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**JOINT FAO/WHO EXPERT COMMITTEE
ON BRUCELLOSIS**

Second Report

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MAY 1953

JOINT FAO/WHO EXPERT COMMITTEE ON BRUCELLOSIS

Second Session

Florence, 13-18 October 1952

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JOINT FAO/WHO EXPERT COMMITTEE ON BRUCELLOSIS

Second Report¹

In November 1950, a meeting of the Joint FAO/WHO Expert Panel on Brucellosis was held in Washington, D.C. The report on this session dealt with the basic aspects of brucellosis as regards the diagnosis and control of this disease in man and animals.²

During the last two years, appreciable advances have been made in the knowledge of brucellosis and, in order to consider the new information available concerning this disease, a meeting of the Joint FAO/WHO Expert Committee on Brucellosis was held at the Institute of Hygiene of the University of Florence, Italy, from 13 to 18 October 1952.

At this session, the committee discussed in detail questions concerning the isolation and identification of *Brucella* and reviewed the report of the first session with the purpose of modifying recommendations, where required, and of adding other points of importance where further information has accrued. In considering the present report, therefore, it is essential that the reader refer to the report on the first session.² Statements not requiring modification are not repeated in the present report, except for the purposes of clarity or emphasis.

¹ The WHO Executive Board, at its eleventh session, adopted the following resolution :

The Executive Board

1. NOTES the second report of the Joint FAO/WHO Expert Committee on Brucellosis ;
2. THANKS the members of the committee for their work ; and
3. AUTHORIZES publication of the report.

(Resolution EB11.R12, *Off. Rec. World Hlth Org.* 46, 4)

² *World Hlth Org. techn. Rep. Ser.* 1951, 37 (also issued as *FAO Agricultural Studies* No. 14)

INTRODUCTION

During the past two years, considerable progress has been recorded in several countries in the control of animal brucellosis, notably in Denmark, Finland, Sweden, the United Kingdom of Great Britain and Northern Ireland, and the USA. The problem, however, remains very important from the point of view of both its public-health and economic significance. Local surveys carried out by national authorities, and through the network of FAO/WHO brucellosis centres,³ have revealed the presence, and sometimes extensive prevalence, of the disease in areas where hitherto information had been lacking or inadequate (e.g., some parts of Africa, southeastern and western Asia). Another disturbing development has been the recognition of porcine brucellosis in Central Europe, an area where the disease had not been reported for many years.

The basis of prevention of human brucellosis lies in the control and eradication of the disease in animals. The improvement in the control of bovine brucellosis and in the adequate heat-treatment of cows' milk and milk products can be correlated with the lessening of human brucellosis due to *Brucella abortus* in the areas affected. Unfortunately, however, the problem of *Br. melitensis* infection of sheep and goats, and its transmission to human beings, has retained its high level of importance. Although experimental results give promise of progress in the control of ovine and caprine brucellosis, little overall improvement will occur unless farmers and government administrators take active steps to inaugurate control programmes for these animals. One important point which has often been overlooked as regards ovine and caprine brucellosis is the serious economic losses occurring through this disease as the result of abortions, infertility, and lowered milk-production, particularly in countries which can least afford such losses. The committee urged, therefore, that agricultural, veterinary, and public-health authorities should take appropriate steps—with the aid, whenever possible, of FAO and WHO—to bring ovine and caprine brucellosis under control (see also section 3.3, page 14).

Attention was drawn to the increasing frequency of cross-infection with any of the three species of *Brucella* in the various domestic animals.

³ See Annex 4, page 29.

This must be given serious consideration, particularly as regards the infection of human beings with *Br. melitensis* transmitted from infected cattle or swine and spread between the animals themselves. In this connexion, control programmes involving vaccine may be interfered with since there is no reason to think that the vaccines used at present protect cattle against *Br. melitensis* infection.

The committee emphasized the importance of the need to make brucellosis in man and animals a notifiable disease by government legislation, and of the exchange of this information through FAO, the International Office of Epizootics (OIE), and WHO.

1. ROUTES OF TRANSMISSION FROM ANIMALS TO MAN [1]*

This topic was considered in the first report. A few items are dealt with here to stress certain facts.

The transmission of brucellosis by food and food products, in particular meat, milk, and milk products, can be prevented by subjecting them to adequate heat-treatment. Wherever necessary, legislation should be enacted to require pasteurization of milk and milk products. Freshly prepared cheeses produced from unheated milk are consumed in many parts of the world, and this constitutes a great source of infection for the human population concerned. It is recognized that matured fermented cheeses are safe from the standpoint of brucellosis, but little accurate information is available as to the time of ageing necessary to ensure safety. In this connexion, some evidence has been advanced that a period of three months is sufficient for hard, fermented cheeses; but no sweeping statement can be made on this point because of the great variety of methods used for producing these products in different countries. The committee recommended, therefore, that tests using guinea-pig inoculations be made in individual countries on the various cheeses, butter, and other milk products produced locally from unpasteurized milk and that the results of these tests be used as the basis for legislation concerning the products in question.

Nothing written above detracts from the fact that contact with infected animals or materials is, in many areas, the most important means of infection.

* Throughout this report, the numbers in square brackets refer to the corresponding sections in the report on the first session (*World Hlth Org. techn. Rep. Ser.* 1951, 37).

2. BRUCELLOSIS IN HUMAN BEINGS [2]

2.1 Clinical Criteria [2.1]

The clinical features of human brucellosis are adequately described in the literature, but the committee pointed out that the two common forms of the disease are :

- (1) an acute fever of limited duration followed by apparent recovery ;
- (2) long-continued disease with periodic exacerbations.

The onset may, however, be insidious, though followed by long-continued disease.

Brucella infection may persist in the body for long periods without causing clinical manifestations. In patients with non-specific and vague symptoms, a diagnosis of brucellosis should not be made except on the criteria as given below. The committee stressed again that there are no criteria, with the exception of the culture of the organism, which will determine with absolute certainty the existence of *Brucella* infection.

2.2 Diagnostic Criteria [2.2]

2.2.1 Culture [2.2.1]

The tissues from which *Brucella* can most easily be isolated are the blood and bone-marrow. If such attempts are unsuccessful, they should be repeated; and other sources, such as lymph-nodes, cerebrospinal fluid, urine, and any abscesses which may be present, should be investigated. *Brucella* has been occasionally isolated from sputum, placenta, mother's milk, vaginal discharge, seminal fluid, etc. (see section 5, page 17).

2.2.2 Agglutination [2.2.2]

Because of its importance, the committee repeated the statement made in the first report :

“The sero-agglutination test, when carried out with a suitable antigen and a satisfactory technique, almost always gives significantly positive results in the presence of active infection. Repeated tests should be carried out in cases giving low titres or negative reactions before regarding brucellosis as unlikely. In cases suspected of brucella infection, a high or rising agglutination titre constitutes presumptive evidence of infection. While high titres indicate a high probability of infection, this does not exclude the possibility of infection in cases with low or no demonstrable titre.”

It should be noted that positive agglutination against *Brucella* may be produced by cholera, tularaemia, or immunization against either of these diseases. This is particularly important in areas where cholera occurs, or in individuals immunized against cholera. Cholera-induced agglutinins for *Brucella* can be differentiated by the agglutinin-absorption test. In the case of tularaemia, the titre differential and the clinical features of the disease leave little doubt as to the diagnosis.

Attention should be called to the existence in some endemic areas of large numbers of persons without symptoms but with low *Brucella*-agglutinin titres. Therefore, in patients with ill-defined symptoms and low titres, caution should be exercised in attributing such symptoms to *Brucella* infection.

The Committee agreed that, in cases of all kinds, extreme caution should be used in interpreting titres of less than one-tenth of the titre obtained when the methods and antigens concerned are used to test the International Standard Anti-*Brucella abortus* Serum.⁴

Where antibiotics are freely used in febrile conditions, positive diagnosis by haemoculture is less frequently possible, and increasing reliance must therefore be placed on the results of agglutination tests.

2.2.3 Complement-fixation and opsonocytophagic tests [2.2.3 and 2.2.4]

The committee was of the opinion that the complement-fixation test has no practical value at present and that the opsonocytophagic test in all its modifications is not suitable for diagnostic use.

2.2.4 Intradermal test [2.2.5]

A positive intradermal test denotes a specific allergic condition of the individual and should be regarded as free from other diagnostic significance, regardless of the antigen or technique employed; it certainly does not prove the presence of an active infection. The chief value of the test is for epidemiological purposes.

2.2.5 Other laboratory tests [2.2.6]

A normal, total, white-blood-cell count or a leukopenia with, or without, a relative lymphocytosis in a febrile patient should suggest the possible presence of *Brucella* infection and should lead to the carrying-out of other diagnostic tests.

⁴ This standard serum is available to government or other official laboratories upon request to the Ministry of Agriculture and Fisheries Veterinary Laboratory, Weybridge, Surrey, England. Instructions for its use will be sent with the serum. (See also footnote 7 on page 16.)

2.3 Therapy [2.3]

A considerable amount of experience has been gained in certain aspects of treatment since the publication of the first report, but the committee considered it desirable to repeat some of the recommendations given then, with the necessary modifications. The recommendations below are applicable in general to human infection with all varieties of *Brucella*.

2.3.1 General [2.3.1]

Supportive therapy, including bed-rest and adequate diet, during the acute manifestations of the disease is essential.

In view of the self-limiting nature of brucellosis, the effectiveness of any form of therapy should be judged by the following criteria :

- (a) immediate and continued clinical improvement in the patient's condition ;
- (b) the disappearance of *Brucella* from the blood-stream and other tissues ;
- (c) the reduction in the incidence of complications ;
- (d) the reduction in the frequency of relapses, the majority of which occur within six months.

2.3.2 Antibiotics and chemotherapy

2.3.2.1 *Antibiotics* [2.3.2-2.3.7]. Although the antibiotics available at the present time mark a great advance in the treatment of *Brucella* infection, they do not furnish a complete solution of the problem, since relapses have been observed following the use of each antibiotic singly or in combination. If relapse occurs after treatment, the patient can be treated in the same ways as previously.

The use of penicillin for the treatment of *Brucella* infections is unjustified. Streptomycin and dihydrostreptomycin are of no value when used alone. When used either alone or in association with other antibiotics or sulfonamides, they are liable to give toxic side-effects, of which the most important is damage to the eighth nerve. Aureomycin is of value when used alone in the treatment of active *Brucella* infection. A suggested schedule for adults is 2 g daily for 14-21 days. Oxytetracycline⁵ when

⁵ Oxytetracycline is the international non-proprietary name for "Terramycin" (see *Chron. World Hlth Org.* 1953, 7, 41).

used alone is comparable in every way to aureomycin. Chloramphenicol has been shown to be of value in the treatment of brucellosis, but caution must be exercised owing to the occasional occurrence of aplastic anaemia following its use ; for this reason aureomycin or oxytetracycline is to be preferred.

2.3.2.2 *Sulfonamides and other chemotherapeutic agents* [2.3.8-2.3.9]. The sulfonamide compounds alone are of very limited value in the treatment of *Brucella* infections. No other chemotherapeutic agents have yet proved to be of value in the treatment of the disease.

2.3.2.3 *Combination treatment.* The simultaneous administration of streptomycin or dihydrostreptomycin with sulfadiazine is of value. A suggested dosage for adults is 1-2 g daily of streptomycin or dihydrostreptomycin and 4-6 g of sulfonamide for 14-21 days. The simultaneous administration of streptomycin or dihydrostreptomycin with aureomycin or oxytetracycline is superior therapeutically to the preceding treatment. The recommended daily dosage is 1-2 g of streptomycin or dihydrostreptomycin combined with 2 g of aureomycin or oxytetracycline for 21 days. The addition of sulfonamide to the two antibiotics in the previous treatment has given good results. Some workers keep the dose of antibiotics as stated, while others prefer to reduce this dose somewhat.

2.3.2.4 *Vitamin B.* It is recommended that, during oral therapy with antibiotics or sulfonamides, adequate doses of vitamin-B complex should be given to avoid manifestations of avitaminosis.

2.3.3 *Antigens* [2.3.10]

There is no general agreement as to the place to which *Brucella* vaccine therapy is entitled in the treatment of the disease. The committee recognized that, in view of the fact that antibiotic therapy is not freely available in some countries, other forms of treatment are being employed, including therapy with antigens. A common form of such therapy is the parenteral administration of various types of *Brucella* antigen, which may be associated with systemic reactions. Some clinicians claim that benefit results from such therapy, particularly in the more chronic forms of the disease with complications. The information available is insufficient to justify the recommendation of any particular antigen.

It should be noted that intravenous therapy is not free from risks, such as severe systemic reactions followed by collapse.

Long-continued vaccine therapy is never justified for patients with multiple and vague complaints in whom the diagnosis is not proven.

3. BRUCELLOSIS IN ANIMALS [3]

Most aspects of this subject have been fully dealt with in the first report. The committee felt it desirable to emphasize in regard to control, however, that, although sanitation and hygiene are of great importance, the prime basis of control rests on the detection and elimination, or effective isolation, of the infected animal.

3.1 Diagnostic Methods [3.2]

The relative value of the various diagnostic tests referred to in the first report was confirmed. Reference here to the whey agglutination test and to the various milk tests with stained antigens was considered desirable.

3.1.1 *Milk agglutinin tests with stained antigens*⁶

These tests are agglutination tests and depend on the presence of *Brucella* agglutinins in the milk from infected animals. The antigens used are stained so that the agglutination reaction may be observed in the presence of milk or cream.

Antigens produced in different countries are usually standardized by comparison with previous antigens; for this purpose one or a number of positive milk-samples of various titres is used. There is some evidence to show, however, that standardization can be achieved by agglutination tests with a standard serum. It was recommended, therefore, that trials be made, using the International Standard Anti-*Brucella abortus* Serum for this purpose.

3.1.1.1 *Milk ring test* [3.2.3 and Annex 3]. This test was referred to in the first report as the ABR test (Abortus Bang Ringprobe), and this name is still used in many countries. However, it was thought by the committee that, since the test is used for the milk of animals infected with any one of the species of *Brucella* and is specially used for milk, it should in future be referred to as the milk ring test.

Antigens used for the test can be stained with various dyes. The two most used up to the present time are haematoxylin and triphenyl tetrazolium chloride. Another recently introduced dye is diphenyl tetrazolium

⁶ Details of these tests are available on request to either of the joint secretaries of the committee, at the Food and Agriculture Organization, Viale delle Terme di Caracalla, Rome, Italy, or at the World Health Organization, Palais des Nations, Geneva, Switzerland.

chloride. The haematoxylin-stained antigen has the advantage in that it gives a ring of a deep-blue colour when fully developed, and many workers consider that this is more easily read than the colour given by other stained antigens. Its disadvantages are that its preparation is more time-consuming, that the dye used is less stable than the vital dyes, and that, in the preparation of an antigen of the intended sensitivity, difficulties are frequently encountered. The tetrazolium-stained antigens have the advantage of ease of preparation and that, once the cells are vitally stained, the dyes are not easily released. The light-red ring produced by the triphenyl tetrazolium antigen is regarded by some workers as less easy to read than the blue of the haematoxylin ring. Diphenyl tetrazolium antigen is prepared as easily as that made with the triphenyl dye, and it has the advantage that the ring is purple-red in colour.

Information collected since the last report has confirmed the great practical value of the milk ring test as a presumptive or screening test to locate infected herds. It is also useful as an adjunct to the sero-agglutination test for the detection of infection in previously clean herds and in lightly infected areas (under 10% of herds reacting). Accreditation of a herd as *Brucella*-free cannot be made on negative ring tests alone, but must be confirmed by sero-agglutination tests of the individual animals. Re-accreditation of a herd can, however, be made on repeated herd ring tests. The combination of this test with the sero-agglutination test has made it possible to carry out tests for eradication purposes much more cheaply and quickly and in some areas has, in fact, made possible programmes which would have otherwise been impossible on the grounds of lack of personnel.

3.1.1.2 *Milk plate test.* Ring test antigens can be used for the plate test; and for this the darker antigens, particularly the haematoxylin-stained antigen, are more easily read. A more sensitive antigen is, however, desirable. The plate test has the advantage that cream, sour milk, homogenized milk, skim milk, or mastitis milk can be tested directly.

3.1.1.3 *Milk capillary-tube test.* The capillary-tube test is best made with the haematoxylin antigen. It gives similar results to the milk plate test but is generally more difficult to read.

3.1.2 *Whey agglutination test*

The whey agglutination test is a useful diagnostic method for detecting virulent *Br. abortus* infection, particularly in the udder, in individual animals. It also has value in differentiating between vaccinated and infected cattle with suspicious and low positive serum agglutinin titres. A high percentage of whey samples from uninfected strain-19-vaccinated animals

show no agglutinins in the dilution below the minimum diagnostic level, whereas a high percentage of whey samples from infected animals show agglutinin titres above the diagnostic level. The test loses accuracy when it is applied during the early and late stages of lactation.

A description of the technique of the milk whey test is given in Annex 1 (see page 26).

3.2 Bovine Brucellosis [3.5]

It would be of much value if investigations could be started to obtain information on the prevalence and economic importance of bovine brucellosis in countries in which such estimates are not yet available. In certain circumstances this work might be carried out under the aegis of FAO and WHO, and the committee recommended that this be done wherever possible. The incidence of the infection in cattle could be obtained from the results of sero-agglutination tests or milk ring tests, or both, carried out in representative areas of the countries.

The basis for the development of any plan of control and final eradication of the disease in any country centres around an educational programme embracing all sections of the community and includes the training of veterinarians and technicians in laboratory and field procedures. The system adopted for this education must depend upon the facilities available in the country. Where farmers' organizations exist, much use can be made of them, working in full collaboration with the central and regional government authorities. Stress should always be laid on the monetary value of a state of freedom from the disease, both from human and animal standpoints.

The ultimate objective to be aimed at is complete eradication of bovine brucellosis from a country: the actual plan must, however, depend upon the circumstances prevailing in the country.

Veterinary services and legislation for animal-disease control vary from country to country: in some countries they are practically non-existent; in others they are well developed.

While the systems of control outlined in the first report were, therefore, supported, the committee felt that emphasis should be laid on the inauguration of an educational programme to reach all sections of the population of a country, and on the establishment of a satisfactory veterinary service in countries in which it does not exist. When these recommendations have been developed, consideration can be given to the control programme.

3.2.1 *Vaccination of cattle* [3.3]

3.2.1.1 *Strain 19 vaccine* [3.4]. The committee felt that strain 19 vaccine holds its place of pre-eminence among vaccines used in brucellosis control from the standpoints of safety, adequacy of protection, and practicability of production and use.

(a) Vaccination of pregnant animals. In animals vaccinated in the normal way when non-pregnant, strain 19 has not been isolated from the milk or genital discharges. In pregnant animals, on the other hand, although the animal does not become permanently infected in either the uterus or the udder, strain 19 may be excreted for a period of up to a week following parturition. Vaccination of pregnant adults is therefore not generally recommended.

(b) Duration of protection. Information is now available showing that animals vaccinated as calves, and in which there has been no opportunity for increase of the immunity by subsequent exposure to *Brucella*, are still resistant for up to five pregnancies. There is, however, some reason to think that the increased age of the animal may play a part in this continued resistance. The committee felt that this suggested that revaccination after the first or subsequent calving is not likely to be necessary. Further work is already in progress on the possible value of repeated vaccination during calfhood.

(c) Vaccination of bulls. Bulls are seriously exposed to infection where they are running with heavily infected herds. Vaccination of bulls is often followed by persistent agglutination titres; it cannot, therefore, be used where vaccination is being combined with elimination on the basis of the agglutination test. There appears to be no reason why bulls should not be vaccinated in herds or in countries where vaccination is the only method of control in operation. In bulls, as in females, vaccination should be confined to the age-group of 6-8 months.

(d) Dried vaccines. Further work with dried vaccines has confirmed their less perishable nature and their value under field conditions. It is not yet possible to state with assurance what their useful life is; but, while it appears to be at least one year if the material is kept in cold storage, it may be much shorter when the vaccine is exposed to high external temperatures, continuously or intermittently.

(e) Routes of vaccination and doses. Vaccination is normally carried out by the subcutaneous route. It has, it is true, been shown that reduced dosage by the intradermal, intracaudal, or subcutaneous routes appears to give sufficient protection for the subsequent pregnancy. The minimal dose (i.e., content of viable strain 19) necessary to produce an adequate protection by the subcutaneous, intradermal, or any other route, is not,

however, yet known. It is therefore concluded that, at present, the recommendation for a dose of 5 ml (approximately 60,000 million viable organisms— 60×10^9) injected subcutaneously should be adhered to. Further information on these subjects is needed.

The committee emphasized that, at the actual time of the injection, the vaccine must have the full content of viable strain 19 organisms and that these must be in an undissociated state.

3.2.1.2 *Other vaccines.* No reference was made to "M" vaccine in the first report, and published evidence on the efficacy of this vaccine for the control of bovine brucellosis is still insufficient to make a definite judgment. The evidence available, however, would appear to show no superiority of "M" vaccine over strain 19 vaccine. Small-scale experiments are still in progress with ether-killed vaccines with adjuvants; no conclusions can be drawn as yet. The danger of using living vaccines of high or unknown virulence was again stressed because these vaccines may be a method of spreading infection and of severely retarding progress in brucellosis control.

3.3 Brucellosis in Goats and Sheep [3.6]

It has been strikingly demonstrated in the past few years in areas of France and Yugoslavia that virtual eradication of brucellosis in goats and sheep is possible and practicable in highly infected areas by blood tests and the elimination of positive reactors. It is noteworthy that human infection in these areas has been correspondingly reduced. The committee therefore urged that similar campaigns be undertaken in other highly infected territories.

3.3.1 *Diagnostic methods* [3.6.4]

From the evidence now available, and until experiments now in course are completed, the committee recommended that the sero-agglutination test should be interpreted on a herd basis. If one animal in a herd shows a blood titre of one-tenth of the titre obtained when the method and antigen concerned are used to test the International Standard Anti-*Brucella abortus* Serum (or a higher titre), the animal should be regarded as infected; and all other animals in the herd which show any titre whatever should also be considered as infected.

The isolation of *Brucella* from an animal in the herd is, of course, definite evidence of infection. The finding of low titres in a herd should be regarded with great suspicion.

It should be noted, however, that animals proved infected by the isolation of *Brucella* may be negative to a single blood test. Therefore, once infection

has been shown to exist in a herd, repeated tests every three to six months are necessary until infection is eliminated.

The application of the stained antigen test to goats' milk for the detection of brucellosis appears to give promising results and affords the possibility of an easy and practical epidemiological weapon in the control of this disease. It is too early to state the exact significance of the stained antigen test, but it is hoped that this can be done in the near future. Sufficient work has been done, however, to make provisional recommendations for carrying out this test ; these are given in Annex 2, page 27. Modifications of this suggested procedure will undoubtedly be introduced in the future in the light of new knowledge which should be forthcoming shortly. The committee strongly recommended that more work be carried out on this test.

The stained antigen test as applied to sheep's milk cannot be recommended as yet with equal confidence. Preliminary tests done with sheep's milk have revealed certain discrepancies in results, and the committee recommended that the application of this test to sheep should be held in abeyance pending further work in this field.

3.3.2 Vaccination [3.6.3]

No vaccines for use in goats or sheep can be recommended at this time. The committee has examined the results of a few vaccines used on experimental laboratory animals, and these give hope for progress. The objections to the use of living vaccines of unknown virulence in cattle apply equally to sheep and goats.

A large-scale controlled experiment using vaccines in sheep and goats has been formulated, and the committee recommended that FAO and WHO should give all possible assistance in carrying out this work. Results cannot be expected for at least two years. If the results are favourable, it would then take several years before various countries could apply a vaccine in the field. The committee urged, therefore, that national authorities should utilize the recommended procedures of test and segregation or, preferably, elimination, in combination with sanitary measures to reduce the severe problem of brucellosis in sheep and goats.

3.4 Porcine Brucellosis [3.7]

Porcine brucellosis caused by *Br. suis* is an important disease economically and is also responsible for the infection of human beings. Although *Br. suis* is the type of *Brucella* commonly found in swine, *Br. abortus* and *Br. melitensis* also occur ; and swine so infected may be a source of infection to other animals and to human beings.

The disease in swine due to infection with *Br. suis* may run an acute course. However, in some herds, particularly in small herds, the disease may disappear or cease to be an important problem because some infected animals recover and most are removed for slaughter in the course of normal farming practice. On the other hand, especially in the larger breeding-herds, the infection is apt to persist and may continue as a chronic disease, to appear again as an acute condition in the next generation.

The questions of diagnosis, vaccination, and therapy were considered in the first report. As stated there, the sero-agglutination test should be used on a herd basis [Annex 5]. If any animal in a herd shows a blood titre of one-tenth of the titre obtained when the method and antigen concerned are used to test the International Standard Anti-*Brucella abortus* Serum (or a higher titre), the whole herd should be regarded as an infected unit.

4. STANDARDIZATION AND INTERPRETATION OF THE SERO-AGGLUTINATION TEST [4]

The committee expressed its agreement with the recommendations made at the first session.

It noted that, since the publication of the first report, the WHO Expert Committee on Biological Standardization had considered the establishment of an international standard for anti-*Brucella abortus* serum of similar agglutinin content to the OIE standard.⁷ The committee felt it necessary to state that this International Standard was not intended for routine use as a positive control-serum, but that it should be used only for standardizing the test annually in each country.⁸ Routine standardization of antigens should be achieved by means of a local or national standard serum [4(2)]. In addition to those for cattle [4(3)], recommendations have now been made for humans, goats, and swine.

The committee again emphasized that published papers including data based on brucellosis sero-agglutination tests should always indicate the sensitivity of the test used [4(8)] because a statement of the dilution at which 50% agglutination is obtained, when the International Standard Anti-*Brucella abortus* Serum is tested with a given antigen and method, enables a worker in any other country to know at once the sensitivity of

⁷ The International Standard for Anti-*Brucella abortus* Serum was established at the sixth session of the WHO Expert Committee on Biological Standardization (see *World Hlth Org. techn. Rep. Ser.* 1953, 68).

⁸ See footnote 4 on page 7.

the method concerned and, therefore, the real meaning of any diagnostic or other titre mentioned [Annex 6].

A serum may occasionally display the prozone phenomenon in which the antigen fails to agglutinate in the lower dilutions of serum. This effect occurs infrequently, and the possible error of interpretation can be avoided if a sufficient number of dilutions is used.

Failure to demonstrate agglutination may be associated with "blocking antibodies". From all reports it would appear, however, that agglutination is prevented mostly in the lower dilutions and that the phenomenon is rarely of any practical importance in the detection of active brucellosis. Further research will be necessary to clarify this question.

5. ISOLATION OF BRUCELLA

5.1 Bacteriological Culture [5]

Good techniques in the collection and preparation of specimens, inoculation of media, and injection of laboratory animals are a prerequisite for the successful isolation of *Brucella*. All operations should be conducted aseptically.

The most satisfactory method of isolating *Brucella* is by direct inoculation on appropriate solid media. The use of solid media for primary isolation is recommended because the characteristics of the micro-organism are more likely to be retained essentially unchanged and subsequent study of these characteristics is facilitated. It is recognized, however, that liquid media are more practicable for isolation from blood and other body-fluids because of the small number of *Brucella* usually present in these specimens. It is essential to make transplants from liquid to solid media early in order to detect growth and because dissociation of the organism occurs with continued growth in liquid media (see Annex 3, page 28).

The wide and sometimes indiscriminate use of antibiotics at the first signs of any illness has introduced complicating factors into the isolation of *Brucella* from human beings. The organism sometimes grows less readily from individuals recently treated, or under treatment, with antibiotics.

Although most recoveries are made within 7 to 14 days, cultures should be kept at least 35 days before they are discarded as negative. Exceptionally, *Brucella* has not been detectable until after as long as six weeks. Care should also be taken as regards CO₂ requirements where *Br. abortus* is concerned.

5.1.1 Media

The choice of media will frequently depend on the considerations of cost and availability as well as on suitability. Whereas "Albimi"⁹ and "Trypticase-soy"⁹ media have acquired wide acceptance and are preferred by many workers for the isolation of *Brucella*, in areas where these media are not available, other less expensive and more readily available media can be employed, either in liquid form or after the addition of agar. Liver-infusion, veal-infusion,¹⁰ beef-infusion,¹⁰ and potato-infusion¹⁰ media are all known to be of value for the primary isolation of *Brucella*. Tryptose medium has been widely used, but recent evidence indicates that certain batches are unsatisfactory for the growth of *Brucella*. The liquid media preferred for the isolation of *Brucella* from man are Trypticase-soy and Albimi. If these are not available, the media mentioned above may be used.

Regardless of the media employed, each batch prepared should be pre-tested for its ability to support growth of *Brucella*. Contaminants that grow rapidly or produce a marked change in the pH of the media will usually prevent the growth of *Brucella*. The optimum pH of the medium after sterilization is 6.8.

5.1.2 Methods

Amounts of 75 ml of either culture medium, to which is added 1-2% of sodium citrate, are dispensed in cotton-stoppered culture flasks and sterilized in the autoclave. Five to ten ml of blood, or other body-fluid, is introduced aseptically, and the flask is then incubated at 37°C in a closed jar containing 10% added CO₂. Beginning on the fourth day, and at regular intervals thereafter, subcultures are made to the surface of solid agar media, using a pipette and 0.2 ml of the broth culture. If desired, the subcultures can be duplicated at this stage under aerobic conditions and with added CO₂. The initial culture bottle should be incubated for 35 days before discarding unless *Brucella* is recovered prior to that time.

Acceptable alternative methods are the following:

(a) Castañeda technique. Employing the same media, agar may be layered on one side of the culture bottle prior to the addition of the previously sterilized broth media. Subculture to solid medium is then unnecessary. Instead, the bottle is tipped at intervals to allow the broth to flow over

⁹ Details are available on request to either of the joint secretaries of the committee, at the Food and Agriculture Organization, Viale delle Terme di Caracalla, Rome, Italy, or at the World Health Organization, Palais des Nations, Geneva, Switzerland.

¹⁰ Where these media are used in solid form, about 10% of serum (*Brucella*-negative) should be added.

the exposed agar surface. Developing *Brucella* colonies may be detected growing on the surface of the agar. The prime advantage of this alternative method is that the culture bottle need not be opened until *Brucella* colonies are seen on the agar, thus avoiding the danger of contamination from making transfers and also reducing the hazard to technical personnel. If visible growth of *Brucella* does not occur, subculture to solid medium should be made before discarding the culture as negative.

(b) While cotton-stoppered flasks are preferred for all methods so as to allow adequate oxygenation, a rubber stopper may be employed. In this case, the proper amount of CO₂ is introduced directly into the culture bottle by needle puncture. A small proportion of strains of *Brucella* do not grow under these conditions because of the limitation of oxygen supply.

In making blood cultures from large animals, the same media and techniques are equally satisfactory. However, larger quantities of blood are usually employed, especially with cattle, and the volume of culture medium must be adjusted accordingly.

The isolation of *Brucella* from solid tissue specimens is best accomplished by streaking the specimen directly on the surface of one of the solid agar media mentioned above. The specimen is seared in a flame, sectioned, and the freshly cut surface minced prior to streaking the agar plate; the plates are incubated in an atmosphere containing 10% CO₂. Since *Brucella* grows slowly, all culture plates should be kept and inspected for five to seven days before they are discarded.

5.2 Other Methods

5.2.1 *Animal inoculation*

Guinea-pig inoculation is a valuable method for detecting *Brucella*, although present cultural techniques are superior. Guinea-pig inoculation has particular value when specimens are derived from highly contaminated sources. A penicillin solution (500 units per ml), mixed with the inoculum, is recommended to prevent the effect of some contaminants; inoculations should be made subcutaneously. The animal is killed four to six weeks after inoculation, blood is collected for the sero-agglutination test, macroscopic lesions are recorded, and the spleen is cultured.

5.2.2 *Egg inoculation*

Brucella grows rapidly in the developing chick-embryo. Eggs of 4-7 day's embryonation are inoculated by the yolk sac method. The yolk and liver of the embryo are cultured three days after inoculation. Heavy

multiplication of *Brucella* is obtained even when very few organisms are inoculated. *Brucella* isolated by this method is usually in a dissociated form, but this is not necessarily important where further detailed study of the organism is not contemplated. Although the chicken embryo has been used successfully in isolating *Brucella* from blood, blood clots, and spinal fluid, the method is applicable principally in central laboratories testing many specimens, and in research laboratories.

6. IDENTIFICATION OF SPECIES OF BRUCELLA [5]

6.1 Type Strains

In accordance with the recommendations made at the first session, type strains were exchanged between certain laboratories, and reports on these were considered by the committee.

It was decided that the following strains should be designated as type strains: *Br. abortus* 544 (Weybridge), *Br. melitensis* 16 M (Beltsville), and *Br. suis* 1330 (Minneapolis). Further, it was decided that the Weybridge Centre should be requested to prepare a sufficient batch of dried samples of each type strain and to distribute these to the other brucellosis centres, and to other laboratories requiring them. Emphasis was laid on the importance of maintaining these in the smooth state and of ensuring that the dried cultures were such as to give rise to a preponderance of smooth colonies when they are grown under proper conditions.

6.2 Differentiation : Recommended Methods

The committee also considered reports on identification methods from a number of centres with, in some cases, protocols regarding the identification of large numbers of strains isolated in various places and from various animal species. It was noted that most strains, when freshly isolated, had all the characteristics, biochemical and serological, of one or another of the recognized *Brucella* species. A number of strains with other combinations of characters have been reported, e.g., strains which had all the biochemical characters of *Br. abortus* but the serological characters of *Br. melitensis* (first described by the Montpellier workers, and since found in Great Britain, India, and Israel), or which had the biochemical characters of *Br. melitensis* but the serological characteristics of *Br. abortus* (reported from southern France, Italy, and Tunisia). These differences rest primarily on serological tests with monospecific sera.

There are also strains which differ in some minor respects from the accepted characteristics, e.g., some strains, which were otherwise typical of *Br. melitensis*, produced considerable quantities of H₂S for several days, while 60% of nearly 600 strains of *Br. abortus* isolated in France were reported to have grown in primary culture without added CO₂.

The committee emphasized the fact that dissociated strains may give aberrant results and that, therefore, the colony characters of all aberrant strains should be carefully examined.

It was decided that it was desirable that biochemical methods should be used in all centres, and serological methods where possible. Laboratories which do not use serological methods should send a small number of representative strains to a brucellosis centre where serological differentiation is made. It was recommended that aberrant strains of any kind should be exchanged with other brucellosis centres.

The committee compared the methods used in different laboratories for the various differential tests; one method for each test is detailed below. It was recommended that these selected methods should be used in all laboratories so that results are easily comparable, but that any laboratory which prefers another existing method, or which develops a new method, should use it as an additional method and exchange information and strains with other laboratories, and, at an appropriate stage, circulate a paper to all brucellosis centres.

Cultures which are isolated and suspected of being *Brucella* should be confirmed by the following tests: typical colonial appearance, a motility test, the Gram stain, and agglutination in a known anti-*Brucella* serum. A non-motile Gram-negative organism having the typical colonial appearance of *Brucella*, and agglutinating to titre in a known anti-*Brucella* serum but not in normal serum, is presumably *Brucella*.

All strains should be typed as to species. Unfortunately, all the tests available for differentiating the species of *Brucella* are quantitative, and not qualitative, in nature. None of these tests is perfectly dependable by itself. All the available tests should be employed in determining the species to which a strain may belong. The following tests are employed in species differentiation.

6.2.1 CO₂ requirement

The requirement of additional CO₂ by freshly isolated *Br. abortus* is the most constant and dependable of these tests, but after subculture the organism may lose its CO₂ requirement. Hence, it is important that this test be performed either on initial isolation or as soon after isolation as possible. In the latter case, a 48-hour culture is employed. Growth is

suspended in broth or saline, a loopful is transferred to agar medium, and the culture is incubated in an atmosphere in which 10% of the air has been replaced by CO₂. A control, likewise inoculated, is incubated in a normal atmosphere and a known CO₂-requiring *Br. abortus* used for comparison. (It should be borne in mind that it is impossible to obtain this concentration of CO₂ by the use of a candle burning in a closed container. Although this method may yield enough CO₂ to allow growth of many strains of *Br. abortus*, there are other strains which will not grow.)

6.2.2 *Production of hydrogen sulfide*

Production, or lack of production, of hydrogen sulfide depends on the quantity and types of sulfur-bearing compounds in the medium. Hence, it is important that a standard medium and method be employed. Liver agar and potato agar are considered the best media for this purpose. The test should be done on slanted tubes of media. Forty-eight-hour cultures are employed, and a heavy inoculum is spread over the surface of the slant with a loop. Lead-acetate paper is prepared by soaking absorbent filter-paper in 10% neutral lead-acetate solution. The filter-paper is then dried and cut into strips. A strip of lead-acetate paper is inserted in the mouth of each test-tube and held in place by the cotton stopper (the paper is not moistened). Cultures are incubated at 37°C and inspected daily for blackening of the lead-acetate paper. If any degree of blackening occurs, the strip is replaced daily with a fresh strip. In general, *Br. melitensis* does not produce blackening or, if so, only a trace; *Br. abortus* produces moderate blackening for two days or longer; *Br. suis* commonly produces blackening for four or five days, except for the Danish variety which does not produce H₂S.

6.2.3 *Inhibition by dyes*

A very valuable test is the determination of the ability of *Brucella* to grow in the presence of certain dyes, the most useful being basic fuchsin and thionin. Various media may be used, e.g., liver agar, Albimi agar, Trypticase-soy agar, serum dextrose agar, or potato agar. It is essential, however, that the proper dye concentration be determined for the particular medium used. If, when employing the batches of dye furnished by WHO (basic fuchsin, Certificate No. NF58; thionin, Certificate No. NT16), only one concentration of each dye is used, the final dilutions likely to be satisfactory are: in Trypticase-soy or Albimi media, a final dilution of 1/80,000 of either dye; in liver agar, 1/50,000; in potato agar, 1/25,000. There is considerable strain variation with regard to the ability to grow in the presence of these dyes. In instances where a culture is not typed satisfactorily by the use of the above concentrations, a retest (using a fresh culture from a medium containing no dye) should be done, employing

two or three dilutions of the dye to determine on which it may grow best. *Control tests using known cultures should always be done simultaneously with the unknown.* While stock solutions (1% in distilled water) of the dyes are relatively stable and may be stored for long periods, dilutions employed in making the media should be freshly prepared each time media is made and added subsequent to sterilization of the media. Freshly prepared media should be used. The surface should not contain excess moisture (incubate at 37°C for 2-3 hours).

When using batches of dye other than those mentioned above, the concentrations for trial should be calculated on the basis of the dye content of the sample.

The test is done on Petri plates divided into segments. Using a 48-hour culture grown on solid medium without dye, a 2-mm loopful of culture is suspended in 0.1 ml of saline; and a loopful of this suspension is streaked over the surface of the agar. The plates are incubated at 37°C for 72 hours or longer. Cultures requiring CO₂ should be incubated in an atmosphere containing 10% CO₂. All other cultures should be incubated in a normal atmosphere.

Br. abortus should grow only on the fuchsin medium, *Br. suis* only on the thionin, and *Br. melitensis* on both media.

6.2.4 Urease production

A semi-quantitative determination of urease activity of *Brucella* cultures is of some, but limited, value in species differentiation. A recommended method is that of Bauer. The substrate consists of 5% urea dissolved in M/8 NaH₂PO₄ adjusted to pH4 (using 10% HCl) and containing 0.0015% phenol red as an indicator. Using a 48-hour culture grown on a solid medium, a loopful (2 mm) of organisms is suspended in 1 ml of the substrate dispensed in test-tubes. The tubes are incubated at 37°C in a water-bath and readings taken at the end of 15 minutes, 30 minutes, 1 hour, and thereafter, hourly, until a positive test (pink or red) is obtained. In general, *Br. suis* yields a positive reaction immediately or within 15 to 30 minutes; most *Br. abortus* strains require two hours or longer. With *Br. melitensis*, some strains act like *Br. suis* and others like *Br. abortus*. Thus, the test has limited value, being of greatest aid in distinguishing between *Br. abortus* and *Br. suis*. Even here it often yields equivocal results. It is, however, of some help when correlated with the results of the other tests.

6.2.5 Preparation of monospecific sera

Monospecific sera as an aid in typing *Brucella* are of value if satisfactory sera are available. However, it is not easy to prepare such sera, and many workers have never obtained satisfactory results. Unfortunately, there is

no empirical rule that can be stated which, if followed, will guarantee a satisfactory product. In the preparation of each lot, a research procedure must be followed.

Cultures used in the production of sera, as well as for absorption, should be in the smooth phase. Active infection is produced in rabbits by the injection of approximately 100 million organisms from a 48-hour culture into an ear vein. Prior to use, an agglutination test should be performed on each rabbit to eliminate the possibility of prior infection with *Brucella*. The animals are bled at intervals of a few days; and, as soon as a titre of 1/640 or higher is obtained, they are bled from the carotid artery. An adequate titre is usually obtained within a week with a single injection, but a second injection may be given if necessary. The serum is separated from the clot and 0.5% phenol added. Small aliquots of each serum are taken and absorbed with the heterologous organism, using 48-hour cultures. The growth is suspended in 0.85% saline and the cells recovered by centrifugation. Measured amounts of packed cells are added to the serum aliquots, suspended, and the mixtures incubated at 37°C for two hours. The absorbing cells are removed by centrifugation and the sera tested for ability to agglutinate both *Br. abortus* and *Br. melitensis*. If satisfactory differentiation (a 4-tube difference in a doubling series) is not obtained, the absorbing process is repeated one or more times. From the results of this experiment, the proper amount of absorbing cells and the number of absorptions necessary are determined; and this procedure is then performed using the entire lot of each serum.

Br. melitensis and *Br. abortus* are employed. The necessity of employing smooth cultures is re-emphasized; for, if partially dissociated cultures are used, satisfactory sera cannot be obtained. The use of such improperly prepared sera leads only to confusion. After preparation, the sera should be tested on a number of wholly smooth cultures of all three species and, unless they perform as expected, should be discarded.

7. FAO/WHO BRUCELLOSIS CENTRES AND FUTURE RESEARCH

The committee noted with satisfaction the valuable work carried out during the past two years by these centres and co-ordinated by FAO and WHO (see Annex 4, page 29). The centres have made important contributions in research, in the unification of field and laboratory procedures, and in the stimulation of brucellosis control in their regions. The committee strongly recommended, therefore, that FAO and WHO should continue to give support to these centres.

In the present report several recommendations have been made for future research on important unsolved problems in brucellosis. A list of major problems is given below, and it is hoped that the FAO/WHO brucellosis centres, and other brucellosis laboratories, will undertake work on these questions and will communicate the results obtained to FAO and WHO.

(1) Vaccine for sheep and goats. The development of an effective vaccine for use in these animals would be of great benefit (section 3.3).

(2) Stained antigen tests for milk. Further studies on the use of these tests in the control of bovine brucellosis are advisable. The application of this test to sheep- and goat-milk would be a very useful weapon in the detection and control of bovine and caprine brucellosis (section 3.1.1).

(3) Therapy in human beings. Further studies are required (section 2.3).

(4) *Brucella* in milk products. The persistence of *Brucella* in milk products made with unheated milk should be investigated with reference to local procedures of manufacture (section 1).

(5) Isolation, identification, and differentiation of *Brucella*. Refinement of procedures and clarification of present problems are desirable (section 6).

(6) Vaccines in cattle (section 3.2.1).

(7) Porcine brucellosis. Improved diagnostic procedures are required. Surveys on the prevalence of this disease should be undertaken (section 3.4).

Annex 1

WHEY AGGLUTINATION TEST

The whey agglutination test can be conducted by either the tube or the plate method. Dilutions employed for testing whey are the same as those employed for testing blood serum. The whey-antigen mixture is incubated for 48 hours at 37°C before reading the reactions.

Udder secretion should not be employed for testing during the early and late stages of lactation because of the high percentage of false-positive reactions occurring at these times. Whey is obtained by adding several drops of a commercially prepared solution of rennin to 6.0 ml of milk. The milk and rennin mixture is incubated for 12 hours at 37°C. This procedure results in the separation of milk solids from the liquid and fat. Whey can be clarified by centrifugation or the addition of carbon tetrachloride or chloroform.

The whey agglutination test has been of considerable value in identifying cattle with localized *Brucella* infection of the udder and shows promise of differentiating the blood serum agglutinin titres in cattle produced by strain 19 and virulent strains of *Br. abortus*.

Traum & Maderious¹ reported that 85 of 107 cattle showing agglutinin titres of 1/25 or higher in the whey from one or more quarters also excreted virulent *Brucella* in the milk. Furthermore, *Brucella* was isolated from the milk of 92.8% of the cattle with whey titres of 1/100, whereas *Brucella* was isolated from the milk of only 4 of 235 cattle in which no agglutinins were demonstrated in the 1/25 dilution of whey. All these cattle had been vaccinated as calves or adults. No whey agglutination tests were conducted until three months after vaccination.

Blake & Manthei² reported the results of the whey agglutination test on 978 samples of milk collected from 66 cattle over a period of 18 months. Complete histories were available on all animals. Whey agglutination tests were negative on 100% of the milk samples from non-infected, non-exposed cattle, and negative on 98.7% of the milk samples from non-infected, exposed cattle. The whey titres did not exceed 1/50 in the remaining 1.3% of the milk samples from the latter group. Only 1.1% of the milk samples from calf-vaccinated cattle showed a whey titre of 1/25 or higher,

¹ Traum, J. & Maderious, W. E. (1947) *Amer. J. vet. Res.* 8, 244

² Blake, G. E. & Manthei, C. A. (1952) *Proceedings book: American Veterinary Medical Association. Eighty-ninth meeting, Atlantic City ... 1952, Chicago, Ill., p. 98*

whereas 10.3% of the milk samples from adult-vaccinated cattle showed a whey titre of 1/25 or higher. This 10.3% represented the results from 2 of 17 cows in this group. No *Brucella* recoveries were made from the milk of vaccinated cattle. In the group of infected cattle that were excreting *Brucella* organisms in the milk, 79.5% of their milk samples showed whey titres of 1/100 or higher, and 15.3% showed titres of 1/25 or 1/50. Although the 5.2% of the milk samples with negative whey-titres were from only 5 of the 18 infected cattle, none of these animals were consistently negative to the whey test.

Annex 2

STAINED ANTIGEN TEST FOR GOATS' MILK

At the present time this test should be used only on a herd basis, i.e., mixed milk from several animals should be employed rather than the milk of individual goats. In preliminary experiments the test is apparently sensitive enough to detect the milk of a positive animal mixed with nine negative milk-samples. A positive test indicates herd infection, and the interpretation of low blood serum agglutination titres (see section 3.3, page 14) should then be applied to the herd.

From results obtained thus far on individual animals, the test appears to give approximately 20% false-positive reactions when correlated with the blood-serum test (negative to blood sero-agglutination test but positive to the milk test). With definitely infected animals (high titres obtained with the sero-agglutination test), the results of the milk test agree to the extent of approximately 94-97%.

Two stained test antigens have been tried—namely, haematoxylin and triphenyl tetrazolium chloride. The latter antigen appears to give slightly more distinct reactions and is slightly more sensitive.

As regards the application of the test to sheeps' milk, there is, as yet, insufficient data available to make any definite statement, although preliminary observations indicate that this test, or modifications of it, may be useful in the future.

Performance of the Test

Milk is obtained from the goats as aseptically as possible in sterile tubes. The milk is mixed thoroughly immediately before performing the test and then pipetted into tubes in 2-ml amounts. Two drops of stained antigen are added to each sample, and the tube is shaken thoroughly and

placed in a hot-air incubator or water-bath at 37°C. Readings of the test are made after 6 to 12 hours' incubation. (Note: This is an important difference from the stained antigen test as used with cows' milk.) The test is interpreted on the basis of three factors: (a) clearing of the milk column; (b) presence or absence of ring formation; and (c) agglutination of the antigen in the bottom of the tube. As will be noted in the table below, (b) or (c) may be present or absent in positive tests. Agglutination in the bottom of the tube presents a picture similar to that seen in the haemagglutination test commonly used in virus techniques, i.e., the antigen is dispersed evenly in a thin, agglutinated layer extending along the curved bottom of the tube. Partial sedimentation of non-agglutinated antigen occurs in the form of a solid button occupying only the centre of the curved bottom.

Table I represents the recording and interpretations of reactions.

TABLE I. TEST READINGS

Gradation of the test	Appearance in the tube		Agglutination
	clearing	ring	
++++ } +++ } positive ++ } + } doubtful - } negative	complete almost complete distinct slight absent	++++, +++ ++, +, or - - -	may be absent ++++, ++ ++ + -

Annex 3

DISSOCIATION OF BRUCELLA

All *Brucella* cultures have a tendency to undergo dissociation which is manifested by changes in cellular and colonial morphology with accompanying changes in infectivity, agglutinability, antigenicity, degree of inhibition by dyes, and other characteristics. Therefore, it is extremely important that smooth cultures be employed for the production of antigens and when performing typing procedures. Unfortunately, there is no simple method for maintaining *Brucella* in the smooth phase. Smooth cultures can be maintained only by constant and critical examination of the cultures and by the selection of smooth colonies for transfer at frequent intervals. All persons working with *Brucella*, and especially those preparing

antigens, must become competent in the recognition of smooth cultures in order to avoid costly and confusing errors.

The detection of the various phases of *Brucella* is accomplished best by the examination of the colonial form through a broad-field binocular microscope, using 45°-angle incident reflected light. The heat and acriflavine tests are not adequate to differentiate the smooth forms from all others. Considerable training and study is required for dependable recognition of the smooth phase and its distinction from the various dissociants, particularly the smooth-intermediate, intermediate, and mucoid forms. However, owing to the importance of the problem and the real necessity of employing smooth cultures in nearly all phases of *Brucella* work, it is strongly urged that such competence be developed in the FAO/WHO brucellosis centres, all laboratories producing antigens for the agglutination tests or strain 19 vaccine, and brucellosis research laboratories in general.

Since the tendency to dissociate is less when cultures are grown on solid media than when grown in liquid media, solid media should be used wherever possible (moisture of condensation should be avoided). Cultures should be incubated for no longer than four days (preferably less) and then kept in the refrigerator; standing at room temperature is detrimental.

Annex 4

WORK OF THE FAO/WHO BRUCELLOSIS CENTRES

In 1950 and 1951 twelve FAO/WHO brucellosis centres were established for the purpose of forwarding work on an international basis in the field of brucellosis. It is hoped shortly to establish an additional centre at the Indian Veterinary Research Institute, Mukteswar-Kuman, U.P.

These centres function for local and international brucellosis work, as well as for their usual routine duties. They are used: (a) for the preparation and testing of antigens, vaccines, and other biological products; (b) as diagnostic centres; (c) to stimulate studies of brucellosis prevalence in man and animals; (d) to forward programmes of brucellosis control in animals; (e) for research on special problems; and (f) as teaching and information centres for their own and nearby countries.

Information documents on brucellosis are prepared by WHO and FAO and are distributed periodically to the centres. Thus, through the exchange of information and correspondence among the members of the Joint FAO/WHO Expert Panel on Brucellosis and among the centres, the latest

advances in research are communicated to leading brucellosis workers throughout the world.

Highly gratifying results have already been achieved through the activities of these centres. One notable example is the virtual eradication of *Br. melitensis* infection in north-west Yugoslavia, where brucellosis was formerly a serious problem. Of great importance has been the progress in the adoption of unified laboratory procedures, especially in the diagnosis of brucellosis, so that results reported in one laboratory have bases of reference elsewhere. This approach is being extended to include the production of vaccines and other biological products. Where difficulties or divergent results have been experienced by one centre, it has been possible to enlist the aid of a second centre for checking purposes. Thus, centres concentrating on a particular problem are used to assist other centres unable to cope with a specific question.

The work on brucellosis accomplished in co-operation with these centres and other brucellosis laboratories has been published from time to time in brucellosis documents circulated by WHO and FAO and obtainable, upon request, from these organizations. An indication of the activities of these centres apart from routine diagnostic work is given below.

Argentina

Division of Brucellosis and Tuberculosis
Department of Zoonoses
Ministry of Agriculture and Animal Husbandry
Buenos Aires
Dr. B. L. Moran, Chief

Local surveys of the prevalence of caprine brucellosis in conjunction with epidemiological surveys in man ; the use of the ring test for detecting bovine and caprine brucellosis ; study of the M vaccine (Huddleson) for the prevention of caprine brucellosis ; popular educational campaigns ; studies on the agglutination test with reference to standardized procedures, the effect of strain 19 vaccination, and infection with foot-and-mouth disease.

Australia

Commonwealth Serum Laboratories
Parkville N. 2
Victoria
Dr. F. G. Morgan, Director

Production of strain 19 vaccine ; standardization of biological products and related problems ; research on immunity and human diagnosis.

Denmark

State Veterinary Serum Laboratory
Bülowsvej 27
Copenhagen

Dr. A. Thomsen, Chief, Brucellosis Department

The development of tetrazolium-chloride ring-test antigens; production of strain 19 vaccine; studies of sero-agglutination test antigens for standardization purposes; training of brucellosis workers from other countries.

France

Centre de Recherches sur la Fièvre ondulante
Institut Bouisson-Bertrand
Montpellier (Hérault)
Professeur L. Carrère, Directeur

in collaboration with

Clinique des Maladies infectieuses
Clinique Pasteur
Montpellier (Hérault)
Professeur M. Janbon

Studies on blocking antibodies in the sero-agglutination test; differentiation of *Brucella* types; field trials of vaccines in sheep; diagnosis in sheep and goats utilizing skin tests and ring tests on milk; diagnostic and therapeutic studies in human beings; training of brucellosis workers from other countries.

Greece

Veterinary Microbiological Institute
Botanikos
Athens
Dr. C. Melanides, Director

in collaboration with

Institute and Museum of Hygiene
University of Athens
Ambelokipi 6
Athens
Professor G. P. Alivisatos, Director

Epidemiological studies on the prevalence of brucellosis in man and animals in different parts of the country; the adaptation and use of the

ring test for sheep and goats ; comparative studies on antigens for sero-agglutination and ring tests ; preparation of strain 19 vaccine.

Italy

Centre for the Study of Brucellosis
Institute of Hygiene and Microbiology
University of Florence
Viale G. B. Morgagni, 48
Florence
Professor G. Mazzetti, Director

in collaboration with

School of Veterinary Medicine
University of Pisa

Virulence, toxicity, and antigenicity of *Brucella* strains ; diagnostic studies in sheep, including ring test and agglutinin-blocking antibodies ; local surveys of prevalence in man and animals ; persistence of *Brucella* in cheese ; diagnosis and therapy in man.

Mexico

Medical Research Institute
General Hospital
Mexico, D.F.
Dr. M. Ruiz Castañeda, Director

Skin test antigens for allergic tests in man ; spot test (agglutination) for rapid diagnosis ; agglutinin titres in active brucellosis ; studies on human therapy.

Turkey

State Veterinary Institute
Etlik, Ankara
Dr. R. Durusan, Chief, Brucellosis Department

in collaboration with

“ Refik Saydam ” Central Institute of Hygiene
Ankara
Dr. S. Bilal Golem, Secretary-General

Local surveys of brucellosis prevalence in sheep, goats, and cattle ; preparation of strain 19 vaccine ; preparation of antigens for skin test,

agglutination test, and vaccine therapy in humans; studies on cross-agglutination reactions in humans vaccinated against cholera.

Union of South Africa

Onderstepoort Veterinary Laboratory
Onderstepoort

Preparation of antigens and strain 19 vaccine; studies on milk ring test; diagnostic centre.

United Kingdom of Great Britain and Northern Ireland

Ministry of Agriculture and Fisheries
Veterinary Laboratory
New Haw
Weybridge
Surrey, England
Dr. A. W. Stableforth, Director

Preparation of International Standard Anti-*Brucella abortus* Serum and monovalent specific typing sera; standardized antigen and sero-agglutination test; preparation of strain 19; studies on *Brucella*-species typing; ring test in vaccinated animals; training of brucellosis workers from other countries.

United States of America

Department of Medicine
University of Minnesota
Minneapolis, Minn.

Dr. W. W. Spink, Director, Brucellosis Laboratory

in collaboration with

School of Veterinary Medicine
University of Minnesota
St. Paul, Minn.

Dr. M. Roepke

Studies on therapy in man; studies on the biology of *Brucella*; studies of skin test, ring test, and sero-agglutination test antigens; *Brucella*-species typing; immunological studies of cattle sera; application of ring test in the eradication of bovine brucellosis.

Yugoslavia

Brucellosis Centre
State Laboratory of Hygiene
Rijeka

Dr. V. Rukavina, Chief, Brucellosis Department

Studies of ring test in sheeps' milk as compared with sero-agglutination and skin test reactions ; local surveys of prevalence in man, sheep, cattle, and swine ; eradication campaign in sheep ; studies on therapy in man.

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