Brucellosis is a bacterial zoonosis caused by microorganisms belonging to *Brucella*, a genus of gram-negative bacteria that behave as facultative intracellular pathogens of ruminants, suidae, canids, and several wildlife species. Some of these bacteria are highly contagious as well as zoonotic; humans can acquire brucellosis readily from animals and their products, even though humans are not themselves contagious. Brucellosis is a complex disease, due to the variety of *Brucella* species involved that, although having species-specific disease syndromes, can sometimes cross-infect. To date, 8 species are members of the *Brucella* genus: *B abortus* (infecting mainly bovines), *B melitensis* (ovines and caprines), *B suis* (swines), *B neotomae* (desert rats), *B ovis* (ovines), *B canis* (canines), *B ceti* (cetacean), and *B pinnipedialis* (pinnipeds). Using a combination of several microbiological, serologic, and molecular tests, several biovars have been identified in some of the main *Brucella* species, including 3 biovars in *B melitensis*.

*B melitensis* is the main etiological agent of brucellosis in sheep and goats. It is also the main agent responsible for human brucellosis, known as Malta fever. Abortion and infertility are the predominant clinical signs in small ruminants. Although there is a paucity of specific studies, it is recognized as a source of significant financial loss to both industries. Its incidence is very high in countries at the south and east of the European Union and in many low-income countries. Altogether, these affected...
countries contain more than 70% of the susceptible world livestock, making brucellosis internationally important. Bovine brucellosis has been successfully eradicated in many developed countries after significant investment and many years of vaccinating and culling. However, *B. melitensis* infection in sheep and goats has been traditionally neglected, because small ruminant production represents generally a low-income activity practiced by landless farmers from marginal rural areas in the developing world. Due to these marginal and usually nomadic farming systems, the control and eradication of this infection is extremely difficult. The infection is practically of worldwide distribution; many countries are suffering a reemergence of the disease in sheep and goats and, accordingly, also in humans.

The global incidence of human brucellosis is not well known because of the low reporting figures, great variations existing between different geographic areas even within the same country. Whereas the reported incidence in most developed countries where infection is present is generally smaller than 1 case per 100,000 inhabitants, in endemic areas, such as some Arab countries, reports reach up to 200 cases per 100,000 inhabitants. However, because of the deficiencies in Health Services of many countries where brucellosis is endemic, there are no reliable data on the global status of the human disease. Nevertheless, a figure of 500,000 new cases per year is usually accepted as a global estimate, reflecting the difficulties in recognizing a disease that, although grave, lacks pathognomonic symptoms and is thus underreported.

The reasons of this high prevalence may result from sociocultural factors, but compounded by the lack of adequate control measures being applied in small ruminant production systems. Contact with animals and occupational exposure, as well as food habits and lack of hygienic measures, represent the main risk factors for *B. melitensis* infection in humans. Because human-to-human transmission is rare, small ruminants are the main reservoir for human cases. Humans can be infected directly by contact with the conjunctival or oronasal mucosae of infected animals, or indirectly by the ingestion of contaminated animal products (mainly dairy products). Human brucellosis is predominantly an occupational disease; professions in direct contact with livestock (farmers, butchers, veterinarians, laboratory personnel, and so forth) are those at higher risk. As there is currently no viable method of preventing human brucellosis, to safeguard people attention must be directed toward effectively controlling the disease in sheep and goats. The diagnostic and prophylactic tools for this disease have been sufficiently validated to effectively fight *B. melitensis* infection in sheep and goats, in most socioeconomic situations. What needs to be improved to assure success is the quality of the national veterinary services and administrative organizations involved. Although sheep and goats are the main reservoirs of infection for humans, in some countries bovines, buffalos, yaks, and camels can also be implicated. Unfortunately, there is a lack of knowledge on the alternatives for controlling *B. melitensis* infection in these species. Accordingly, this review focuses exclusively on the different strategies that could be applied to either control or eradicate brucellosis in sheep and goats.

**PREREQUISITES FOR IMPLEMENTING CONTROL OR ERADICATION PROGRAMS**

When developing a national strategy for control or eradication, the veterinary services must select an approach compatible with the prevailing socioeconomic conditions and infection status. The impact of brucellosis on the livestock economy and human health and the costs of the different control or eradication strategies that could be implemented must be evaluated as part of this strategy. Aspects to consider include: knowledge of the local animal breeding practices and habits, which can vary between different regions of the country; agreement regarding the principles
of the strategy with the local administration; and, in particular, the availability of adequate human resources to carry out the strategy. Finally, because of its zoonotic importance, cooperation between all related stakeholders is of paramount importance and should be promoted. Epidemiologic surveillance to detect human brucellosis in medical centers should be reinforced and notification of cases should be compulsory for both veterinary and public health services involved.

Improved collaboration between the public health and veterinary services can be encouraged, through the reinforcement or the establishment of national zoonoses committees, in which the relevant producer and consumer organizations should be also represented. Provided that the national veterinary service organization is adequate, prevalence of disease and economic resources will dictate the approach. Test and slaughter (T/S) based programs are often unfeasible in developing countries because of the economic cost. In addition, countries that have successfully eradicated \textit{B melitensis} offer monetary compensation to affected shepherds. Provided that the veterinary services organization, farmers’ involvement, and the economic resources are adequate, the final technical elements to select a proper strategy should be the prevalence of disease and the definition of the minimal epidemiologic unit(s) of intervention. A survey should identify the percentage of infected flocks/herds, understanding that differences in prevalence would be expected between different regions placed in the same epidemiologic unit of intervention. Calculating mean prevalence figures for the whole country or particular region considered is a frequent error of decision makers, as those figures may not reflect local conditions. Accordingly, rather than taking generalist sanitary measures, decision makers should be ready to apply different strategies adequate to each of the different epidemiologic situations identified. The minimal epidemiologic unit of intervention should be a given territorial extension with similar epidemiologic situation. In some cases, this can be a couple of isolated flocks/herds in a village and in others, the whole flocks/herds of a given county, but frequently, all flocks/herds in a region or country. The implementation of any brucellosis sanitary strategies requires considerable technical training, and an awareness campaign aimed at the farmers and general population. Once all these elements have been properly defined, 2 possible alternatives exist to fight \textit{B melitensis} infection in small ruminants: (1) control based on mass (whole-flock/herd) vaccination or (2) eradication based on T/S with or without vaccination. In both cases, the use of adequate vaccination procedures and diagnostic tests is of paramount importance.

**DIAGNOSTIC TESTS AND VACCINES**

Eradication of \textit{B melitensis} in small ruminants by applying combined vaccination and T/S is unrealistic in many countries, as they do not have access to the appropriate tests. No serologic tests for \textit{B melitensis} have been developed specifically, and it is widely assumed that the available tests for \textit{B abortus} infection in cattle are also adequate for diagnosing \textit{B melitensis} infection in small ruminants. Accordingly, the Rose Bengal (RB) and the complement fixation (CF) are the most widely used classic tests for the serologic diagnosis of brucellosis in sheep and goats. Both tests detect antibodies raised against the \textit{Brucella} smooth lipopolysaccharide (S-LPS). The RB test was developed originally for the diagnosis of bovine brucellosis and, despite the scant information available, it is also recommended for the screening of \textit{B melitensis} infection in small ruminants. The CF test is also considered suitable for the serologic diagnosis of brucellosis in small ruminants at population level. However, the sensitivity of the CF test is poorer than that of both the RB and indirect enzyme-linked immunosorbent assays (ELISA). In addition, both RB and CF tests lack specificity when testing
sera from sheep and goats recently vaccinated with Rev-1, the only available vaccine against *B. melitensis*.\(^8\text{–}^{10}\) However, specificity of all serologic tests is somewhat preserved (see later discussion), if the Rev-1 is applied by conjunctival route.\(^9,^{10}\) Several reports have confirmed the adequate sensitivity of the different ELISAs for the diagnosis of brucellosis in sheep. In general, the indirect ELISAs are good tests for surveillance purposes in countries in the latter phases of eradication and in which vaccination is no longer used. However, these ELISAs lack specificity when used in vaccinated animals, particularly when Rev-1 is used in adult animals. In these conditions, only the Native Hapten (NH) gel precipitation test\(^1,^{11}\) is useful for determining infection in vaccinated animals. Although the competitive ELISA is promising, this test lacks specificity in vaccinated animals and those infected with *Yersinia enterocolitica* O:9.\(^12,^{13}\)

Low-income countries would profit from improved vaccines and simple, specific, and inexpensive diagnostic tests. However, it is unlikely that these tools will be developed by richer countries, as they prefer eradication using automated surveillance tests to reduce labor costs. Therefore, interest in brucellosis research is waning in first-world countries, despite the disease imposing a severe burden elsewhere. Because of this, the World Health Organization has recently classified brucellosis among the 7 top “neglected zoonoses,” a group of diseases that are simultaneously a threat to human health and a cause of poverty perpetuation.\(^14\) The live-attenuated *B. melitensis* Rev-1 vaccine is the only vaccine available, and has been proved to be effective for prevention of *B. melitensis* infection in sheep and goats.\(^15\) However, when administered by the classic subcutaneous method (individual doses of \(1 \times 10^9\text{–}2 \times 10^9\) cfu), a long-lasting serologic response is induced, which makes an eradication program based on combined T/S impractical. When the same vaccine is administered by the conjunctival method (at the same dose, but applied by conjunctival instillation in a smaller volume), the immunity conferred is similar to that induced by the classic subcutaneous method, but the serologic responses evoked are significantly reduced, making this program fully compatible with the application of an eradication program based on vaccination combined with T/S.\(^15\) However, this type of program is still out of the reach of many countries that have only elementary veterinary services and limited economic resources. In these cases, a mass vaccination strategy is the only reasonable alternative to be applied to control brucellosis. Unfortunately, the vaccination of pregnant animals with Rev-1 administered subcutaneously can induce high numbers of abortions and the excretion of Rev-1 strain in milk.\(^15\) Reduction of the Rev-1 dose (individual doses ranging from \(10^3\) to \(10^6\) cfu administered subcutaneously) has been reported as a method avoiding these significant adverse reactions while maintaining effective protection.\(^16\) However, field and experimental data suggest otherwise,\(^15\) so that the reduced doses of Rev-1 should never be recommended as an alternative to the vaccination with standard doses. Due to the risk of abortion, there is no entirely safe strategy for mass vaccination. Even conjunctival vaccination is not safe enough to be applied regardless of the pregnancy status of the animals.\(^15\) It is recommended that Rev-1 not be used in mid-gestation animals, the main critical period for abortion as a consequence of vaccination.\(^15\) However, this is impractical under field conditions, and some of the risks have to be assumed if the objective is to control the disease. Conjunctival vaccination of animals before the start of the mating season, during the late stages of the lambing season, or during lactation seems to be the safest approach to performing a whole-flock/herd vaccination program.\(^15\) Another small but potential risk with the modified-live vaccine (proven minimal after >50 years of widespread use worldwide) is that this strain can infect humans\(^17\) and is resistant to streptomycin, an antibiotic that in combination with doxycycline constitutes the most effective treatment of
brucellosis in humans. Accordingly, some minimal individual biosafety measures (protection glasses and gloves) and awareness campaigns addressed to people involved in vaccination procedures should be implemented to lessen the infection risks in humans. In the case of accidental infection with Rev1, a combined doxycycline-gentamicin (or doxycycline-rifampin) treatment should be administered.\(^{17,18}\)

The diagnostic interference generated by vaccination hampers T/S eradication programs. The diagnostic epitopes involved are located in the O-polysaccharide section (a homopolymer of N-formylperosamine) of the \(B\ melitensis\) S-LPS immunodominant surface antigen, the genetics of which have been recently elucidated.\(^{19}\) Research to improve the classic vaccines by removing these S-LPS epitopes (ie, to develop rough—R—vaccines) has been conducted. Among the live rough \(Brucella\) strains obtained by classic attenuation methods, is the \(B\ abortus\) RB51 vaccine. However, its efficacy and safety with regard to bovine brucellosis is questioned\(^{20,21}\) and it is not effective against \(B\ melitensis\) or \(B\ ovis\) infections in sheep.\(^{20}\) Finally, human infections due to RB51 have also been described\(^{22}\); this mutant is resistant to rifampin, an antibiotic widely used in the treatment of human brucellosis.\(^{18}\) Therefore, RB51 should never be recommended for vaccinating small ruminants. Other research efforts in developing R vaccines resulted in candidates of low overall efficacy.\(^{20,23}\) Whereas R candidate vaccines do not interfere with the classic serologic tests (RB and CF), this cannot be said for ELISA. Using the S-LPS or its hydrolytic polysaccharides as antigens, it has been proved that an important proportion of ewes vaccinated with R candidates were detected to be seropositive in an indirect ELISA.\(^{23}\) This result is not unexpected, because 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 classic RB and CF tests), but they can become exposed on adsorption to ELISA plates and, therefore, prevent a clear-cut distinction of the antibody responses to S and R brucellae. This problem is likely to affect all R vaccines, including RB51, because the authors have found that a significant proportion of cows that aborted as a consequence of vaccination with RB51 develop antibodies reacting in ELISA tests.\(^{21}\) As a conclusion, the potential advantages claimed for R vaccines have been seriously questioned and there is increasing evidence showing that these vaccines interfere in S-LPS–based ELISAs; flaws include lack of safety in pregnant animals, possible excretion in the milk of vaccinated animals, potential for human infection, and reduced efficacy compared with the classic Rev-1 and S19 vaccines against brucellosis in small ruminants and cattle.\(^{20}\)

Other approaches to develop new-generation vaccines, such as the construction of recombinant strains deleted in relevant diagnostic proteins or DNA-based vaccines, are being also investigated.\(^{24}\) In fact, a Rev-1 vaccine strain deleted in the gene coding for BP26 protein (that can be used as a differential marker) resulted in the same protective efficacy as Rev-1 in sheep.\(^{25}\) Its efficacy was also evidenced against \(B\ ovis\) infection in rams, but evaluation of the performance of the BP26-based differential diagnostic test is limited.\(^{26}\) Up to now, none of these new-generation vaccines have been found to provide an improvement over efficacy and safety of the classic Rev-1 vaccine. Therefore, Rev-1 should continue to be the reference vaccine for prevention of brucellosis in sheep and goats.\(^{24}\) Independent of their origin, the Rev-1 vaccine and the diagnostic tests to be used should be always submitted for quality control to internationally recognized laboratories, and should fulfill the minimal requirements described by the World Organization for Animal Health.\(^{1}\)
CONTROL STRATEGIES

Independent of the prevalence of infection, a whole flock/herd vaccination of all susceptible sheep and goats is the only reasonable strategy to control brucellosis in many low-income countries. To avoid the risk to pregnant animals, the focus should be on vaccinating young replacement animals (3–4 months old) exclusively. The hypothesis is that if 100% of young replacements (representing usually 20%–25% of the total population, depending on the animal species and breeding systems considered) are vaccinated yearly, the whole population would be fully immunized after only a moderate period of time (4–5 years). To be successful, all young replacements (both males and females) should be vaccinated and, ideally, also identified for successful follow-up. However, because of practical difficulties in vaccinating 100% of replacements, this strategy fails to control brucellosis even in developed countries and it is generally inapplicable in the developing world. In the characteristic extensive husbandry conditions of small ruminants, several veterinary visits would be required to locate and vaccinate 100% of these animals. This practical problem, coupled with the difficulty of localizing all flocks/herds in nomadic breeding systems, results in frequent failures in adequate vaccination coverage. Therefore, the mass vaccination of all susceptible animals irrespective of age is the only suitable strategy to control brucellosis in sheep and goats in many countries. This mass vaccination could be complemented with an individual ear tagging (or alternative identification procedure) of vaccinated animals to facilitate appropriate follow-up of animals in subsequent years. However, ear tagging is not considered permanent, is expensive, and can predispose animals to fly-strike.

To be effective, any whole-flock/herd vaccination program should be maintained over time. The ideal follow-up procedure to minimize Rev-1 side effects could be vaccinating exclusively the young replacements every year and for at least 5 to 6 years following the first mass vaccination campaign, which should include ear tagging. The characteristic annual replacement figures for small ruminants in extensive breeding systems usually range from 20% to 25%. Therefore, the next year after the one when the first mass vaccination program takes place, only 20% to 25% of the population would be new and susceptible to the disease. Because of a flock/herd immunity effect, transmission to this relatively low percentage of unvaccinated replacements—already low risk as they are not pregnant—is much smaller, making the need for an annual mass vaccination unnecessary. By contrast, 2 years after mass vaccination, around 40% to 50% of the entire flock/herd population would be fully unprotected and would contain a high proportion of animals at risk (pregnant and lactating). Therefore, a practical and cost-effective recommendation would be to repeat the mass vaccination of the entire flock/herd animals by using Rev-1 every 2 years. To minimize the Rev-1 side effects, the ideal window of opportunity (i.e., before the start of the mating season, during the late stages of the lambing season, or during lactation) should be selected. This approach is especially feasible when taking into account the characteristic seasonal breeding of sheep and goats. In fact, many mass vaccination campaigns, covering several million of sheep and goats in many countries, have been applied using this strategy and very few adverse effects have been reported.

ERADICATION PROGRAMS

Vaccination itself is a suitable measure to control brucellosis, but additional measures are required for eradication. Once the disease is controlled, and provided that the veterinary services and economic resources of the concerned country have been also improved, eradication could become feasible. Eradication could be achieved
through implementation of a very complex and expensive program based on the combination of vaccination of young replacements (3–4 months old, both males and females, and exclusively by the conjunctival method) with the T/S of adult animals found to be seropositive. The basic principle for eradication is avoiding the introduction of infected animals into healthy flocks/herds. Accordingly, as complementary tools, the effective control of all animal movements and the adequate individual identification would be implemented in the selected epidemiologic unit of intervention. The control of animal movements is probably one of the most problematic issues faced by the veterinary services involved in any brucellosis eradication program. The successful application of this complex combined eradication program for at least one entire generation (5–6 years) could lead to a generalized brucellosis-free status in the epidemiologic unit involved. In a final eradication step, ban of Rev-1 vaccination and application of an exclusive T/S program (applying either partial of full depopulation of infected flocks/herds) could lead to complete eradication of the disease and granting of official brucellosis-free status for the epidemiologic unit considered.

Once brucellosis has been controlled by Rev-1–based mass vaccination, a combined eradication program could be selected. Because the serologic interference caused by Rev-1 in vaccinated adult animals is of higher intensity and duration than that induced in young replacements, the interpretation of serologic results during the passage from mass vaccination to a combined eradication program is critical to avoid the unnecessary culling of healthy but seropositive animals. With this in consideration, 2 effective possibilities could be recommended when finishing a mass vaccination control strategy and starting a combined eradication program.

The first possibility could be to avoid the serologic screening of the mass-vaccinated animals for a period of at least 2 years after finishing mass vaccination. Vaccinated adults, particularly those having contact with field B melitensis strains, are at risk of developing persistent antibody responses. To prevent these specificity issues, it is recommended that during the 2 first years after stopping mass vaccination, veterinary services (1) maintain the conjunctival vaccination of the whole replacements, and/or (2) individually identify the entire sheep and goat population and establish a system for controlling animal movements. Effective control of the animal movement is very expensive and requires suitable identification, perfect administrative organization of the veterinary services involved, and the active collaboration of farmers. Once this recommended period of 2 years is finished, it is expected that the serologic background of the vaccinated population will be reduced significantly. Then the individual testing (RB test as screening test) of all adult animals, that is, older than 12 to 16 months (with, at least, the first pair of permanent incisors erupted), and culling of those detected as positive in the CF test (≥30 IU) could be recommended as complementary to the aforementioned interventions (1) and (2). The flocks/herds having at least one CF-positive animal should be retested as many times as necessary, until 2 negative consecutive whole-flock CF tests result. This outcome would allow the certification of the flock/ herd as “brucellosis-free.”

The second possibility could include, in addition to the interventions (1) and (2), testing and culling of seropositive animals soon after mass vaccination is finished. As indicated, the serologic background of adult vaccinated animals living in infected contexts is complex, and none of the S-LPS–based immunologic tests available (ie, RB, CF, or ELISA) is 100% sensitive nor specific. However, as mentioned previously, the NH gel precipitation test has superior sensitivity and specificity in vaccinated animals. Accordingly, 6 to 12 months after the last mass vaccination has been performed, NH gel precipitation testing and culling of seropositive animals could be implemented in those older than 12 to 16 months. Not only would this eliminate
infected animals but would lower the challenge to vaccinated animals (anamnestic response), making their serologic responses easier to interpret. This NH testing should be repeated as frequently as possible in each flock/herd identified as infected, until at least 2 consecutive negative tests are obtained. Then the testing schedule could be performed using the classic RB and CF testing already indicated. This strategy could be applied for several years, until arriving at null prevalence and obtaining a generalized brucellosis-free status in the epidemiologic unit of intervention. This brucellosis-free status is the most recommendable technical strategy, because the disease is eradicated yet, simultaneously, the population is immunized, thus being able to facing new infections caused by accidental reintroduction from neighboring epidemiologic units still infected.

When a brucellosis-free situation is maintained for many years, an exclusive test and slaughter program with banning of vaccination could be applied with the objective of a country or region obtaining the brucellosis “officially-free” status. This status is required for international animal trade and is considered erroneously by many veterinarians as the highest sanitary standard. However, it is difficult to understand why the veterinary services from many countries where *B. melitensis* has not been eradicated are in favor of the generalized officially-free rather than the brucellosis-free status, even in the absence of farmers exporting live animals to international markets. Vaccination should be banned only when a generalized brucellosis-free status has been firstly obtained in the whole epidemiologic unit involved, and this situation has been maintained for many years. Premature cessation of vaccination is the most frequent error of decision makers during the late stages of a *B. melitensis* eradication campaign. As a general rule, the Rev-1 vaccine should be never abandoned until 4 requisites are fulfilled simultaneously: (1) existence of a generalized need of farmers to access international markets, (2) the prevalence is null in the whole epidemiologic unit, (3) this eradication situation is maintained in absence of new cases during at least one entire generation (4–6 years), and (4) risks of transmission or reintroduction of infection from infected neighboring epidemiologically related units are negligible. Once Rev-1 vaccination is stopped, the detection and immediate culling of positive animals in an adequate repetitive context by means of the proper diagnostic tests (ie, association RB + CF, indirect ELISAs + CF, or indirect ELISAs alone), could allow the generalized officially-free status. During these final eradication steps, it is recommended that test results have a collective rather than an individual interpretation. The entire stamping out of flocks/herds detected as infected is frequently more practical and effective than the partial culling of only the seropositive animals identified. As prevalence drops, even a test with acceptable specificity will have a low predictive value of a positive test (PVPT), meaning that most test-positive individuals are actually healthy. By way of example, with a test with 99% sensitivity and 99% specificity and a disease prevalence of 20% (1 in 5 animals infected), the PVPT is 96%, meaning that of 100 positive tests 96 animals will actually be infected. But at a prevalence of 1%, the PVPT is 50%, meaning half of all test-positive animals will be healthy. This problem has increased significantly in many officially-free countries as a consequence of the false-positive serologic responses caused by *Y. enterocolitica* O:9 and other bacteria sharing cross-reactive epitopes with the *Brucella* S-LPS.

When the disease has been eradicated, a surveillance program has to be implemented for early detection of new outbreaks or reintroduction. Passive surveillance systems based, for example, on the compulsory declaration of abortions by farmers are not sensitive enough and have proved ineffective for the early detection of disease. Accordingly, an active surveillance system is preferred that can be based on the regular serologic screening of a representative sample of the population (RB or
indirect ELISAs could be suitable tests for this purpose. Use of generalist and empiric sampling rules (as an example, some European Union countries test only 25% of adult females in a 3-year interval to maintain the officially-free status) should be avoided. It is more recommendable to test regularly (once a year should be a minimum) a representative sample of the population considered, the composition of the sample being calculated using adequate epidemiologic software, by taking into consideration the number of flocks/herds, the average number of animals per flock/herd, the threshold level of expected prevalence, and the confidence level of the expected results.

**SUMMARY**

*B. melitensis* is the main responsible causal agent of brucellosis in sheep and goats and is the primary cause of brucellosis in human beings in many countries. Several strategies to control and eradicate this infection in small ruminants have been proposed and used by national and international animal health organizations. This article reviews the different control and eradication strategies used in small ruminants in different socioeconomic and epidemiologic situations.

**REFERENCES**