficulty in controlling this infection. Moreover, *B. melitensis* is highly zoonotic and the responsible for the vast majority of human brucellosis cases around the world. Despite the considerable increase in scientific knowledge of brucellosis in the recent past, many aspects concerning this disease in sheep and goats remain yet unknown, unclear, and are frequently controversial. However, even though some basic aspects of brucellosis pathogenesis require additional research, the diagnostic and prophylactic tools have been sufficiently validated and standardised, and are widely available to control the disease in most parts of the world. In my opinion, success in eradicating brucellosis in small ruminants is largely dependant on the quality of the veterinary services and administrative organisations involved, rather than on any deficiencies of either diagnostic or prophylactic tools. The aim of this review is to describe the different strategies that could be applied to either the control or eradication of brucellosis in sheep and goats.

Epidemiology and clinical aspects

B. melitensis infection appears to occur naturally in the Mediterranean region, but infection is widespread world-wide. Canada and the USA are free of

this disease, as are Northern Europe, Southeast Asia, Australia and New Zealand [1]. *B. melitensis* infection of small ruminants is quite similar in both pathological and epidemiological standpoints to *B. abortus* infection of cattle. The main clinical manifestations of brucellosis in ruminants are reproductive failure (*i.e*. abortion and birth of offspring that do not thrive), orchitis and epididymitis. Arthritis is an infrequent sign. *B. melitensis* biovars 1 and 3 appear to be the most frequently isolated strains in small ruminants in Mediterranean and Middle-East countries. There is no evidence according the different biovars isolated that either the epidemiological or clinical features of infections in ruminants can be variable. Only when the animals excrete the bacterium do they become dangerous to other animals and human beings. In most circumstances, the primary (and more relevant from the epidemiological standpoint) excretion route of *B. melitensis* is the placenta, foetal fluids and vaginal discharges expelled by infected animals after abortion or full-term parturition. Shedding of *B. melitensis* is also common in udder secretions and semen. *Brucella* may be isolated from various tissues, such as lymph nodes from the head and those associated with reproduction, and from arthritic lesions [2]. As happens in *B. abortus* infection of cattle, *B. melitensis* can be transmitted congenitally in sheep and goats. Most latent infections in cattle take place through *in utero* transmission. However, only a small proportion of lambs and kids are infected *in utero*, and the majority of *B. melitensis* latent infections are probably acquired through colostrum or milk [3]. It is also probable that a self-cure mechanism similar to that suggested in cattle takes effect in most perinatally infected lambs [3]. Despite the low frequency of transmission, the existence of such latent infections increases the difficulty of eradicating this disease, as the bacteria persist in the animal without inducing detectable immune responses. The exact mechanism of the development of *B. melitensis* latent infections remains unknown [3].

In many parts of the world, small ruminants and cattle (and frequently also camels, yaks and buffaloes) are reared together. In these production systems the existence of cross-infections is very frequent with *B. melitensis* being the most common cause of infection when the above animal species are reared together.

Control and eradication programmes

An important problem faced by the veterinary authorities in countries affected by brucellosis is to select the sanitary strategy to be applied against the disease. Adequate organisation of veterinary services is, without any doubt, the most important element to be taken into consideration by decision-makers previous to any potential selection of a sanitary programme. The recent experience in the EU demonstrates that no sanitary strategy can be applied in the absence of

a minimum of capacity, quality and adequate organisation in the veterinary services involved. Even for implementing the simplest control strategy (*i.e.* a mass vaccination programme), the veterinary services involved should have a proper organisation to identify all flocks present in the country and apply the vaccine to the whole population in a very short time interval. In the case where the organisation of veterinary services is adequate, the strategy to be applied should then be decided according the economic resources available, the degree of involvement of farmers, and the extent and prevalence of the disease.

The economic costs of eradication programmes are very important, and financial resources should be allocated to support the programme as an essential requisite prior to the selection of any eradication strategy. The practical experience of many countries that succeed with *B. melitensis* eradication also demonstrates that adequate economic compensation (*i.e.* equivalent to the real market value of animals culled) to affected farmers has to be provided. Moreover, to be successful, the full operative costs of interventions have also to be covered by the public administration concerned.

The adequate organisation and involvement of shepherds is also another essential requisite for success in the implementation of even the simplest control strategy based on mass vaccination. Therefore, in addition to implementing adequate compensation (*i.e.* subsidising vaccination and operative costs, indemnisation of animals slaughtered, etc), adequate awareness campaigns should be addressed to the affected farmers with the objective of getting full agreement with the strategy decided. No success in implementing any control or eradication programme should be expected without the active involvement of the affected owners.

Provided that the veterinary services organisation, farmers' involvement and economic resources are fully adequate, the final elements to be considered by decision-makers are *i)* assessing the real situation of brucellosis (*i.e*, identifying the bacterial and animal species involved, and also the collective prevalence), and *ii)* defining the minimal epidemiological unit of intervention. The bacterial species and biovars involved should be identified though an active bacteriological search, in close collaboration with national and international laboratories with proven competence in *Brucella* typing. The collective prevalence (*i.e.* percentage of infected flocks) of disease has to be determined following a technically adequate epidemiological survey, and always taking into consideration the important differences in prevalence that should be expected between different regions within the same country or epidemiological unit of intervention. A frequent error of decision-makers is tending to "homogenise" the level of prevalence calculating "mean prevalence" figures for the whole country or particular region considered. However, prevalence is infrequently homogeneous and, in most cases, the territorial extension considered is repre-

sented by a combination of different epidemiological situations. Accordingly, decision-makers should avoid the use of generalised measures, and be ready to apply different strategies adequate to each of the different epidemiological situations identified. A given territorial extension with a similar epidemiological situation should be considered as the minimal epidemiological unit of intervention. In some cases this unit could be a couple of isolated sheep and camel flocks in a village, sometimes the whole sheep and goat flocks in a county, and more frequently, the whole flocks of all animal species involved in a country or region. It is essential to take into consideration that *B. melitensis* infection has no administrative borders, and decision-makers should consider this when implementing control or eradication strategies in the neighborhood of other countries affected by the disease. On many occasions, particularly in extensive and transhumant breeding systems, the minimal epidemiological unit of intervention is of a transboundary nature.

Once the professional organisation and the economic resources are fully adequate, the epidemiological unit of intervention should be defined. Whenever the collective prevalence (percentage of infected flocks) in this unit be uniformly very low (always less than 1% of flocks infected), a strategy based on a *test and slaughter* programme and a ban on vaccination could be applied to eradicate the disease in the short to medium term in that particular epidemiological unit. In the case where prevalence is uniformly moderate, a *combined eradication* programme based on the simultaneous application of vaccination in young replacements (3–4 months old animals) and a test and slaughter in adult animals could be recommended to eradicate the disease in the medium to long term. However, when the disease is highly prevalent (more than 10% of flocks are infected), even though the professional organisation and the economic resources be fully adequate, the *mass (whole-flock) vaccination* of all animals from all animal species involved in the epidemiological cycle is the only reasonable strategy that can be applied to control the disease.

For success in the application of the last two strategies, the use of adequate vaccines and vaccination procedures is of paramount relevance.

Vaccines and vaccination procedures

1. Classical vaccines

Despite its important drawbacks, the live *B. melitensis* Rev 1 vaccine is considered the best vaccine available for the prophylaxis of *B. melitensis* infection in sheep and goats. When used in well conducted whole-flock vaccination programmes repeated in time, a great decrease in brucellosis prevalence is obtained in most situations [4–6]. However, when this vaccine is administered by

the classical method (individual doses of 1–2 × 10^9 CFU applied subcutaneously), a long-lasting serological response is induced in vaccinated animals, making it difficult to interpret serological tests applied after vaccination. Accordingly, this classical vaccination method makes the combined eradication programme mentioned above practically inapplicable. However, when Rev 1 is administered by the conjunctival method (the same 1–2 × 10^9 CFU individual standard dose but applied by conjunctival instillation in a small volume -30/50 microlitres), the immunity conferred is similar to that induced by the classical subcutaneous method but the serological responses evoked are significantly reduced. In fact, this conjunctival procedure is the only available tool fully compatible with the successful application of the combined eradication programme in small ruminants [6, 7].

As commented above, a whole-flock vaccination programme is the only feasible alternative to control *B. melitensis* infection in high prevalence situations, and under the poor socio-economic conditions and extensive breeding systems characteristics of many countries. In these situations, the serological interferences caused by Rev 1 are fully irrelevant, and then the administration route would be of little importance. However, the vaccination of pregnant animals with standard doses (1–2 x 10^9 CFU) of Rev 1 administered subcutaneously is followed by huge numbers of vaccine-induced abortions and the excretion of Rev 1 strain in the milk of many animals. These side-effects are reduced but not abrogated when Rev 1 is administered conjunctivally [6, 8]. Reducing the dose of Rev 1 has been suggested as a method of minimising or fully avoiding these important side-effects and accordingly, a reduced dose vaccination procedure (undefined individual doses ranging 10^3–10^7 CFU and administered subcutaneously) has been widely used and reported as safe and effective enough for controlling brucellosis in small ruminants [4, 9, 10]. However, field and experimental experience in sheep has demonstrated beyond doubt that this is not true. Due to the induction of abortion in pregnant animals and the low degree of immunity conferred (for a review see ref. 6 – Blasco 1997), the reduced doses of Rev 1 – even when administered conjunctivally – should never be recommended as an alternative to vaccination with the standard doses (1–2 × 10^9 CFU).

A mass vaccination including adult animals (males and females) is the simplest control method, and frequently the only reasonable strategy to be applied in high prevalence situations and extensive breeding conditions. Unfortunately, due to the induction of abortions when vaccinating pregnant sheep and goats, there is no entirely safe strategy for using Rev 1 in these conditions. Even considering that conjunctival vaccination with Rev 1 is safer than subcutaneous vaccination for mass vaccination purposes, this procedure is not safe enough to be applied regardless of the pregnancy status of the animals (for a review see ref. 6 – Blasco, 1997). Accordingly, Rev 1 vaccine should be used only under

restricted conditions avoiding whenever possible vaccination during mid pregnancy, which is the main critical period [6, 8, 11]. This, however, is impractical under field conditions, and some of the above side-effects have to be assumed by both decision-makers and farmers. Field experience in many countries has demonstrated that conjunctival vaccination with standard doses of Rev 1 ($1–2 \times 10^9$ CFU) during the prebreeding period, late lambing season and lactation is the optimal window of opportunity to perform a whole-flock vaccination programme with the minimum of induced side-effects (for a review see the ref. 6 – Blasco, 1997). Another drawback of Rev 1 (that has been proven of little human health significance after more than 50 years of widespread use of this vaccine) is that it can infect humans [12] and, moreover, this strain is resistant to streptomycin, the antibiotic that combined with doxycycline provides the most effective brucellosis therapy [13]. Accordingly, awareness campaigns addressed to people involved in vaccination and minimal individual biosafety measures have to be implemented during vaccination to lessen the Rev 1 risks in human beings. In the case of accidental human infection with Rev 1, a combined doxycycline-gentamicin (or doxycycline-rifampin) has to be used [12, 13].

Despite the above drawbacks of Rev 1, it is important to stress that several million animals all over the world have been repeatedly mass vaccinated with this vaccine, and no Rev 1 epidemics in both animals and human beings have been reported in any country.

2. Other vaccines

New vaccines can be classified depending on the generation method used, either by classical techniques or by mutagenesis or genetic engineering. Among the live rough *Brucella* strains obtained by classical attenuation methods is the *B. abortus* RB51 vaccine. This strain has been considered to be as effective as the classical *B. abortus* S19 vaccine in protecting against *B. abortus* in cattle, with the additional advantage of not inducing anti – O polysaccharide antibodies interfering with classical serological tests (Rose Bengal – RB – and Complement Fixation – CF – tests), whereas it does in enzyme immunoassays (ELISAs) [14]. Moreover, the protective efficacy and safety of this vaccine in cattle are controversial, and remain to be properly established [14, 15]. In sheep, it has been clearly shown that RB51 is not effective enough against *B. melitensis* [16] or *B. ovis* [17] infections. Moreover, even though the risks are low, human infections due to RB51 have also been described [18], and this mutant is resistant to rifampin, the antibiotic that, combined with doxycycline, is widely used for treating brucellosis in humans [13]. Taking all the above comments into consideration, RB51 should never be recommended for vaccination in small ruminants.

VTMR1 and VTSR1 are two live rough strains obtained by transposing mutagenesis from *B. melitensis* 16M and *B. suis* 2579, respectively. Mutagenesis in these strains results in mutation of the *wbo*A gene coding for mannosyltransferase, an enzyme required for the S-LPS O-polysaccharide synthesis. Accordingly, these strains do not induce antibodies against the O-polysaccharide and, similarly to RB51, do not interfere with classical serological tests (RB and CF tests). Both strains are attenuated in mice but VTMR1 resulted in poor protective efficacy against *B. melitensis* in goats [19, 20].

Recently, *B. melitensis* R mutants in all main lipopolysaccharide (S-LPS) biosynthetic pathways have been obtained [21] and evaluated for ability to induce anti-O-polysaccharide antibodies, persistence and innocuousness, and efficacy against *B. melitensis* in sheep [22]. All these R mutant induced antibodies caused interference in *B. melitensis* ELISA tests, and moreover, protection by the best R vaccines was 54% or less whereas Rev 1 afforded 100% protection against a challenge able to infect 100% of unvaccinated control ewes [22].

The main reason for developing R brucellosis vaccines is the lack of interference that these vaccines cause in classical serological tests (RB and CF tests). However, this advantage is not so clear when classical tests are replaced by immunosorbent assays using the S-LPS or its hydrolytic polysaccharides as antigens. An important proportion of R mutant vaccinated ewes became positive in an indirect ELISA [22]. This is not unexpected since R mutants elicit antibodies to the core epitopes also present in the wild-type S-LPS and its hydrolytic polysaccharides. Core epitopes are not readily accessible on the whole S brucellae (used as antigen in the classical RB and CF tests), but they become exposed upon adsorption of those molecules to ELISA polystyrene plates and, therefore, prevent a clear-cut distinction of the antibody responses to S and R brucellae in immunosorbent assays. This is likely to be a problem affecting all R vaccines because we have found that a significant proportion of cows that aborted as a consequence of vaccination with RB51 develop antibodies reacting in an indirect ELISA performed with the S-LPS hydrolytic polysaccharide as antigen [15]. As a conclusion, the great potential advantages claimed for R vaccines have been seriously questioned, and there is increasing evidence showing that these vaccines interfere in S-LPS based immunosorbent assays, lack safety in pregnant animals, can be excreted in the milk of vaccinated animals, can infect humans, and are less effective than classical Rev 1 and S19 vaccines against brucellosis in small ruminants and cattle.

Other approaches to develop new generation vaccines, such as the construction of recombinant strains deleted in relevant diagnostic proteins or DNA based vaccines, are also being investigated. A Rev 1 vaccine strain deleted in the gene coding for BP26 periplasmic protein (that can be used as a differential marker) resulted in the same protective efficacy as Rev 1 in sheep [23]. This

good efficacy was also evidenced against *B. ovis* in rams, but the performance of the BP26 based differential diagnostic test was only very limited [24].

Up to now, none of these new generation vaccines have been found to improve the immunity or fully resolve the problems caused by the classical Rev 1 vaccine. Accordingly, until new, safer and more effective vaccines are developed and tested properly, Rev 1 should continue to be the reference vaccine for the prophylaxis of brucellosis in sheep and goats.

Control programmes

In many countries having organised but only elementary veterinary services and limited economic resources, the Rev 1 based mass vaccination programme is the only feasible alternative to be applied and maintained for many years, independently of the level of prevalence determined in the epidemiological unit identified (usually the whole country). As commented above, the main practical problems generated when applying this programme are due to the drawbacks of Rev 1 vaccine when used in pregnant animals. An alternative safest control strategy classically recommended to avoid problems in adult vaccinated animals is based on the annual vaccination of young replacements (3–4 months old) exclusively, a fully innocuous method lacking relevant side effects [25]. The hypothesis of this conservative but safest control strategy is that if 100% of young replacements (which can usually represent 15–25% of the total population, depending on the breeding systems considered) are vaccinated each year, the total animal population will be fully immunised after a moderate period of time (usually 4–8 years, according to the animal species and the husbandry systems considered). To be successful, the whole of the young replacement population (both males and females) should be vaccinated annually and, ideally, identified with a distinctive ear tag or suitable mark for adequate follow-up in the later years. However, due to the practical difficulties of getting full vaccine coverage of the whole population, this strategy has failed to control brucellosis even in favorable conditions in developed countries [6], and it is frequently inapplicable in the developing world. Finally, it has been proved unsuccessful in controling the disease in high prevalence conditions. In the characteristic extensive husbandry conditions of ruminants, owners can keep young replacements all through the year according to grazing resources and market prices. Therefore, several veterinary visits are required each year to achieve whole vaccination coverage of these young replacements. This important practical problem and the great difficulty in identifying flocks reared in nomadic or semi-nomadic breeding conditions, results in a failure of adequate vaccination coverage for the whole population, and the ensuing maintenance of the disease.

Therefore, the whole-flock vaccination programme is the only feasible alternative to control brucellosis infection in ruminants under the extensive breeding practices characteristic of many countries. This mass vaccination could be accompanied or not with individual ear tagging (or alternative suitable identification procedures) of vaccinated animals. This individual identification could facilitate the adequate follow-up of the control programme in subsequent years (only unmarked animals should be vaccinated the next year). Unfortunately, ear tagging is not exempt from inconveniencies. First, it is not a fully suitable procedure for the permanent identification of the animals. Moreover, it is expensive (sometimes the cost of tags exceeds that of the vaccine), and it can be a cause of important problems of miasis when implemented in hot climates such as those of Mediterranean and SEE countries.

To be effective, any control programme based on mass vaccination has to be maintained over time. Once the first mass vaccination campaign has been applied in the whole population of the epidemiological unit considered, the ideal follow-up procedure to minimise the side effects of Rev 1 should consist of vaccinating exclusively the young replacements in the following years, and at least for the 8–10 years following the first mass vaccination. A distinctive ear tagging (or suitable individual identification) should also be recommended as the adequate follow up. Without any doubt this is the safest procedure because of the lack of side effects of Rev 1 when used in both young male and female replacements [25]. However, as has been indicated above, due to the practical difficulties of vaccinating 100% of young replacements each year, this alternative can fail to control brucellosis even in the best socio-economic conditions [6].

Another more practical alternative could be applied to reach the adequate vaccination coverage of the population in the years following the first intervention. The characteristic annual replacement figures for sheep and goats in extensive breeding systems usually range from 15–25%. Therefore, the next year after the application of the first mass vaccination a total of 15–20% of the population should be composed of new replacements, then unvaccinated and susceptible to the disease. However, it is highly improbable that *B. melitensis* infection would be maintained and extended by transmission to this relatively low percentage of unvaccinated young replacements (most of them unpregnant, and at that time out of the period of maximal risk of excretion and spreading). Accordingly, considering these low replacement figures and the relatively low risks of disease maintenance and spreading among this unvaccinated young replacement population, it could be acceptable to avoid repeating a new mass vaccination every year to maintain a 100% vaccination coverage. However, two years after the first mass vaccination has been performed, and no complementary vaccination has been applied to new young replacements (the safest alternative), around 30–50% of the animal population would be composed of unvacci-

nated replacements, then fully unprotected, and with a relatively high proportion of animals at risk of the ensuing danger of disease maintenance and spreading. Therefore, a practical way to apply a cost-effective Rev 1 based mass vaccination control strategy could be based on repeating vaccination in the whole population every two years. Obviously, this repeated vaccination should again be conducted exclusively in the ideal window of opportunity (see above) trying to minimise Rev 1 side effects [6]. When combined with individual ear tagging (or suitable individual identification procedures), this could facilitate the avoidance of repeated vaccination in previously vaccinated (tagged) animals. In fact, several mass vaccination campaigns covering millions of animals have been or are being currently applied to control the disease in many countries, using this methodology, combined or not with individual tagging.

Independently of the strategy chosen, the evaluation of the efficacy of mass vaccination cannot be established by means of serological surveys conducted at a short time interval after vaccination. First, the serological response induced by Rev 1 in adult animals is of high intensity and duration, being practically impossible to discriminate from that induced in infected animals. Moreover, taking it into consideration that infected animals are not culled but maintained in contact with healthy vaccinated animals, the serological background becomes even more complicated to interpret because of the anamnestic responses of vaccinated animals after having antigenic contacts with infected materials. In these conditions, a significantly higher number of reactors than before vaccination should be evidenced when conducting a serological survey after vaccination. The most practical way of assessing the efficacy of the mass vaccination strategy implemented is to demonstrate a decrease in the human cases in the following years. However, a serological survey conducted soon after vaccination in a representative sample of the vaccinated population is recommended to assess that vaccination has been properly conducted. Reactors in the RB test should range from 80–100% when animals vaccinated adequately are tested 15–20 days after vaccination [6].

Eradication

When any of the above basic mass vaccination strategies has been successfully applied and maintained for at least one entire generation (around 6–12 years, depending on the animal species and breeding systems considered), the disease would be controlled effectively and the prevalence decreased to minimal levels. In this situation, decision-makers can then move to a more advanced eradication strategy. In that case, the sequence of events should always follow a logical trail avoiding backward steps.

1. Bucellosis-free status

Once brucellosis prevalence has been decreased to minimal levels by the repeated mass vaccination of the population over the years, and provided that the veterinary infrastructure and economic resources are also improved in parallel, eradication may then be feasible. This could be achieved through the implementation of a very complex and expensive programme based on the combination of the vaccination of young replacement animals (3–4 months old, by the conjunctival method exclusively) with the test and slaughter of adults found seropositive. As basic but imperative complementary tools, the adequate individual identification of all animals and the effective control of all animal movements would be implemented in the defined epidemiological unit of intervention. The basic principle of eradication is to avoid the entry of infected animals into healthy flocks. Accordingly, the strict control of animal movements is of paramount importance, and probably one of the most problematic issues faced by the veterinary services involved in eradication programmes applied to any of the relevant animal diseases. The successful application of this sophisticated and very expensive combined programme for at least one entire generation (6–12 years, depending on the animal species and breeding systems considered) could lead to a zero or close to zero prevalence, and then to a generalised brucellosis-free status in the epidemiological unit involved. In a final step, the ban on Rev 1 vaccination and the application of an exclusive test and slaughter programme (applying either partial or full depopulation of infected flocks) could lead to obtaining brucellosis officially-free status.

Once brucellosis has been controlled by Rev 1 based mass vaccination, the implementation of a combined eradication programme should be the next logical step. Since the serological response induced by Rev 1 in adult vaccinated animals is of higher intensity and duration than that induced in young replacements [26], the interpretation of serological results during the passage from mass vaccination to a combined eradication programme is critical to avoid the unnecessary culling of healthy but seropositive animals. Taking this important fact into consideration, two technical possibilities could be applied when moving from a control programme based on mass vaccination to a combined eradication programme, whose efficacy has been demonstrated in some regions in Spain (JM Blasco, unpublished results):

A. Avoid serological testing of the animals for a period of two years after stopping mass vaccination

The main objective is to avoid the excessive culling of healthy but seropositive adult vaccinated animals. This is not a trivial point since the serological background of mass vaccinated animals, living in an infected environment,

it is not easy to interpret. Even when applied conjunctivally, the serological response induced by Rev 1 in adult animals is of significantly higher intensity and duration than that induced in young replacements, making the interpretation of serological results extremely cumbersome [26]. Moreover, as indicated above, this situation becomes even more complicated because the Rev 1 immunised animals living in an infected environment can produce anamnestic responses after having antigenic contacts with *B. melitensis* infected materials, with the ensuing development of high and persistent antibody titers even in the case of being protected against these repeated field challenges. In these conditions, very high numbers of animals should be culled when implementing a serological testing using the classical RB and CF tests. Therefore, this strategy is unfeasible (depending on the prevalence of prior vaccination, figures as high as 15–50% of the population would result in positive CF titers several months after vaccination), and this is the reason to recommend avoiding the serological testing of the population for a given time (at least two years) after a mass vaccination is implemented. Accordingly, the only interventions to be applied during these two first years after stopping mass vaccination would be the following:

a) the exhaustive individual identification and vaccination (conjunctival procedure) of 100% of young replacements (both males and females, and ideally when 3–4 months old – it is never recommended to vaccinate animals older than 4 months because the older the animals vaccinated, the longer the serological response induced), with the compulsory implementation of owner registries, on which the new replacement animals should be compulsorily recorded.

b) the entire individual identification of the whole population and the establishment of a compulsory system for movement control. This seems an easy task, but experience in many countries demonstrates that succeeding in the effective control of the movements of the entire animal population is extremely difficult and requires suitable identification procedures, a perfect administrative organisation of the veterinary services involved and, of course, the active collaboration of farmers.

Once this period of two years is over, it would be expected that many infected animals (vaccination has no therapeutic effects in previously infected animals) would have disappeared by natural replacement, and moreover, the serological background in the population should have been significantly reduced. Then, the compulsory individual testing of all adult animals (over 12–16 months of age: *i.e.* the first central pair of permanent teeth are present; therefore, the new born and the recently vaccinated young replacements should not be tested) using the RB test as screening, with the ensuing culling of animals positive in the CF test (considering 30 IU as the cut-off), would be recommended as complementary to measures i) and ii) indicated above. All flocks having at least one CF positive animal should be retested as quickly as possible and as many times

as necessary until at least two consecutive negative CF test results are obtained in a reasonable interval. This can allow the certification of the flock as brucellosis-free.

B. Immediate testing and culling of seropositive animals after stopping mass vaccination

As indicated above, the serological background of mass vaccinated animals is not easy to interpret due to the serological responses induced by Rev 1 and the secondary anamnestic responses produced in infected environments. In these conditions, none of the immunological tests available is able to identify with 100% accuracy the responses induced exclusively in the truly infected animals. Only gel precipitation tests with Native Hapten (NH) antigen [27, 28] are able to identify with an acceptable level of accuracy the serological responses induced by the truly infected animals [26]. Accordingly, 6–12 months after mass vaccination is stopped, the NH gel precipitation testing and culling of seropositives could be implemented in all animals of over 12–16 months of age, with the main objective of accelerating the elimination of infected animals and lowering the serological background of the population (the lower the number of infected animals present in the environment, the fewer anamnestic responses will be produced, then the serological background of the population will be reduced progressively). This serological testing should be repeated as frequently as possible (and at the shortest intervals possible) in each flock identified as infected until at least two consecutive negative NH tests are obtained. Of course, measures a) and b) indicated above should also be implemented compulsorily. Once the percentage of animals seropositive in NH gel precipitation tests is zero in at least two consecutive samplings, the testing schedule could be modified using the classical RB and CF testing. This strategy could be maintained for years (at least one entire generation) until reaching and maintaining a prevalence close or equal to zero, and then reaching the brucellosis-free status.

Without any reasonable doubt, this brucellosis-free status obtained using either A or B possibilities above, is the most recommendable eradication strategy from the technical standpoint since the disease could be fully eradicated but the animals will yet be immunised, thus being capable of resisting infection caused by accidental reintroduction from neighbouring epidemiological units still infected.

When this extremely favourable situation is maintained for at least one entire generation, an exclusive test and slaughter programme with a ban on vaccination could then be applied with the objective of getting the brucellosis officially-free status.

2. Bucellosis offically-free status

This status is required by many countries for international animal trade purposes, and it should be recommended only in the case where farmers need that trade for an adequate income. It is quite difficult to understand that many countries have implemented eradication via this officially-free strategy rather than via brucellosis-free, in a total absence of farmers dedicated to exporting live animals to international markets. This is even more surprising when this situation happens in countries which have not fully eradicated the disease and, therefore, banned Rev 1 prematurely, accepting important risks of reintroduction of brucellosis infection in animals from infected to free regions. The recent example of Greece should be taken into consideration: after Rev 1 was banned bearing in mind the apparent good epidemiological situation, mass vaccination had to be implemented again several years later due to an important increase in brucellosis prevalence. Vaccination should be banned only when a generalised brucellosis-free status has been obtained in the whole epidemiological unit considered and, importantly, when this situation remains unchanged for many years. The premature banning of vaccination is the most frequent error of decision-makers in many countries during the latter stages of combined eradication programmes (as an example, the Rev 1 and S19 vaccines were recently banned in some Spanish regions, despite collective prevalence in some bordering regions being high, and this caused many relapses). As a general rule, vaccination should never be abandoned in a given epidemiological unit: *1)* there is a generalised need of the officially-free status for access to international markets, *2)* the collective prevalence is zero in the whole epidemiological unit, *3)* this favourable situation is maintained with an absence of new cases during at least one entire generation (6–12 years, depending on the animal species and breeding systems considered), and *4)* the risk of transmission or reintroduction of the disease from infected neighbouring epidemiologically related units is also negligible.

Once vaccination is forbidden, the detection of positive animals in an adequate repetitive context by means of the proper diagnostic tests (*i.e*. associations RB + CF, indirect ELISA + CF, or indirect ELISA alone) and their immediate culling, could allow the generalised officially-free status. In this advanced stage, it is recommended that test results have a collective rather than an individual interpretation. The whole culling of flocks detected as infected is frequently more practical and effective than the partial culling of only the infected animals identified. These latter stages of eradication also have plenty of technical difficulties: particularly remarkable is the over-killing of healthy animals as a consequence of the lack of specificity of serological tests in low prevalence situations. Moreover, this problem has dramatically increased in many officially-free countries as a consequence of the false positive serological responses due to *Yersinia enterocolitica* O:9 and other bacteria inducing cross-reactive antibodies against the S-LPS of *Brucella* [29].

It is important to take into consideration that to be successful in obtaining either the brucellosis-free or the officially-free status, a perfect administrative organisation, the individual identification of each animal and the exhaustive control of animal movements, an adequate budget, and the active cooperation of farmers will be compulsory.

Surveillance

When the disease has been fully eradicated from a given epidemiological unit (thus obtaining free or officially-free status), a surveillance system has to be implemented to detect eventual new outbreaks or reintroduction. Passive surveillance systems based, for example, on the compulsory declaration by farmers of abortions produced are not suitable and have proved ineffective for the early detection of new outbreaks of disease. Therefore, an active surveillance system is always recommendable. In the case of small ruminants, active surveillance could be based on the regular serological screening (RB or i-ELISA could be the most recommendable tests for this purpose) of a representative sample of the population. The use of generalist and empiric sampling rules (for example, some EU countries test only 25% of adult females in a three-year interval for maintenance of the officially-free status) should be avoided. It is always recommendable to test regularly (once a year should be the minimum) a representative sample of the population considered, whose composition should also be calculated using adequate epidemiological software having in mind the number of flocks, the average number of animals per flock and, importantly, the threshold level of prevalence expected, and the level of confidence in the calculations made.

Conclusion

B. melitensis infection is responsible for brucellosis in sheep and goats, and the primary cause of brucellosis in human beings. Whenever possible, the eradication of this infection in sheep and goats should be the final objective of any control programme to be implemented, no matter what the extension of the epidemiological unit considered. In absolute terms, "eradication" means the total elimination of the pathogen involved from the country or region considered. Accordingly, eradication is frequently inapplicable in high prevalence situations and extensive breeding systems, and a control programme is then the only feasible strategy. We can define "control" as the reduction of the prevalence to a minimum, with the objective of limiting the main consequences of disease. Therefore, the concept "controlled" could be equivalent to "minimisation of the disease effects". The selection of an eradication or control strategy is of paramo-

unt relevance, and a frequent cause of controversy among decision-makers. Before any decision can be taken, and as a condition *sine qua non*, the quality and organisation of the veterinary services involved should be properly assessed (otherwise, no strategy can be applied). Whenever the organisation of veterinary services is adequate, the final strategy should be established according to the economic resources available and the extent and prevalence of disease. The economic costs of eradication programmes are very high, and plenty of money has to be available along with other elementary conditions required for the selection of any eradication strategy. Experience in countries that succeed with eradication has demonstrated beyond doubt that the eradication programmes cannot be implemented without farmers' cooperation and if adequate economic compensations to affected farmers are not provided. Only when the above conditions are fulfilled, and whenever brucellosis prevalence in the epidemiological unit concerned is very low and uniform, can short term eradication through an exclusive test and slaughter programme without vaccination be considered. In the same favorable socioeconomic situation but having a moderate or not homogeneous disease prevalence, a combined eradication strategy based on young replacement vaccination (using Rev 1 by the conjunctival route) and a test and slaughter programme in adult animals could be recommended. However, when brucellosis is highly prevalent, and independently of the socioeconomic situation considered, the application of eradication programmes is generally unfeasible. In these conditions, the mass (whole-flock) vaccination of all susceptible animals from all animal species involved in the epidemiological cycle is the only reasonable alternative to control the disease. Moreover, this mass-vaccination programme is the basic strategy to be applied over probably several generations in countries with elementary veterinary services and few economic resources, with the main objective of lowering the prevalence of the disease. Once the disease has been controlled effectively (*i.e*. prevalence has been decreased to a minimum and this favourable situation maintained for at least one entire generation), and when the veterinary services organisation and economic resources are improved in parallel, a shift towards eradication may be recommended. This can be achieved through a combined eradication programme based on the vaccination of young replacements and the test and slaughter of adult animals as above. The successful application of this combined programme for at least one entire generation (6–12 years, depending on the breeding systems and animal species involved) could lead to a collective prevalence (percentage of infected flocks) close to zero, and then to a generalised brucellosis-free status in the epidemiological unit considered. Once this has been accomplished, and this favourable situation maintained for at least one additional entire generation, the ban on vaccines and the application of an exclusive test and slaughter programme could lead to a generalised officially-free brucellosis status. Once the effective control of the disease has been accomplished and eradication becomes feasible, the

premature ban on vaccination to obtain this officially-free status (frequently required for international trade) is one of the most common errors of decision-makers. As a general rule, vaccination should be abandoned only when the collective prevalence is zero, this situation maintained during at least one entire generation, and also the risk of transmission or reintroduction of the disease from infected neighbouring epidemiologically related units is negligible. In addition to the availability of huge amounts of money and the full agreement of the farmers involved, to be successful in obtaining either brucellosis-free or officially-free status, a perfect administrative organisation, full individual identification of animals and exhaustive control of animal movements will be compulsory. Once the disease has been fully eradicated from a given epidemiological unit, a surveillance strategy has to be implemented for the early detection of eventual new outbreaks or disease reintroduction.

It is important to stress that in many countries with extensive or transhumant breeding systems, both small ruminants and cattle (and frequently also camels, yaks and buffaloes) are reared together, and that these animal species can be infected by *B. melitensis*. In these epidemiologically complex situations the application of any sanitary strategy should cover the different animal species involved in the epidemiological cycle. Unfortunately, knowledge of the value of the diagnostic and prophylactic tools for brucellosis in camels, yaks and buffaloes is non-existent or very limited. It is frequently considered that the diagnostic and prophylactic tools applied in small ruminants and cattle are equally effective in these animal species, but this has never been adequately proved. Accordingly, a research effort should be made in the coming years to determine the precise role played by these animal species in *B. melitensis* epidemiology and the most effective diagnostic and prophylactic tools in these species.

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Резиме

СТРАТЕГИИ ЗА КОНТРОЛА И ЕРАДИКАЦИЈА НА ИНФЕКЦИИТЕ СО BRUCELLA MELITENSIS КАЈ ОВЦИТЕ И КОЗИТЕ

Blasco JM.

Единица за здравје на животните CITA (Unidad de Sanidad Animal, Gobierno de Aragón) Сарагоза, Шпанија

Цел: Да се опишат различни стратегии за контрола и ерадикација на инфекциите со *B. melitensis* кај овците и козите.

Методи: Предностите и лошите страни на различни стратегии се дискутираат врз основа на личното искуство на авторот, како и со преглед на релевантна литература.

Резултати: Програмите за вакцинација во различни комбинации може да се применуваат за намалување на преваленцата на инфекцијата помеѓу животните, или може да се комбинираат со адекватни дополнителни ерадикациони мерки за да се постигне искоренување на бруцелозата.

Заклучок: Контролирањето на болеста треба да биде примарната цел на вклучените ветеринарни служби и институции. Сепак, ерадикацијата треба да биде конечната цел на секоја имплементирана програма за контрола. Одбирањето на стратегија за контрола или ерадикација е од клучна важност, а често е причина за контроверзи помеѓу носителите на одлуки. Конечната стратегија би требало да се направи согласно квалитетот и организацијата на ветеринарните служби, расположливите финансиски средства и раширеноста и преваленцата на болеста. Соработката со фармерите е клучна за постигнување успех дури и при спроведувањето и на наједноставните контролни програми. Кога бруцелозата е многу застапена, масовната (на цело стадо) вакцинација е најдобар избор, независно од социоекономската ситуација. Кога ќе се постигне ефективна контрола на болеста, ерадикацијата е остварлива. За успешна ерадикација, неопходна е адекватна и квалитетна организација на ветеринарните служби, строга контрола на движењето на животните и задолжително обезбедување на адекватна економска компензација на засегнатите фармери. Кога болеста е комплетно ерадицирана, мора да биде имплементирана стратегија за следење/надзор заради рано откривање на евентуално повторно разгорување на болеста.

Клучни зборови: бруцелоза, *Brucella melitensis*, зооноза, вакцинација, стратегии за контрола/ерадикација

Corresponding Author:

Prof. Dr. José M. Blasco
Animal Health Unit
CITA *(Unidad de Sanidad Animal, Gobierno de Aragón)*
50059 Zaragoza, Spain.
Phone: +34 976716460

E-mail: jblasco@unizar.es