Brucellosis in cattle, sheep and goats: diagnosis, control and vaccination*

R. FENSTERBANK**

Summary: Reports received from forty-eight countries constitute a representative sample which demonstrates the universality of the problems created by brucellosis, and the wide range of control measures which have been adopted.

Among the forty-eight countries, thirteen have obtained complete eradication of the disease, seventeen are in the process of more or less advanced clearance, eight have a vaccination programme primarily designed to decrease prevalence of the disease and ten do not yet have well-defined applicable measures.

For diagnosis, the two serological tests used most frequently are the Rose Bengal test and the ring test, with recourse to complement fixation and serum agglutination in doubtful cases. There is a need for more sensitive and more specific complementary serological tests and for bacteriological examination in countries free from brucellosis, where serological cross-reactions create problems.

Twenty countries apply strict disease control measures, and eighteen apply a combination of vaccination and disease control measures. Strain B19 is almost exclusively used for vaccination, either just for heifers, or for adults as well, using reduced doses of the vaccine administered by subcutaneous or conjunctival routes.

Brucellosis of small ruminants is less widespread than bovine brucellosis, but it is more contagious, and is controlled in seventeen countries by means of slaughter and vaccination with Rev. 1 vaccine, with the exception of one country which is on the verge of eradication through strict application of a slaughter policy.

Contagious epididymitis of rams, due to B. ovis, is of no danger to man, and occurs in eight of the countries which supplied reports. These countries apply either strict sanitary control or a combination of control measures.

KEYWORDS: Brucella - Brucellosis - Cattle diseases - Diagnostic techniques - Disease control - Goat diseases - Reviews - Sheep diseases - Vaccination - Veterinary Services.

INTRODUCTION

Forty-eight countries (eleven African, eight belonging to the Americas, eleven of Asia and Oceania and eighteen European) and the European Common Market

---


** INRA, Centre de Recherches de Tours, Station de Pathologie de la Reproduction, Nouzilly, 37380 Monnaie, France.
have made reports on the prevalence of brucellosis and the means of obtaining its eradication or protecting brucellosis-free herds. This selection, taken from nearly half the Member Countries of the OIE, is highly representative, both at the geographic and epidemiological level, thus expressing the diversity of health situations and control methods used.

### Table I

<table>
<thead>
<tr>
<th>List of OIE Member Countries the reports of which have been analysed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Argentina</td>
</tr>
<tr>
<td>Australia</td>
</tr>
<tr>
<td>Austria</td>
</tr>
<tr>
<td>Burkina Faso</td>
</tr>
<tr>
<td>Canada</td>
</tr>
<tr>
<td>Central African Rep.</td>
</tr>
<tr>
<td>Chile</td>
</tr>
<tr>
<td>Colombia</td>
</tr>
<tr>
<td>Cuba</td>
</tr>
<tr>
<td>Cyprus</td>
</tr>
<tr>
<td>Czechoslovakia</td>
</tr>
<tr>
<td>Ethiopia</td>
</tr>
<tr>
<td>Finland</td>
</tr>
<tr>
<td>France</td>
</tr>
<tr>
<td>German (Dem. Rep.)</td>
</tr>
<tr>
<td>Germany (Fed. Rep.)</td>
</tr>
</tbody>
</table>

Human beings become infected by contact with infected animals or by consuming food of animal origin. Consequently brucellosis is a public health problem, particularly serious because the human disease is often debilitating if not correctly cared for at an early stage. Brucellosis in animals is also a serious economic problem, bringing about considerable losses, and leading to restrictions in international trade in livestock.

These reasons have led each country to introduce control measures, or at least to consider what action is compatible with their resources. The result is that many countries are now declared to be free from brucellosis, or in the course of eradication, while others have not yet taken coordinated action, although they are aware of the problem. It must be recognised that this is a difficult procedure because of uncertainties about detection, prolonged because of reinfection, and costly through the implementation of control measures and associated slaughtering.

As with each infectious disease, brucellosis has its own features, the most important of which is contagiousness. An abortion leads to the massive discharge of $10^{12} - 10^{13}$ bacteria, while just 15 million *Brucella* instilled into the conjunctiva are capable of infecting 95% of pregnant heifers. The quantity of *Brucella* excreted during an abortion could infect 60,000 to 600,000 pregnant heifers. The disease also presents very clear multiplicative characteristics since $15 \times 10^6 Brucella$ inoculated into pregnant heifers can provoke the excretion of $10^{12}$ to $10^{13}$ bacteria in case of subsequent abortion. A lower excretion, but not less dangerous, should also be noted at the moment of normal calving in affected cows the infection of which had not been detected by serological testing. In addition, it should be remembered the danger of calves born of brucellic dams, and which can be found to be infected themselves at the moment of their first calving, after an often prolonged seronegative phase.
Such contagiousness explains the reinfection which may occur in herds believed to be disease-free. Without reinfection, control by slaughter of reactors would be effective very rapidly. On the other hand, if vaccination was 100% effective, eradication would be achieved very rapidly by injecting the vaccine into all the animals. This is not the case, and a campaign for the eradication of brucellosis pursues a course between its elimination by slaughter and its propagation, which may be inhibited by vaccination.

The methods for controlling the disease are diagnosis and control, which may include or exclude vaccination. These three aspects will be examined by combining the information provided in the reports, and comparing them with the latest scientific knowledge.

BOVINE BRUCELLOSIS

DIAGNOSIS

The purpose is to search for brucella infection, to reveal prevalence and distribution, and (in countries where eradication has been achieved) to monitor freedom from reinfection. Techniques employed are serological and allergic tests, and isolation of the agent by bacteriological methods.

Serological diagnosis

This utilises basic tests suitable for large-scale testing, coupled with additional tests for clarifying doubtful results. The samples tested are blood, taken from live or slaughtered animals (e.g. Canada), and milk. The tests detect antibodies, which are evidence of infection, and make their appearance after a variable incubation period, after which they tend to persist. The relative proportion of antibodies in the immunoglobulin classes IgG1, IgG2 and IgM (32) differs according to the stage of evolution of the disease. In addition, vaccination (which is often resorted to) is also responsible for inducing antibodies of the same classes. An ideal serological test would establish an early diagnosis, identify chronic infections, and distinguish between the antibodies of vaccination and those of infection. A test also needs to be economical, simple and rapid, and possible to repeat on numerous occasions. No serological test possesses all these qualities.

The slow tube agglutination test on serum (SAT), the ancestor of all serological tests, is still widely used, for it is the basic test in twelve countries, and a supplementary test in seventeen others. Evidence of its efficacy comes from the countries from which the disease has been eradicated. It is often used in conjunction with the complement fixation and ring tests. SAT detects antibodies of the classes IgG2 and IgM (32). Shortcomings are its inability to detect recently infected animals and an inability to detect chronic infection, which may be accompanied by low titres which are difficult to assess (26, 39). The last-named fault is particularly serious, bearing in mind the habitual chronic nature of the disease.

Complement fixation (CF) is the basic test in two countries (Malaysia and New Zealand — automated in the latter country), and a complementary test in practically all the others. It detects IgG1 and IgM antibody classes, and is considered to be the most sensitive and the most accurate, enabling a distinction to be made between antibodies of vaccination and those of infection (2, 39). Disadvantages of CF are its delicate and long procedure, requiring the services of a trained technician, which unfortunately often rules out its use as a basic test.
The *Rose Bengal test* (RBT) is a rapid plate agglutination test on pure serum using a stained antigen at pH 3.6. This is the basic test for twenty-three of the thirty countries where it is used. There is therefore a large measure of agreement on the use of this test, which is justified to the extent that the RBT is economical, simple and rapid, and gives few false negative or false positive results, requiring verification by CF (with or without SAT) (13). The immunoglobulins responsible for the reaction are IgG1 (9), as for CF, and sometimes IgM, depending on the type of antigen preparation (D. Levieux, personal communication). The test can detect infection at an earlier stage than SAT (13), and at the same time (or perhaps sooner than) CF (39, 20).

The *ring test* (RT) is also widely used. It detects immunoglobulins present in milk, whether originating from the blood by filtration (IgM) or locally within the mammary gland (IgA), an organ which is among those most frequently infected. It is efficient, easy to perform and economical (35, 48). The RT can be performed frequently (monthly) both to detect infected dairy herds (being the basic diagnostic test, particularly in Switzerland), and to provide continuous surveillance of brucellosis-free herds. Its success seems to be due more to the frequency of testing than to its sensitivity (Great Britain), which diminishes with increasing herd size. It can be used as an early alerting test, because it is not possible to repeat serological testing so frequently. Addition of formaldehyde (to a final concentration of 0.2%) preserves milk samples for up to 14 days, and seems to augment the sensitivity of the test rather than reduce it (Great Britain). Finally, the RT can be used on individual cows.

The detection of serologically positive herds is easy at the commencement of a control scheme. With the progress of eradication, or when eradication is almost complete, new problems arise which have to be solved in a more elaborate way, often at the level of individual cases. In addition to tests which have become established, such as the Rivanol, mercaptoethanol (39, 42) and Coombs’ antiglobulin tests, new tests are under investigation (16, 44, 51). Among those still being tried out is ELISA, performed on blood serum or milk whey (Great Britain), which is very promising.

Other difficulties involving SAT and CF are due to cross-reactions between *Brucella* and other bacteria, such as *Yersinia enterocolitica* serogroup 09, various salmonellas and *Escherichia coli*, etc. (11). Here the RBT seems to be more specific because of its pH of 3.6 (13). Addition of EDTA (43) seems to overcome this snag in the case of SAT (Great Britain).

**Allergic diagnosis**

*Brucella* infection creates a state of sensitisation which can be detected by hypersensitivity reactions of the delayed type, provoked by the injection of allergen extracted from *Brucella*. Among a large number of allergens, those prepared by the method of Bhongbhibhat *et al.* (6) have the advantage of not provoking the formation of antibodies detectable by serological tests, and of not inducing sensitisation. Therefore this test can be repeated without upsetting subsequent serological or allergic tests. This test has been proposed (21) for routine diagnosis and as a supplementary test in problem herds, and it is used in four countries (New Zealand, Czechoslovakia, FRG and the USSR). New Zealand claims a specificity of 100% and a sensitivity of 60-68%, and the intention is to use this type of test for the simultaneous diagnosis of tuberculosis and brucellosis.
**Bacteriological diagnosis**

Most countries employ bacteriological techniques to detect *Brucella*, mainly to investigate abortions, the notification of which is often compulsory. Vaginal discharge, colostrum, the aborted fetus and the placenta from an infected cow contain large numbers of *Brucella*. Excretion of the bacteria in milk is tested either on individual cows (those which are serologically negative but RT positive), or on a large scale in combination with other tests (USA). Lymph nodes and other organs taken at slaughter may also be tested for *Brucella* (Canada) or fluids from hygromas (15), which occur frequently in infected African animals (Burkina Faso). This testing has the advantage of providing direct evidence of the disease, if the bacteria are isolated. It requires good laboratories and well-trained technicians. Most laboratories use selective media. However, it must be borne in mind that biovar 2 of *B. abortus* requires the addition of serum to the medium. Many reports mention the use of the medium of Kuzdas and Morse, and the WE medium of Renoux, but these will not detect this biovar. It would be better to use the medium of Farrell (19), which contains serum. The bacteriological identification of *Brucella*, in addition to the above-mentioned methods, is indispensable to obtain an accurate evaluation of the epidemiological status of herds or of communities of people. Identification of species and biovars of *Brucella* has made considerable progress, thanks to the use of a set of bacteriophages, and is done in many countries. Correct identification can be made by non-specialised laboratories. Information obtained from the typing or recognition of rare markers, makes it possible to identify and trace sources of infection.

**CONTROL**

In thirty-two countries the detection of brucellosis has been made compulsory throughout the country or in specified parts of it (provinces or natural regions). In the sixteen other countries it is partial, usually depending on voluntary effort or application to certain zones (dairying areas close to large towns, state farms or experimental farms). A scheme for disease-free status, defining the criteria which have to be fulfilled before a herd can be recognised as free from the disease, is in operation in twenty-six countries. In most countries all cases of abortion have to be notified, and are followed up by a range of diagnostic and disease control measures.

The forty-eight countries can be placed into five groups according to the type of control and their current disease status (Table II).

It can be seen that thirteen countries have been officially declared to be free from brucellosis (column 1). Vaccination is prohibited or is not used in these countries, although it was used at the start of eradication in at least five of them. Diagnostic tests are still performed with vigilance. Some countries in which the movement of cattle is strictly controlled, or which are geographically isolated (Norway) practise a reduced degree of testing. In seven other countries (column 2), some of which are on the verge of eradication, vaccination is no longer performed, with the sole exception of Greece.

Eighteen countries operate a mixed scheme for control. In ten of these countries (column 3) the accent is on compulsory slaughter of infected animals, vaccination only being practised in certain areas. For the eight other countries (column 4) control is primarily based on the vaccination of heifers, and even of adults, slaughter of infected animals only being compulsory in well-defined areas.
<table>
<thead>
<tr>
<th>Method of control</th>
<th>Sanitary</th>
<th>Mixed</th>
<th>Not yet established</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slaughter of reactors</td>
<td>Compulsory</td>
<td>Compulsory</td>
<td>Compulsory in some zones</td>
</tr>
<tr>
<td>Vaccination</td>
<td>None</td>
<td>Recommended in certain zones</td>
<td>Compulsory in certain zones or everywhere</td>
</tr>
<tr>
<td>Prevalence</td>
<td>Very low to average</td>
<td>Very low to average</td>
<td>Variable according to country and zone</td>
</tr>
<tr>
<td>Austria</td>
<td>Australia</td>
<td>Chile</td>
<td>Argentina</td>
</tr>
<tr>
<td>Canada</td>
<td>Cyprus</td>
<td>Cuba</td>
<td>Colombia</td>
</tr>
<tr>
<td>Czechoslovakia</td>
<td>Greece</td>
<td>France</td>
<td>Iran</td>
</tr>
<tr>
<td>Finland</td>
<td>Korea</td>
<td>Italy</td>
<td>Mexico</td>
</tr>
<tr>
<td>FRG</td>
<td>Taiwan</td>
<td>Malaysia</td>
<td>Saudi Arabia</td>
</tr>
<tr>
<td>GDR</td>
<td>Vanuatu</td>
<td>New Zealand</td>
<td>Sri Lanka</td>
</tr>
<tr>
<td>Ireland</td>
<td>Yugoslavia</td>
<td>Portugal</td>
<td>Uruguay</td>
</tr>
<tr>
<td>Japan</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norway</td>
<td></td>
<td>USA</td>
<td></td>
</tr>
<tr>
<td>Poland</td>
<td></td>
<td>USSR</td>
<td></td>
</tr>
<tr>
<td>Romania</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
In the ten last countries, brucellosis has been known to occur for some time. They all face considerable difficulties in human resources and/or finance, and brucellosis may be downgraded to second place (because it does not kill cattle) in favour of the "major" diseases like rinderpest, pleuropneumonia and anthrax (Ethiopia, Sudan). Often, as in Sudan, nomadism renders detection illusory, even by surveys. The presence of wildlife reservoirs (Vanuatu) raises problems which have been only partly, or not at all elucidated. Vaccination is practised in all these countries, and more as a result of individual initiative than a concerted plan of action, with some hesitation about the type of vaccine (Ethiopia) or its mode of administration, the conjunctival route having been chosen in Burkina Faso.

This classification is a rather artificial one. It would be of interest to study the diversity of the measures employed and the results obtained. It is impossible to apply everywhere one and the same control scheme because of the very variable conditions in different countries. A strategy has to be chosen after profound and accurate consideration (39), which is apparent from examination of the various reports, taking into primary consideration the human, technical and financial resources available.

People play a decisive role in control schemes. The veterinarians and technicians in charge have to be capable of correct interpretation of tests and of intervening wisely in the progress of the operation. Farmers will not support the constraints imposed on them and will not be able to understand the difficulties of diagnosis and eradication unless they are adequately informed and motivated, a point which has been made particularly by the reports from Canada, Cyprus and Czechoslovakia.

The technical procedures require laboratories, and lack or inadequacy of laboratories will invalidate the best conceived plan. Mention should also be made of certain special strategies, such as the definition of a disease-free zone suitable as a source of replacement animals (Chile).

The financial aspect is extremely important, for the cost of control is very high for a long period. Before any decision is made, an economic study should be made by using a simple model (14) or a more elaborate one (5, 27). The concern with obtaining a good cost-benefit ratio is evident in most of the reports. On the other hand, the lack of sufficient means is cited by many developing countries to explain the current lack of a control scheme.

The prevalence of the disease is a basic element which affects the choice of a mode of control, whether by disease control measures alone, medical (immunisation) measures alone, or a combination of the two. By taking into account the variations in prevalence which occur within a given country, the operations can be adjusted to suit the zones of differing prevalence (Chile, Portugal). Type of husbandry, patterns of commerce, the grouping or dispersion of farms, size of herds, proportion of animals vaccinated, and segregation of calving cows (GDR) are all important parameters (27, 30, 40, 53), which in some countries are given the same importance as the control of animal imports and the control of movements of animals; these measures are strictly applied in most countries in which control is compulsory. Finally there is a problem of wildlife reservoirs, about which little or nothing is known (Vanuatu).

During the course of operations, changes may be necessary to reflect the progress of eradication (for example, prohibition of vaccination) or the appearance of
new techniques (such as adoption of the Rose Bengal test, vaccination of adults, vaccination by the conjunctival route). Any such change to the original plan means that all the participants have to be kept well informed.

This leads us to discuss the choice between the three modes of intervention:

a) Strict application of disease control measures, including eradication by slaughtering infected animals. This is the most radical method and there is no doubt that it is the most economical when prevalence is low and conditions favourable. It should be carried out rigorously and rapidly in order to diminish the risk of reinfection as much as possible. The reports submitted provide a partial answer to the question often asked, about the threshold of prevalence below which this sort of control is technically and economically feasible. Three countries achieved eradication after starting with 10.5-11% of herds infected (Finland), 20% (Czechoslovakia) and 5% infected animals (Switzerland). Unfortunately this information does not include data on the size of herds, their dispersion, or geographical obstacles to the spread of the disease, all of which have considerable influence on the progress of eradication.

b) Medical control by general vaccination leads to a decrease in prevalence of this disease and its maintenance at a very low level. In fact, none of the reporting countries use this method alone, without at least some compulsory or recommended slaughtering, even though it is the principal method adopted by many countries of Central and South America.

c) Mixed control is a simultaneous or successive combination of the two above methods, utilising the slaughter of reactors and the protection of healthy herds by vaccination. Compulsory or voluntary vaccination is chiefly aimed at heifers, by inoculating strain B19 vaccine at 3-8 months of age, except in Saudi Arabia where strain H38 vaccine is used.

The results of vaccination are plain: after ten years of intensive vaccination in certain zones, the prevalence of the disease fell from 7 to 3% in Chile, where the maximum proportion of heifers protected by vaccination was estimated at 72.9%, and from 17 to 3% in Argentina, where the maximum protection was 79.4%. Overall prevalence in Uruguay is 0.4% after 20 years of intensive vaccination practised on 95% of heifers. In addition, in South Africa "the lowest prevalence was observed in a province where strict supervision of the vaccination of heifers was maintained for a number of years", which is precisely the same sort of observation made in the USA (29). Thus vaccination diminishes the prevalence of the disease and the rate of reinfection to a point at which fewer cattle will have to be slaughtered, making the project less costly. In this respect, vaccination by the conjunctival route (46), with the possibility of booster injections to increase the level of protection, should give still better results. It is, therefore, particularly recommended in developing countries to obtain control of the disease.

On an experimental basis and for some years, in order to avoid recontamination of herds in the process of becoming disease-free, vaccination of adults has been carried out. A reduced dose of strain 19 vaccine is used, either by the subcutaneous route (Cuba, New Zealand, South Africa, Sri Lanka and the USA), or by the conjunctival route (France, Italy). The results are very significant (42, 46).

In this mixed type of control, the problem is when to stop vaccinating in order to conclude eradication by slaughtering the last reactors. There is a threshold of prevalence below which vaccination becomes less profitable because of the slaugh-
ter of heifers which possess residual antibody induced by vaccination. This problem was raised by Great Britain, which believed that more heifers had been slaughtered than necessary. WHO experts proposed that the threshold should be 1 to 5% of herds, or even 0.5% under difficult conditions (large-size herds). Once again, the determination of this threshold depends on the conditions of husbandry.

**VACCINATION**

Three vaccines are available: inactivated 45/20 (37) and H38 (47) vaccines and the live strain *Brucella abortus* B19 (8). Inactivated vaccines, in principle, have the advantage of keeping better than live vaccines. The 45/20 vaccine used in addition to the B19 vaccine in Ethiopia, France, Ireland, Malawi and Portugal is non-agglutinogenic, but raises problems of control and efficacy, which varies with the method of preparation. H38 vaccine used in Saudi Arabia and Ethiopia is very effective but agglutinogenic, which is a major drawback posing insoluble detection problems among vaccinated animals.

Consequently it is not surprising that strain B19 is employed in every country which practises vaccination, with the exception of Saudi Arabia. This large consensus is justified by the extensive research conducted on this vaccine since the 1930's, which has given rise to large numbers of publications concerning its efficacy (29, 33, 34, 36), method of use (42, 46) and testing (45).

The resistance induced by strain B19 is not complete, but it remains constant throughout the economic life of a cow (34, 36), although some doubt has been cast on this opinion. Its use has enabled many countries to reduce the prevalence of the disease to a point at which eradication becomes feasible (29), as is shown by the results submitted by Argentina, Chile and Uruguay.

The disadvantage of using strain B19 is the persistence of vaccinal antibodies in 1-2% of animals, including those vaccinated very young (4 months). Curiously, none of the presented reports mention this disadvantage, with the exception of Great Britain, where it is believed that the presence of such antibodies resulted in more animals being slaughtered than necessary.

Two new methods of vaccination can overcome this disadvantage using reduced doses of B19. The first was developed by Nicoletti *et al.* (42) to avoid frequent contamination, making eradication by slaughter impossible in very large American herds. It consists of injecting $3 \times 10^8$ to $3 \times 10^9$ B19 vaccine subcutaneously into heifers of 4 to 12 months in the USA and, in Canada, into animals to be exported to the USA, and $3 \times 10^8$ (New Zealand) and $5 \times 10^8 \times 10^8$ (USA) into adults. The serological response is decreased in intensity and duration, and the immunity conferred appears to be similar to that provided by the standard dosage.

The other procedure, developed by Plommet *et al.* (46), consists of inoculating $5 \times 10^9$ B19 vaccine by the conjunctival route twice at an interval of 6-12 months. Antibodies induced by vaccination disappear completely within 4 months at the most, regardless of the age of the animal, or any previous vaccination with B19. This procedure abolishes all restrictions on the age at vaccination. In case of need, it can be used as a booster for animals which received the conventional vaccination at 4-8 months of age. This repetition induces an immunity better than that provided by conventional vaccination.
For all the contagious diseases, the extent to which a group of animals is protected depends on the percentage of animals vaccinated, but for some diseases a percentage of 60-80% provides adequate protection against the spread of the disease. This does not seem to be the case with brucellosis, because of the high contagiousness of the disease. Research by Vanderwagen et al. (53) has shown that any shortfall in the number of animals vaccinated, however small, permits the disease to develop very rapidly.

Finally, vaccination should be carried out regularly during a period long enough to produce a fall in prevalence, until disease control measures become technically and financially feasible by themselves. It is quite possible that vaccination of nearly all of a cattle population by the conjunctival method (including a booster dose to reinforce and extend the immunity) can lead, by itself, to practically complete control of the disease after a few years.

Current research has abandoned the whole bacteria, whether live or inactivated, for vaccines and is concentrating instead on the isolation of a fraction of bacteria, or even the antigens in such a fraction, which are responsible for protection, with the object of producing a “chemical” vaccine. A further development would be to characterise the determinants (epitopes) of these protective antigens. Those of polysaccharide nature could be coupled to a macromolecule to give a “semi-synthetic” vaccine. Those of proteic nature would be analysed to determine the amino acid sequence of the peptides obtained by enzymatic cleavage, leading to a “synthetic” vaccine after similar coupling to a macromolecule. Finally, vaccines may be obtained by genetic engineering after cloning the genes of protective antigens, and incorporating them within a non-pathogenic bacterium (such as Escherichia coli). In any event, research continues into a non-ambiguous way of distinguishing the antibodies of vaccination from those of infection.

BRUCELLOSIS OF SMALL RUMINANTS DUE TO BRUCELLA MELITENSI

Infection with *B. melitensis* seems to be less widely distributed in the world than *B. abortus*. Seventeen countries report its presence on their territory (Argentina, Cyprus, Ethiopia, France, Greece, Iran, Italy, Mexico, Nigeria, Oman, Portugal, Saudi Arabia, Sri Lanka, Sudan, Uganda, USSR and Yugoslavia), and thirteen countries report its absence (Australia, Canada, Cuba, Finland, German Dem. Rep., Germany (Fed. Rep.), Great Britain, Ireland, New Zealand, South Africa, Sri Lanka, Switzerland and the USA). Eighteen countries do not state whether it is present or absent.

Diagnosis is obligatory nationwide in ten countries, and in certain parts of three countries. The SAT, CF and RB tests are used in the same way as for cattle. The CF test is considered to be the most sensitive and most specific, while the Rose Bengal test is generally considered to be less sensitive, but is widely used because of its simplicity and low cost. In specific countries, CF is regarded as the principal diagnostic test (Cyprus) or complementary to the RBT (France). SAT is still widely used, despite the fact that it is considered to be less sensitive (41, 54), particularly in brucellosis cases of long standing (31), even when it is performed with 5% NaCl solution instead of 0.85%, or with antigen prepared from *B. melitensis* (1).

These three tests, used singly or together, give good results at the flock level, but permit the identification of barely 70% of individual infected animals (41). This
fact explains the difficulties encountered in diagnosis, and consequently in eradication.

Allergic diagnosis (22) is used in three countries (Cyprus, Switzerland and USSR). It is well suited to large-scale testing and avoids the collection of numerous blood samples and their testing in the laboratory. Although it did not detect as many infected animals as the serological tests, it has given most satisfactory results in Cyprus.

Strict application of disease control measures, carried out competently and diligently in Cyprus, has given impressive results: infection was reduced between 1973 and 1984, the percentage of reduction being 99.7% in sheep and 99.4% in goats. It is one of the apparently rare countries to be on the point of eradicating brucellosis from small ruminants solely by using disease control measures. Some significant results have been obtained locally in other countries by this method of control. However, brucellosis of small ruminants is particularly refractory to this control method, with a contagiousness greater than bovine brucellosis, and the detection of individual cases less satisfactory than in cattle.

All other countries which report the occurrence of B. melitensis infection practise vaccination with strain Rev. 1, with very encouraging results (Greece). The vaccine strain Rev. 1 (18) confers excellent immunity (3, 17, 31). Its use has even permitted elimination of the disease from very large flocks of sheep in the USSR (52). However, there are two disadvantages attached to vaccination with Rev. 1 (4):

a) It sensitises the animal for a long time, upsetting subsequent allergic tests.

b) It provokes the formation of antibodies which disappear rapidly in most animals, but which persist in a few for a long time. This disadvantage can be eliminated by using the conjunctival route for administering the vaccine (24).

CONTAGIOUS EPIDIDYMITIS OF RAMS DUE TO BRUCELLA OVIS

This disease was reported in eight countries: Australia, Canada, France, New Zealand, Romania, South Africa, Uruguay and the USA. The reports from other countries do not mention it. So far there has been no record of human infection, and control measures against the disease are justified only on account of its effects on sheep breeding (reduced fertility of rams, some abortions in ewes).

It is detectable by CF or gel immunodiffusion (29). These tests seem to be lacking in sensitivity, and research is in progress on the development of an ELISA test (50, 55). The serological tests should be accompanied by clinical diagnosis by means of palpation. Brucella ovis can be isolated readily on the medium of Brown (7) from semen or from portions of organs and lymph nodes taken at the abattoir.

Prophylaxis solely by application of disease control measures is currently being applied in Australia, Canada and Romania; it is difficult but effective (49). Other countries practise mixed control, using vaccines prepared from killed B. ovis (38) (New Zealand and the USA) or strain Rev. 1 vaccine (23, 25) (South Africa, France).
CONCLUSION

Reading the 48 reports reveals the universality and the importance of the problems created by brucellosis. Every country is involved whether they have infected herds or not, for in the latter case a continuous surveillance is necessary. In brucellosis-free countries, the smallest failure to detect and control the movement of infected animals could compromise their disease-free status.

The tests used most often for serological diagnosis are the RBT, RT and CFT. This choice is justified, for experts acknowledge the efficacy (sensitivity and specificity) of the three tests. This applies particularly to the CFT, but unfortunately this is unsuitable as a basic test because the procedure is delicate and long, and requires adequately equipped laboratories and well-trained technicians. Consequently the CF test is generally used as a complementary reference method, when other tests have given doubtful results. By contrast, the RBT and RT are very easy to perform, do not cost too much and do not require sophisticated equipment. Accordingly they are very suitable for large-scale testing.

However, there is a problem with regard to the Rose Bengal antigen. The present antigen, prepared from the \textit{B. abortus} 99 (A > M) strain, appears to detect the antibodies to \textit{Brucella} (M > A) \textit{(B. abortus} biovar 5, \textit{B. melitensis} biovar 1) poorly (12). The problem with regard to the strain or strains of antigen preparation or of antigens, and of standard control sera, is currently being studied.

The serum agglutination test is being used less and less since the introduction of the RBT. This is also justified because it is less sensitive and less specific than the other three tests. Curiously there is a certain trend to retain this test, particularly in countries which have completed eradication after having used it extensively, although it was used in combination with CFT and RT.

Other tests, such as the Rivanol and EDTA tests, and those currently still under investigation (such as ELISA and IHLT, etc.) are used less extensively, sometimes being reserved for special cases (problem herds and problem animals). Above all they are suitable for differentiating vaccinal or non-specific antibodies from those of infection, and to improve the sensitivity of detection.

Allergic diagnosis is not used much. It has certainly given very good results in Cyprus. It could be used more often, particularly in small ruminants.

Bacterioscopy is too imprecise and is being progressively abandoned. On the other hand, new selective media and the new methods of identifying and typing \textit{Brucella} are being used more and more to provide a precise diagnosis, which may provide a basis for future epidemiological investigations.

While awaiting the vaccines of the future, mentioned above, and with the almost universal abandonment of the inactivated vaccine strains 45/20 and H38, the B19 and Rev. 1 vaccines are practically the only ones in use. There may be fresh interest in the use of these vaccines in view of the development of modern methods of vaccination, at reduced dosage or by the conjunctival route, which may render them more compatible with the requirements of diagnosis, and more effective as a result of conjunctival booster doses.

There are still many gaps to be filled in our knowledge of brucellosis. Numerous scientific teams are continuing their research work. There are also stan-
dardisation problems to be resolved concerning diagnostic antigens and vaccines, their preparation and testing.

Nevertheless, currently available knowledge and experience of the disease should enable us to control brucellosis with even better chances if an adequate strategy has been given greater thought and been more carefully chosen. The success of countries notified free is sufficient to convince us of this.

*  *

Appendix

54th GENERAL SESSION OF THE OIE
RESOLUTION No. XII
BRUCELLOSIS IN CATTLE, SHEEP AND GOATS: DIAGNOSIS, CONTROL AND VACCINATION

CONSIDERING THAT

* Brucella abortus* and *Brucella melitensis* infections are of public health significance and create economic loss in livestock;

* several countries have commenced or completed eradication programmes;

* no serological test can completely differentiate between antibodies produced by natural infection from those produced by vaccination;

* for diagnosis in cattle, standard tube agglutination, acidified antigen agglutination such as Rose Bengal plate in combination with complement fixation are proven tests;

* for diagnosis in small ruminants the Rose Bengal, complement fixation and allergic tests are proven tests;

* vaccination programmes can maintain the disease at a low level;

* live vaccines such as Strain 19 and Rev. 1 have proved effective,

THE COMMITTEE

RECOMMENDS THAT

1. A programme to control brucellosis would depend on the disease status, the economic situation, the species of animals involved and the livestock management practices of the relevant country.

2. Countries wishing to initiate a programme of brucellosis control should adopt established diagnostic procedures.

3. More specialised diagnostic procedures such as ELISA, IHLT, EDTA modified agglutination, could be used in the final phases of eradication and for post-eradication surveillance.
4. Test and slaughter is the method of choice for elimination of this disease.

5. When economic, epidemiological or other circumstances preclude strict test and slaughter policy, vaccination should be used to reduce the prevalence of infection. Although live vaccines should be used, further studies of dosage levels and route of administration are required.

6. For control of this disease, a functional veterinary infrastructure is necessary and should take account of the following:

   a) adequate resources (personnel, funds, transportation);
   b) control of import and movement of livestock;
   c) surveillance and reporting systems.

(Adopted by the International Committee of the OIE on 29 May 1986.)

**REFERENCES**

(see p. 600)