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JOINT FAO/WHO EXPERT PANEL ON BRUCELLOSIS

Report on the First Session

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JOINT FAO/WHO EXPERT PANEL ON BRUCELLOSIS

First Session

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JOINT FAO/WHO EXPERT PANEL ON BRUCELLOSIS

Report on the First Session [1]

Early in 1950, FAO and WHO, upon request of their member countries, undertook a joint programme for combating brucellosis. A Joint FAO/WHO Expert Panel on Brucellosis was formed, and 12 FAO/WHO brucellosis centres were designated in various countries to forward work in this field.[2]

The Third Inter-American Congress on Brucellosis was held in Washington, D.C., USA, in November 1950. In view of the presence of many members of the Joint FAO/WHO Expert Panel on Brucellosis at the Congress, meetings of the panel were convened in Washington from 6 to 13 November, during and after the Congress.

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[1] The WHO Executive Board, at its seventh session, adopted the following resolution:

The Executive Board

1. NOTES the report of the Joint FAO/WHO Expert Panel on Brucellosis on its first session;

2. THANKS the panel for its work;

3. AUTHORIZES the publication of the report;

4. RECOMMENDS that the activities of the FAO/WHO brucellosis centres be developed, where possible, in research and training and in the co-ordination of field and laboratory procedures used in brucellosis;

5. REQUESTS the Director-General to report on these centres to the next session of the Executive Board;

6. NOTES the recommendation that a worldwide survey should be made under the aegis of FAO and WHO on the prevalence of brucellosis in livestock and the measures being taken in various countries to combat this disease;

7. RECOMMENDS that, where possible, this survey should be carried out through the FAO/WHO brucellosis centres;

8. DRAWS the attention of the competent authorities to the advisability of systematic reporting of incidence of brucellosis in man and animals, and

9. REQUESTS the Director-General to give assistance, in collaboration with other specialized agencies and international organizations, wherever possible, to governments in controlling and eradicating brucellosis in countries where this disease is prevalent.

(Resolution EB7.R81, *Off. Rec. World Hlth Org.* **32**)

[2] See Annex 1, page 23.

Introduction

Brucellosis has proved to be one of the most difficult disease problems. A great amount of research still needs to be done for a better understanding and solution of some phases of the disease. It is felt by the panel, however, that there is now sufficient knowledge and practical experience to undertake satisfactory programmes of diagnosis, control, and eradication in the various areas of the world.

The important aspects of brucellosis as a world problem are essentially twofold :

(1) the public-health significance ; and

(2) the economic loss to animal industry.

The public-health significance of brucellosis includes not only the direct or indirect transmission of the disease from infected animals to man, and the consequent illness, physical incapacity, and loss of manpower, but also the serious diminution of needed foodstuffs, especially animal protein, which are essential to human health and well-being. Since brucellosis is not generally transmitted from person to person, the prevention of human infection depends upon the control and elimination of this disease in animals.

In many areas of the world brucellosis contributes largely to a low standard of living. The economic losses attributable to brucellosis in animals are caused by abortion or premature birth, decreased milk flow, and temporary or permanent infertility in infected livestock. Where a vigorous campaign against brucellosis in animals has been carried out, great savings to the national economy have resulted. In the USA, for example, it is reliably estimated that the reduction of the incidence of bovine brucellosis by one half has resulted in a saving of 50 million US dollars annually to the livestock industry. In Norway, where brucellosis of cattle has been almost completely eradicated, the cost of the entire programme was less than the estimated annual loss formerly caused by this disease.

Although *Brucella abortus* commonly infects cattle, *Br. suis*, swine, and *Br. melitensis*, goats, infections with any of the three species of *Brucella* may occur in all domestic animals and in man.

The reporting of animal and human brucellosis is of major importance as a basis for the control of the disease. Accurate reporting and statistics enable proper evaluation of the disease problem, which will provide the justification for necessary research and control procedures. Statistics also enable governments to measure the progress of their control efforts. In order to develop a good reporting system based on statistical methods,

properly trained personnel are necessary for the collection and interpretation of data. Education of the public and of the medical and veterinary professions, together with legislation, are necessary to inaugurate such a system. The panel recommends, therefore, the enactment by all governments of legislation to make brucellosis in man and animals a notifiable disease. Governments collecting data on brucellosis should exchange information on reported brucellosis with FAO, OIE (International Office of Epizootics), and WHO, and should distribute this information to the interested agencies within their countries.

1. Routes of Transmission from Animals to Man

There are many animals which can serve as sources of infection for man, among which the most important are cattle, swine, goats, and sheep. The routes of transmission of brucellosis from animals to man are ingestion, contact, inhalation, and inoculation.

Infection by ingestion may occur by the gastro-intestinal route, and by penetration of the mucous membrane of the oral cavity and throat. All food products made from raw milk originating in infected herds are potentially dangerous. Viable brucella may be present in the viscera, flesh, and lymph-nodes of infected carcasses for periods in excess of one month. It has been shown that the organism can survive pickling but not commercial smoking. Human cases of disease have on occasion been traced to meats and meat products which were not properly cooked. Wild greens used in salad, contaminated with sheep and goat urine and faeces, have been found to produce human infection. The contamination of water-supplies, such as cisterns and wells, by animal excreta has also been reported as a source of the disease.

Contact with infected materials is an important cause of brucellosis. In some countries it has been reported that 60%-70% of cases are the result of contact with infected materials, such as the aborted foetus, placenta, urine, manure, carcasses, and salvaged animals, and in the laboratory. Entry of the organism is through the skin, mucous membranes, and eyes. Infection by contact is common among veterinarians, farmers, rendering-plant workers, packing-house employees, and animal handlers.

Airborne infection occurs when dust- or droplet-borne organisms come into contact with the mucous membranes of the eye, respiratory tract, or digestive tract. Infection by this method can be presumed possible wherever dried animal substances or excreta are found. Dust from the wool of sheep, from railroad cars and trucks which have transported infected animals, and in brucella laboratories have all been reported as being contaminated, in addition to samples obtained in and around farm premises.

Infection by accidental inoculation has been recorded among veterinarians and laboratory workers. Laboratory workers should take special precautions because brucellosis is one of the most readily acquired of laboratory infections.

Prevention of animal-to-man transmission can be achieved in many instances by the heat treatment of food products which are ingested by man, especially the effective heat-treatment of milk and milk products. There is insufficient evidence to recommend the optimal duration of the ageing period of cheese that will destroy brucella. Further investigation of naturally infected cheese is recommended.

It is recommended that the carcasses of animals known to be infected be handled with special care and supervision. Investigations should be continued on the handling and utilization of such carcasses.

Personal hygiene and environmental sanitation are prerequisites for the prevention of any disease. Personal hygiene should be scrupulously observed by farmers, animal handlers, and workers. Washing of exposed body-surfaces and the removal and cleaning of soiled clothing and footwear is essential. Environmental sanitation requires that the surroundings be kept free from any contaminated substances and dirt which may shelter bacteria. This is very important in preventing airborne infection.

2. Brucellosis in Human Beings

2.1 *Clinical criteria*

By " brucellosis " is meant infection of man or animals with any of the three common species of *Brucella : Br. melitensis*, *Br. abortus*, and *Br. suis*.

Clinical manifestations in man vary, in general, according to the invading species, although in individual cases such distinctions may not be valid.

Although in the majority of cases the interval between infection and the onset of clinical manifestations does not exceed four weeks, these clinical manifestations may be delayed up to many months after infection.

The disease may take many forms, of which the following are the most common :

(1) an acute fever of limited duration followed by apparent recovery ;

(2) long continued disease with periodic exacerbations ;

(3) an insidious onset, followed by long continued disease ;

(4) a course in which no history of an acute stage can be obtained and in which multiple and varied symptoms are observed.

Brucella infection may persist in the body without causing clinical manifestations.

There are no criteria, with the exception of culture of the organism, which will determine with absolute certainty the existence of brucella infection, nor are there any infallible criteria for the absence of infection.

2.2 *Diagnostic criteria*

2.2.1 *Culture* (see also section 5). If suitable methods are employed, brucella can often be isolated from various sources, especially the blood, bone-marrow, lymph-nodes, urine, abscesses, placenta, and vaginal discharge. Repeated attempts at culture should be made in all cases.

2.2.2 *Agglutination* (see also section 4). 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.

2.2.3 *Complement-fixation test.* The complement-fixation test has no practical value at present, but it deserves further investigation from both basic research and clinical aspects.

2.2.4 *Opsonocytophagic (bacteriotropic) test.* The opsonocytophagic test is not suitable for routine diagnostic use, but further basic and clinical studies should be carried out on it.

2.2.5 *Intradermal test*

(*a*) Regardless of the antigen or technique employed, the results of an intradermal test should be interpreted as determining a specific allergic condition of the individual, and should be regarded as free from other diagnostic significance.

(*b*) In view of the evidence of wide variations in the results obtained with the intradermal test according to the antigens employed, the methods of using them, and the interpretation of the results, the panel recommends strongly that a planned investigation be carried out using the various antigens available in a number of different countries in which different conditions prevail, in order to establish the basic conditions governing this test and the uses to which the test may be put.

(*c*) Indiscriminate use of allergenic agents in intradermal tests for epidemiological purposes should be discouraged because of possible interference with subsequent serological tests.

2.2.6 *Other laboratory tests.*[3] In view of the urgent need for more satisfactory criteria of infection, the panel recommends that laboratory tests, including Castañeda's spot test, agglutinin-blocking antibodies, surface fixation, smooth-selecting (SS) factor, bactericidins, and protective antibodies in sera should be further investigated and, where necessary, that funds should be made available for this purpose.

2.3 *Therapy*

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

2.3.2 *Penicillin.* The use of penicillin for the treatment of brucella infection is unjustified. Penicillin may be indicated where simultaneous bacterial infection due to penicillin-susceptible organisms is encountered.

2.3.3 *Streptomycin*

(*a*) The use of streptomycin or dihydrostreptomycin is of value in combination with a suitable sulfonamide, such as sulfadiazine.

(*b*) Streptomycin, or preferably dihydrostreptomycin, is also of value in combination with other antibiotics, such as aureomycin and terramycin, with or without the addition of a sulfonamide. A suggested dosage schedule (for adults) is 1-2 g daily of streptomycin or dihydrostreptomycin for 14 to 21 days, provided no toxic signs or symptoms develop. Simultaneous administration of aureomycin or terramycin is advised in a dose of 2 g daily for 14 to 21 days. There is some evidence that the addition of a suitable sulfonamide enhances the therapeutic effect if given in a dose of 3 g daily for 14 to 21 days. The panel recognizes the variability in dosage schedules now being used in the chemotherapy of brucellosis, and anticipates revisions in the foregoing recommendations as further information is obtained.

(*c*) Caution should be employed when using streptomycin or dihydrostreptomycin in the treatment of brucella infection on account of the damage to the eighth nerve which may be produced.

2.3.4 *Aureomycin.* Aureomycin is of value when used alone in the treatment of active brucella infection. A suggested schedule is 2-4 g daily for 14 to 21 days. Relapses may be similarly treated.

2.3.5 *Chloramphenicol.* Some experimental and clinical evidence indicates that chloramphenicol is of value in the treatment of brucellosis.

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[3] For references, see Annex 2, page 25.

2.3.6 *Terramycin.* Terramycin used alone appears comparable in efficacy to aureomycin.

2.3.7 *Antibiotics in general*

(*a*) 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. In relapses, similar treatment may be undertaken to that outlined above.

(*b*) The use of antibiotics in conjunction with other forms of therapy should be further investigated.

2.3.8 *The sulfonamide compounds.* The sulfonamide compounds alone are of only limited value in the treatment of brucella infections.

2.3.9 *Other chemotherapeutic agents.* No other chemotherapeutic agents have yet proved to be of value in the treatment of the disease.

2.3.10 *Vaccines.* There is no general agreement as to the place to which brucella vaccine therapy is entitled in the treatment of this disease. In this connexion, the panel recommends that:

(1) where the appropriate antibiotics are available, they should be employed in the first instance;

(2) where the appropriate antibiotics are not available, vaccine therapy should be considered;

(3) in using vaccine therapy, only well-established and safe methods should be employed;

(4) investigations should be carried out to establish the utility of brucella vaccines and the ways in which they should be employed, special attention being paid to their effects in proved cases of the disease, particularly in those cases in which the use of antibiotics has not resulted in a complete cure.

3. Brucellosis in Animals

3.1 *General*

The control and eventual eradication of brucellosis in animals is dependent upon two general principles: the prevention of exposure of animals to infection, and the increase of resistance of the susceptible animal. In order to accomplish these objectives, three broad methods may be utilized:

(1) elimination of infected animals based on diagnostic tests;

(2) vaccination;

(3) a combination of (1) and (2).

In all these methods sanitation and hygiene are essential. The terms hygiene and sanitation as employed in this report denote the physical methods and management by which the environment is freed and maintained free from infectious materials. These procedures as applied to the control of brucellosis include the removal of the aborted foetus, placenta, excreta, and contaminated materials (hay, straw, dirt, etc.) which may provide a harbourage for brucella organisms. The isolation of the infected animal at parturition and for ten days thereafter, or until the vaginal discharge ceases, is also an important hygienic measure. In addition, the management of the male animals, especially those used in artificial inseminations, should be supervised, and infected animals should be removed. Infectious materials should be disposed of or disinfected in such a manner as to prevent the contamination of water supplies and courses, pastures, farm premises, and other physical objects. Recommended methods of disposal are burial or burning.

Regional differences in incidence of infection and economic and educational status require that the methods of choice be specifically adapted to countries, areas, or herds.

The feasibility of eradicating the disease in countries, areas, or herds solely by eliminating reactors has been demonstrated. Similarly, a highly significant reduction of disease incidence by vaccination alone has been proven, but the advantages and limitations of vaccination procedures must be carefully weighed before this method is employed (see also sections 3.3 and 3.4). Regardless of the method of control employed, the ultimate goal should be elimination of the disease.

In herds and areas showing a high incidence of acute disease, vaccination is the method of choice, but it should not be considered as a permanent solution. In herds and areas with relatively few positive reactors (under 10%), method (1) above (test and elimination) or method (3) (combination of test and elimination, and vaccination) should be employed, according to circumstances in the herd or area (see section 3.5).

3.2 *Diagnostic methods*

3.2.1 *Sero-agglutination tests* (see also section 4). The tube sero-agglutination test is considered to be the most reliable diagnostic method for the detection of infection in individual animals. The rapid or plate test, if properly adjusted to the tube test and carried out with due care, may be employed as a substitute in field work where the use of the tube agglutination test is impractical.

3.2.2 *Complement-fixation test*. The results of the complement-fixation test are somewhat comparable to those of the agglutination test, but since

the former generally does not furnish more accurate diagnostic information and is more cumbersome to perform than the latter, the agglutination test is preferable as a routine or standard diagnostic blood test. The complement-fixation test is subject to greater technical errors.

3.2.3 *Ring or ABR test (Abortus Bang Ringprobe).*[4] The recently developed ring or ABR milk and cream test is a valuable presumptive or screening test to locate infected herds. It is also useful as an adjunct to the sero-agglutination test for the control of the disease in clean herds and lightly infected areas (under 10% of the herds reacting). In areas where it is impracticable to carry out sero-agglutination tests, the ABR test may be used to determine the approximate extent of infection.

It is suggested that this test may be of some use as a presumptive test in epidemiological studies for tracing sources of human disease.

3.2.4 *Other diagnostic tests.* Other diagnostic procedures, such as blood cultures, complement-fixation tests on placental material, bacteriological and biological examination of suspected materials, and microscopical examination, preferably by use of the Køster stain or one of its modifications,[5] are recommended where feasible. Further research on other diagnostic tests is desirable.

3.3 *Vaccines*

An ideal vaccine for the prevention of brucellosis in animals :

(1) confers adequate protection ;

(2) is safe, i.e., is dead or relatively avirulent and shows no tendency to increase in virulence in the animal body ;

(3) causes a minimum of interference with the sero-agglutination test ; and

(4) is easy to produce, preserve, and distribute.

Many attenuated vaccines have been produced and tested in various countries. Of these, strain 19 vaccine has proved to be the most valuable from the standpoints of safety, adequacy of protection, and practicability of production.

Living vaccines of high or unknown virulence have been used in the past, but they may be the means of spreading infection and should not be used.

Dead vaccines in saline solution are of no value. Dead vaccines in oily excipients have been shown to confer considerable protection, but they

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[4] See Annex 3, page 26.

[5] See Annex 4, page 27.

are costly to produce and are inferior to strain 19 in the protection induced against heavy infection. Ether-killed vaccines with adjuvants and some brucella extracts have given promising results in experimental animals, but further work is needed before an assessment of their value can be made.

Although, to date, strain 19 most nearly satisfies the criteria for an ideal vaccine, continued research on this subject is desirable.

The use of vaccine should be limited to uninfected animals.

3.4 *Strain 19 vaccination in cattle*

Although the value of strain 19 vaccine is generally recognized, it is important that certain factors regarding the product and its use be considered if maximum benefits are to be obtained.

The protection induced by strain 19 vaccination is relative and not absolute. The resistance-inducing properties of strain 19 are directly dependent on the colony type and the viability of constituent cells. The protection afforded by strain 19 vaccination of adult cattle is considered comparable to that obtained in calves vaccinated at recommended ages.[6] However, because of the recognized disadvantages of vaccinating mature cattle, this practice should not be employed except in emergency conditions where preferred measures cannot be adopted. Persistent blood agglutinin reactions are usually observed in vaccinated adult animals, and an occasional abortion may be caused by vaccination of pregnant animals. There is not sufficient evidence to indicate whether the vaccinal resistance in cattle of comparable ages is influenced by the state of pregnancy.

There are a number of factors encountered under field conditions that will modify the duration and serviceability of vaccinal resistance. Included among these are :

(1) degree of exposure ;

(2) virulence of the infecting strain ; and

(3) the immunity response of the individual animal to vaccination with strain 19.

Because any one of these will modify the resistance level, it is impossible to forecast the limits of vaccinal resistance under varying conditions existing in different herds. Data available from a limited number of controlled vaccination experiments indicate that the resistance induced

[6] Calves are usually vaccinated when they are between 6 and 8 months of age. Vaccination after the 8th or 9th month often results in prolonged positive reaction to the sero-agglutination test, while vaccination before the 4th or 5th month may result in insufficient immunity.

in calves by vaccination with potent strain 19 vaccine will provide serviceable protection against moderate exposures to *Br. abortus* for a period of at least two to three years.

Because many bulls are prone to retain vaccinal agglutinin titres for indefinite periods, it is considered inadvisable to vaccinate these animals as a routine practice.

Vaccination of suspect or reactor animals with strain 19 has no measurable effect on the normal course of bovine brucellosis.

There is no evidence that strain 19 is spread from vaccinated animals to unvaccinated animals.

Controlled studies have failed to support field reports concerning the relationship between vaccination and the occurrence of either temporary or permanent infertility.

Strain 19 vaccination must not be accepted as a substitute for good sanitation and herd management. Its maximum value is realized only when these principles are rigidly observed.

Transient excretion of strain 19 in the milk occasionally occurs, but no human cases have been traced to this origin ; on the other hand, human infections have been reported from accidental inoculation of, or heavy laboratory exposure to, this vaccine and great care should therefore be exercised by those handling it.

3.4.1 *Production and control of strain 19 vaccine.* To ensure maximum benefits from strain 19 vaccination, certain precautions in the production and handling of the vaccine must be observed to avoid the occurrence of undesirable changes in the final product. Otherwise variants of low immunogenic potency may arise. It is of primary importance that cultures used for production purposes be carefully selected from the standpoint of desirable characteristics.

Standardized methods for selective subculturing, vaccine production, and control have been established and must be followed.[7]

Preliminary studies indicate that desiccated strain 19 vaccine is less perishable than the liquid product and may be preferable for use under adverse climatic or field conditions. However, the vaccine in either form must be handled with care to avoid deterioration.

It is recommended that one central laboratory distribute seed cultures to regional centres which will in turn be responsible for the distribution of proper subcultures to the vaccine producers.

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[7] Details of these methods are available on request to the Joint Secretary, Joint FAO/WHO Expert Panel on Brucellosis, World Health Organization, Palais des Nations, Geneva, Switzerland.

3.5 *Bovine brucellosis*

Since no successful treatment of bovine brucellosis is known, all control measures must centre around prevention of infection of brucellosis-free cattle.

Conditions existing in the different countries of the world vary so much that the universal use of any one technique or procedure for solving the brucellosis problem is impossible. The panel realizes that it will be very difficult to inaugurate active programmes in some areas and countries; but the panel believes very strongly that such control measures as can be put into practice should be adopted immediately. The complete elimination of brucellosis from herds, cattle, and countries should be the final goal.

As a step to achieve this goal the panel recommends that a worldwide survey be made under the aegis of FAO and WHO to determine:

(1) The present prevalence of brucellosis in large animals. This should include blood or ring tests from representative areas in countries which do not have satisfactory statistics available.

(2) What laws, rules, and regulations for the control and prevention of brucellosis are in existence.

(3) The programmes of control and prevention which are under way, and the results which are being obtained.

Following step (1), the different countries which do not now have active programmes for control and prevention are urged to initiate such programmes as soon as practicable. The panel suggests the following steps should be taken in countries in the different categories listed below:

(1) Countries with inadequate veterinary services and inadequate laws, rules, and regulations:

(*a*) The enactment of satisfactory laws.

(*b*) The appointment of livestock sanitary personnel to enforce such laws. Such personnel should be professionally trained persons with permanent status.

(*c*) The immediate activation of control and preventive measures. In areas of low infection these should include:

(i) Rigorous control of imports. In so far as is possible, it is preferable to restrict importations to unbred heifers tested for brucellosis and coming from brucella-free herds. If animals of breeding age are imported, they should originate from brucella-free herds, and these should be tested for brucellosis, both in the country of origin and upon arrival in the importing country.

(ii) Restriction of movement of cattle of breeding age. If the incidence of brucellosis is high, vaccination of heifer calves should be practised in infected herds and areas.

Note : Vaccination in these recommendations refers to the use of strain 19 vaccine.

(iii) Inauguration of an educational programme to reach all segments of the population, and to include the training of veterinarians and technicians.

Note : It should be clearly borne in mind that the final aim in countries in this category is elimination of brucellosis. When adequate veterinary personnel becomes available, such countries automatically fall into the category described below.

(2) Countries with adequate veterinary personnel and livestock sanitary laws. Under any conditions in this category, the programme should include :

(*a*) the control of distribution of vaccine

(*b*) the permanent identification of reactors

(*c*) the control of movement of reactor and untested cattle.

Control and elimination of brucellosis may be approached by any one of the procedures given below, the choice being determined by the conditions encountered :

(1) The following type of programme is usually preferable in areas and countries with a low prevalence of infection (10% or less of the herds infected, and 3% or less of the individual animals infected).

(*a*) Test with elimination of reactors, with or without vaccination of heifer calves in infected herds ;

(*b*) In some herds under special conditions, test and temporary retention of reactors, with vaccination of heifer calves and possibly of mature negative animals. Any reactors not immediately eliminated should be permanently identified and kept apart, whenever possible, from other animals.

(2) The following type of programme is usually preferable in areas with a moderate to high prevalence of brucellosis (10%-35% of the herds infected, and 3%-10% of the individual animals infected).

(*a*) Test with elimination of reactors, with or without vaccination of heifer calves, in herds with a low incidence of infection.

(*b*) Test with either elimination or retention of reactors, with vaccination of heifer calves and, possibly, of adult negative animals in acutely infected herds, problem herds, and herds with a high incidence of infection. Any reactors not immediately eliminated should

be permanently identified and kept apart, whenever possible, from other animals.

(*c*) Vaccination of heifer calves in negative herds which are so situated or so managed that exposure of such animals is likely to occur.

(3) In areas or countries where the prevalence of brucellosis is high (above 35% of the herds infected, and more than 10% of the individual animals infected), and where there is some shortage of personnel, the vaccination of heifer calves without testing can be employed.

3.6 *Brucellosis in goats and sheep*

3.6.1 *Goats.* Infected goats are a dangerous source of brucellosis, especially since the disease in these animals assumes a chronic and often asymptomatic form. It is recommended that wherever possible goats reacting to the sero-agglutination or intradermic tests be slaughtered. If radical elimination of infected animals is impossible, every effort should be made to evolve sanitary and isolation measures, including destruction of infected corrals, disinfection of all potentially infectious material, and segregation of infected animals and herds.

3.6.2 *Sheep.* Brucellosis in sheep, in distinction to the disease in goats, is usually self-limiting. Thus, the slaughter of infected sheep is not as essential as the slaughter of infected goats, and the segregation of reacting sheep can be used for control purposes with more confidence.

Note: Where infection of sheep and goats has been introduced into an area for the first time, total slaughter of all infected and in-contact animals is strongly recommended.

3.6.3 *Vaccines.* Strain 19 vaccine has been shown to be of no value in sheep and goats, but the panel notes the encouraging results obtained recently with other vaccines in these animals and strongly urges further research on this problem, so that economically underdeveloped countries, where caprine and bovine brucellosis are important problems, may obtain a valuable weapon for control purposes.

3.6.4 *Diagnosis*

(*a*) *Sero-agglutination test.* Even low sero-agglutination titres are of diagnostic significance, especially in herds where infection is known to exist. Research, however, is needed on the titre levels indicative of infection. Study is also needed to determine the possible importance of cross-reactions due to Q fever, although it should be kept in mind that both infections may exist concurrently.

(*b*) *Intradermal or allergic tests.* Positive reactions to skin tests indicate either present or past infection. A comparative study of the various antigens now being employed for intradermic tests in sheep and goats would be useful.

(*c*) *Ring test (ABR)*. The possible adaptation of this test to the milk of sheep and goats should be investigated.

(*d*) *Other diagnostic methods*—see subsection 3.2.4.

3.7 *Brucellosis in swine*

Brucellosis in swine, caused by *Br. suis*, is usually a self-limiting disease; this is especially true in females. However, this does not diminish the importance of the economic losses and public-health hazards caused by this disease. Since all three species of *Brucella* have been isolated from swine, these animals are a source of infection for both man and domestic animals.

3.7.1 *Diagnosis*

(*a*) The sero-agglutination test is generally accepted as being effective in determining the presence or absence of brucellosis in the herd, but has its limitations in detecting the brucellosis status of individuals. In the interpretation of the test, it is considered necessary to recognize low-titre agglutinin reactions.

(*b*) Intradermal or allergic tests, at present, have more limitations than the sero-agglutination test. Further research to improve methods of diagnosis of brucellosis in swine is recommended.

3.7.2 *Vaccination and therapy*. Vaccination and therapy have not proved satisfactory. Thus, the control of swine brucellosis is dependent on segregation and eventual elimination of infected animals. The most satisfactory procedure has been complete herd disposal and replacement of the infected parent herd with brucella-free progeny.[8]

4. Standardization and Interpretation of the Sero-Agglutination Test

The panel, recognizing the wide variation which exists in the method and antigens used for the diagnosis of brucellosis with the tube sero-agglutination test in man and animals, and the fact that steps to unify the test have been taken by FAO, OIE, PASB (Pan American Sanitary Bureau : WHO Regional Office for the Americas), and WHO, recommends that international uniformity should be secured through the bodies mentioned.

In this connexion, the panel further recommends that uniformity be sought through the following steps :

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[8] See Annex 5, page 28.

(1) Correlation should be achieved by adoption of the OIE standard dried serum, and by periodic distribution to national laboratories of this serum and such other sera as may be decided upon from time to time for check test purposes.

(2) Each country should arrange for the designation of a central laboratory to be made nationally responsible for unification and standardization of materials and methods used within its own territory. The importance of using completely smooth cultures for antigen production is emphasized.

(3) In the diagnosis of bovine brucellosis, the minimum positive titre should be between 1/10 and 1/12 of the titre obtained when the OIE standard serum is tested with the antigen and by the methods of the country concerned. (It is already known that in most countries the test is now in alignment with these criteria.)

(4) The titre considered as indefinite (suspect or doubtful) should be approximately one-half of the minimum positive titre.

(5) The titre for diagnosis of brucellosis in lower animals other than cattle, not having yet been agreed upon, should be the subject of further study.

(6) On the basis of (2) and (3) above, workers in brucellosis in man should exchange information in order to establish the minimum titre for human beings indicative of a high probability of infection.

(7) Materials and methods for the plate or rapid agglutination test should be adjusted to give results in accordance with those of the tube test.

(8) Published papers including data based on brucellosis sero-agglutination tests should always indicate the sensitivity of the test used by stating the dilution at which 50% agglutination is obtained when the antigen and methods employed are used to test the OIE standard dried serum.[9] This information would normally be supplied by the laboratory producing the antigen.

5. Bacteriological Culture and Typing

The ideal method of isolation of brucella cultures from infected hosts should permit the recovery of a small number of cells and the subsequent multiplication of these cells without changes in characteristics. Cultivation on solid media most nearly meets these requirements and should be employed wherever feasible. Thus, isolations from milk, vaginal discharges, many body-fluids, and all solid tissues can and should be made directly

[9] See Annex 6, page 33.

on solid media. With respect to blood and certain body-fluids, initial culturing in liquid media is usually employed.

For such isolation the following media are considered satisfactory : [10]

(1) *Liquid*

(*a*) Trypticase-soy

(*b*) Tryptose

(*c*) " Albimi "

(2) *Solid*

The above, plus agar; also serum agar, liver-infusion agar, potato-infusion agar, cooked-blood agar, etc. It is essential that the efficiency of every batch of medium should be pre-tested.

Blood samples may be cultured in the above liquid media, but the method of isolation from blood used at present is that described by Castañeda. Liquid cultures should be transferred to solid agar media when not using the Castañeda method. All subcultures should be maintained on solid media or be preserved by freeze-drying. For special investigations on isolated strains, subcultures should be forwarded to regional laboratories. In this instance care must be taken to avoid prolonged culturing in liquid media and storage at unfavourable temperatures. When solid tissues, milk, or certain body-fluids are cultured on solid media, these media should contain crystal violet or other appropriate bacteriostatic agents if there is danger of contaminants. Other methods of isolation, including egg culture, semi-solid media, animal inoculation, etc., are often useful. The limitations of the present methods of isolation are recognized, primarily in respect of growth of small inocula and potential variation of cells during culturing in liquids, and additional research on methods of isolation should be encouraged.

It is suggested that the final identification of all isolated strains be concentrated in regional laboratories. The following tests are recommended :

(1) biochemical tests (CO_2 requirement, H_2S production, urease activity, etc.) ;

(2) bacteriostatic tests (Huddleson's dye tests) ;

(3) serological identification and classification, including detection of non-smooth variants.

Simultaneous use of all these tests, and inclusion of controls with standard strains, is urged. The desirability of using standard media and

[10] Details are available on request to the Joint Secretary, Joint FAO/WHO Expert Panel on Brucellosis, World Health Organization, Palais des Nations, Geneva, Switzerland.

standard techniques for all these tests should be recognized. In this connexion, it is recommended that specific instructions, standard lots of dyes, and standard comparative strains of each species be established by one selected laboratory and be supplied to all approved testing laboratories.

Virulence titration in laboratory animals may also be useful.

In the case of isolation of atypical strains, it is recommended that such strains and all pertinent information be submitted to additional laboratories for confirmation.

6. Role of International Agencies in Brucellosis Work

The panel notes the establishment of 12 FAO/WHO brucellosis centres in various countries throughout the world, and strongly recommends the use of these centres for the furtherance of activities in the control of brucellosis in man and animals. The panel recommends an expansion of the present activities of the centres, particularly in the spheres of research and training, and as regional centres for the unification of field and laboratory procedures. The co-ordination of the activities of these centres through FAO and WHO will provide an appreciable contribution to the advancement of brucellosis work throughout the world.

The panel would like to stress the important service rendered by FAO and WHO, in co-operation with OIE, on brucellosis research, the distribution of stock cultures and standard sera, and in the provision of technical advice to requesting countries.

The panel recommends the increased use of these agencies for the regular reporting of incidence of brucellosis in man and animals, and in the provision of technical assistance where needed.

Annex 1

JOINT FAO/WHO BRUCELLOSIS CENTRES

Veterinary Microbiological Institute
Botanikos
Athens
Greece
Dr. Costas Melanides, Director

State Veterinary Serum Laboratory
Bülowsvej 27
Copenhagen
Denmark
Dr. A. Thomsen, Chief, Brucellosis Department

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

Centre de Recherches de la Fièvre ondulante
Institut Bouisson-Bertrand
Rue de l'Ecole-de-Médecine
Montpellier
France
Professeur L. Carrère, Directeur

Onderstepoort Veterinary Laboratory
Onderstepoort
Union of South Africa
Director of Veterinary Services

Brucellosis Centre
State Laboratory of Hygiene
Rijeka
Yugoslavia
Dr. J. Srakocic, Chief, Brucellosis Department

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

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

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

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Department of Medicine and School of Veterinary Medicine
Minneapolis
Minn., USA
Dr. W. W. Spink

Medical Research Institute
General Hospital
Mexico
Dr. M. Ruiz Castañeda

State Veterinary Institute
Etlik, Ankara
Turkey
Dr. M. Z. Muslu, Director

Annex 2

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Annex 3

RING OR ABR TEST

The ring or ABR test is an agglutination test and depends on the presence of brucella agglutinins in the milk from infected cattle. The antigen used is stained so that the agglutination reaction may be observed in the presence of milk or cream. In milk from infected cows the antigen agglutinates and collects on the fat droplets which carry the antigen to the top to form a cream ring or line having the colour of the stained antigen. In negative milk the stained antigen remains in the skim-milk fraction and the cream ring which forms is white.

The ABR test is so sensitive that in a high percentage of cases a positive test is obtained even though the milk from one infected animal is mixed with that from five to ten negative cows. This marked sensitivity permits the use of the test on mixed-herd milk or cream from each can of milk or cream on the receiving line in a dairy plant or creamery. Thus, in conjunction with the sero-agglutination test, the ring test serves as a simple, rapid, and inexpensive means of locating infected herds.

A single ring test used on an area basis is positive in 70%-80% of the infected herds tested. Approximately two-thirds of the failures to detect infected herds are due to the fact that the infected animals (usually dry cows and heifers) are not in production at the time of the test. This natural limitation of the test is obviated by employing the test at regular intervals of three to six months.

In the USA, approximately 30% of the herds positive to the ABR test were found to be negative to the sero-agglutination test. The cause for these discrepancies between the two tests is still ill-defined. Some of the discrepancies are apparently due to mastitis. Some are believed to be due to milk obtained too early after parturition or late in lactation. In some instances the ABR tests were positive before the sero-agglutination test became positive. Because of its marked sensitivity, when used on a herd, the ABR test tends to remain positive for a period of time after removal of animals positive to the sero-agglutination test. These discrepancies occur in only a very low percentage of the total number of herds and, therefore, do not seriously interfere with the use of the ABR test as a valuable adjunct to the sero-agglutination test.

Details concerning the preparation of ABR antigens and the performance of the test can be obtained on request to the Joint Secretary, Joint FAO/WHO Expert Panel on Brucellosis, World Health Organization, Palais des Nations, Geneva, Switzerland.

Annex 4

MODIFIED KØSTER STAIN

This stain is used for the examination of placental scrapings. In work carried out at the State Veterinary Serum Laboratory in Copenhagen, it was found that the number of positive identifications of brucellosis from placental examinations increased by 10% when the modified Køster stain was compared with the usual carbol-methylene-blue method of staining.[1] The brucella bacilli are stained red in strong contrast to the blue- or violet-stained surroundings.

Of the usual bacilli found in placental smears, only *Brucella* and *Mycobacterium tuberculosis* are stained red.

Staining is performed as follows :

(1) Dry and fix the smear over a flame.

(2) Stain for about 1 minute with a mixture of 2 parts of saturated safranine solution and 5 parts of normal KOH solution. The safranine and KOH solution are mixed just before their use.

(3) Wash under tap water.

(4) Flood with 0.1% H_2SO_4 solution for 10-20 seconds.

(5) Wash thoroughly.

(6) Counterstain with ordinary carbol-methylene-blue solution for 2-3 seconds.

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[1] *Skand. vet. Tidskr.* 1941, **31**, 599

Annex 5

CONTROL OF BRUCELLOSIS IN SWINE

General Recommendations

Unfortunately, proposals for the control of swine brucellosis cannot be substantiated by the vast experience which accompanies proposals for the control of bovine brucellosis. Official recommendations, rules, or regulations for the control of swine brucellosis have not, for the most part, been tried on an area or nationwide basis. In fact, livestock sanitarians, veterinarians, and owners have been prone to ignore the effects of swine brucellosis. Interest in and demand for the control of this disease of swine has only recently been forthcoming. For the most part it has not been recognized that brucellosis in man may be transmitted from swine as well as from infected cows. Human brucellosis caused by *Br. suis* is now known to be one of the serious occupational hazards inherent in those occupations where direct contact between man and swine is inevitable. This disease is just now being recognized in official quarters as an occupational, compensatable disease.

The diagnosis of swine brucellosis is based on the sero-agglutination test, similar to the diagnosis of brucellosis in cattle. As regards swine, it is generally accepted that the test is effective in determining the presence or absence of brucellosis in the herd, but the test has limitations in detecting the brucellosis status of individual animals. In other words, there are some swine from which brucella may be isolated that do not react to the blood test. Thus it becomes necessary to use the agglutination test as a herd diagnostic procedure and to base any attempts for control on entire herds or units rather than on individual swine.

Another factor in diagnosis is the interpretation of the agglutination test. It is now generally considered necessary to conduct the test routinely in serum dilutions of 1/25, 1/50, 1/100 and above.[1] In the interpretation of the test results it is apparent that judgment must be used, since in nearly any sizeable herd of swine low-titre reactions occur without the presence of infection ; while, also, these same low-titre reactions occur in herds where infection is present. Thus, as a practical rule, sero-agglutination reactions at the 1/50 dilution or less are considered negative unless there are definite reactors at the 1/100 dilution or higher in the herd. Here,

[1] These titres refer to agglutination tests utilizing US Bureau of Animal Industry antigen.

again, caution should be exercised in the purchase of individual swine which exhibit a negative or low-titre agglutination response unless the status of the entire herd of origin is known.

Management factors contributing to control difficulties are numerous. The large numbers of swine in a herd, their prolificacy, the community boar, the widespread use of the sale barn, and breeding for two litters a year all have an effect on the control of brucellosis.

In areas where slaughter-house identification of swine is possible, routine sampling of swine blood may be conducted in an attempt to determine foci of infection and thereby to provide a means of control.

Specific Recommendations

Prevention

The most important preventive measure is to prohibit the introduction of infected swine into a brucellosis-free herd. This is best accomplished by purchasing replacements or additions from herds known to be free of brucellosis. If this is not possible, each addition should be tested and no animal showing an agglutination reaction in any degree should be accepted ; replacements from herds of unknown history should be kept in isolation for at least 60 days and re-tested before entry into clean herds is permitted. The practice of assembling a swine herd from many different sources is dangerous. It is safer to purchase fewer animals from one source, if possible, and thus lessen the chances of purchasing an infected hog which does not react to the agglutination test. Herd sires should be purchased well in advance of breeding time in order that a minimum of two blood tests at least 60 days apart can be made on the boar before his use.

Community boars are not conducive to brucellosis control. The practice of lending boars to a neighbouring herd should be prohibited because of the danger of infection being spread both ways.

" Show " swine may spread or contract brucellosis while at fairs and shows. Such swine should be held in isolation upon their return and show a negative test before entering the main herd.

Owners of pure-bred animals should be encouraged to sell breeding-stock only from herds completely free of brucellosis as evidenced by tests of the entire herd. It is known that negative reacting groups of breeding swine from infected herds have been offered for sale. These animals may spread brucellosis although they are negative to the blood test when offered for sale.

Plans of control

Since neither test and immediate slaughter of reactors, nor vaccination, have been satisfactory in the control of swine brucellosis the following two plans of control are presented for consideration :

Plan 1. Sale of entire herd for slaughter

This plan is useful in herds, large or small, where the primary consideration is the production of pork. The plan produces quick results and is easy and economical to undertake. An interval of 3 to 6 months may be necessary to dispose of the entire herd, including feeder pigs, and to clean and disinfect the premises and equipment. Replacement of the infected herd should be from herds free from infection. Contaminated lots should not be used for swine again until a period of at least 90 days has elapsed from the time infected animals were removed. Periodic blood tests should be conducted on the newly purchased herd as a means of detecting infection that might be resident about the premises. Brucellosis is primarily an animal-to-animal contact disease; hence early detection of animals that may become infected from the premises is essential to protect the entire replacement herd.

Plan 2. Test, segregation, and delayed slaughter of infected herd

The details of this plan are:

(1) Perform blood tests on the entire breeding herd.

(2) If infection is present, consider the entire herd as infected rather than remove the positively reacting animals. Manage the herd as a unit.

(3) Raise pigs from this infected unit. Wean and test the pigs when they are eight weeks of age. Isolate the negative pigs on clean premises as far removed as possible from the infected parent herd. Maintain this isolation until the infected parent herd is disposed of.

(4) Test the pigs at 60-day intervals. Immediately before breeding remove all animals that show a reaction of 1/25 or above as they are found. Breed only certified brucellosis-free gilts to non-infected boars.

(5) Dispose of the original infected herd as soon as suitable negative replacements are available, or as soon as it is obvious that the plan is giving satisfactory results.

(6) Premises where the infected herd was kept should be cleaned and disinfected thoroughly and allowed to remain idle for at least 90 days before admission of the clean replacement herd.

This plan provides for the raising of negative pigs from the infected parent breeding stock in such a manner that clean replacements of known

blood lines are available. Ultimate disposal by slaughter of the original infected herd is necessary, but this is delayed until the quality and the disease status of the pigs is known. This plan avoids the necessity of purchasing replacements from unknown sources and also aids the breeder in maintaining desirable blood lines.

Plan 2 has been used under experimental field conditions and has given good results. It is the method of choice for pure-bred herds, or in herds where improved blood lines have been developed, even if the ultimate objective of the owner is pork production rather than the sale of breeding stock.

The time of disposition of the infected herd will depend upon whether the " one-litter " or " two-litter " system is employed. Naturally the " two-litter " system will be more difficult since numbers of swine alone will tend to complicate control. A decrease in numbers of breeding swine is advisable with plan 2. In either the " one- " or " two-litter " system it is necessary to maintain complete and permanent segregation of the infected parent swine from the weaned and tested offspring.

Plan 2 does not necessitate complete cessation of swine production at any time during the operation of the plan, but the chances of success are enhanced if the size of the herd is reduced during the period of segregation.

Suggested plan of agreement between owners and official animal disease control agency

(1) A herd may be designated as certified brucellosis-free when it has passed two successive negative agglutination tests conducted 60 days apart and a final re-test at the end of six months, with no agglutination reactions at 1/100 or over.

(2) A certificate to this effect can be issued to the owner by the official animal disease control agency and shall be valid for a period of six months from date of issue. The certificate is renewable on a subsequent negative test after six months.

(3) Blood samples are to be taken by an accredited and approved veterinarian and shall be submitted for the official agglutination test. The tests shall include all breeding swine or prospective breeding stock.

(4) All animals shall be properly identified in a manner satisfactory to the official animal disease control agency. In the interest of permanency, ear tattoos or notches are recommended in addition to ear tags.

(5) Replacement breeding swine procured directly from certified brucellosis-free herds may be added to the herd without additional tests. All other replacement breeding animals shall be accompanied by a certificate showing they have passed a negative agglutination test, and thereafter shall be isolated from the remainder of the herd until they have passed

a second negative blood agglutination test for brucellosis. Such re-tests are to be made not less than 60 days after arrival on the premises. Bred gilts and sows from non-certified herds should be isolated until they have farrowed and are found to be negative to a post-farrowing agglutination test.

(6) When replacement breeding animals are added to a certified brucellosis-free herd, the official animal disease control agency shall be notified in writing. Such notification shall include the number and class of animals purchased, name of seller, and acceptable identification of the individual animals involved.

(7) Owners shall not allow the use of any biological product for the prevention or treatment of brucellosis in the herd, unless authorized by the official animal disease control agency.

(8) Reacting swine shall not be disposed of for purposes other than immediate slaughter.

(9) In consideration of assistance rendered by the official animal disease control agency, the owner agrees to undertake the eradication of swine brucellosis and to maintain a certified brucellosis-free herd in accordance with one of the foregoing plans.

Annex 6

50 % AGGLUTINATION IN THE STANDARDIZATION AND INTERPRETATION OF THE AGGLUTINATION TEST

In section 4 it is stated that published papers including data based on brucellosis sero-agglutination tests should indicate the sensitivity of the test used, by stating the dilution at which 50% agglutination is obtained when the antigen and methods employed are used to test the OIE standard dried serum.

The 50% agglutination end-point is adopted because it is believed that this is the most suitable end-point for exact assessment. Lower degrees of agglutination are less easily assessed with accuracy, and " complete agglutination " is an unsatisfactory end-point for two reasons : (1) some workers insist on absolute crystal or water clarity (i.e., 100%), while others record degrees of agglutination varying from 75% to 100% as " complete " ; and (2) some antigens clear completely at serum dilutions which are about one-half of that which gives 50% agglutination (i.e., they have a sharp end-point), and others only clear completely at dilutions which are a quarter or an eighth of that at which 50% agglutination occurs. Some never clear completely. Moreover, if several workers are asked to make exact readings of a series of tests, there will be better agreement in regard to the 50% agglutination end-point than above or below it.

For routine testing, it will be regarded by many as more convenient to continue to use their traditional end-point.

For estimation of the sensitivity of a given test, however, it is essential to have an accurately measured end-point and one on which agreement would be reached by workers making observations in different countries ; and for this the 50% end-point is best. It can be determined with considerable accuracy by taking the average of five series of tests with closely spaced dilutions around the expected titre ; e.g., if a preliminary test shows the titre to be between 1/640 and 1/1,280, the dilutions used would be 1/640, 1/800, 1/960, 1/1,120, and 1/1,280.

Opacity standards for reading the degree of clearing (which may be taken as a measure of the degree of agglutination) can be prepared as follows:

Stage 1 (similar for all methods of test)

Tube 1, for	'no agglutination'	undiluted antigen			
,, 2, ,,	25% ,,	3 parts	antigen,	1 part	saline
,, 3, ,,	50% ,,	2 parts	,,	2 parts	,,
,, 4, ,,	75% ,,	1 part	,,	3 parts	,,
,, 5, ,,	100% ,,	saline alone			

Stage 2 (varies according to method)

(*a*) For the method in which antigen is added to an equal volume of serum dilution—add to each tube above, an equal volume of saline [1] or of the appropriate saline dilution of a completely negative serum (i.e., negative at one-tenth to one-sixteenth of the titre regarded as positive).

(*b*) For methods in which antigen is added to small volumes of whole or diluted serum—add to each tube above, saline (or saline dilution of negative serum) equal to the volume of the whole or diluted serum.

(*c*) Make appropriate adjustments for other methods.

For convenience, opacity standards appropriate to the method concerned, prepared as above, are spaced in a rack and the tube to be read is matched between them.

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[1] Saline can be used when the tubes are to be used for reading high dilutions of serum (which have no colour), e.g., when testing the sensitivity of a method by means of the OIE standard dried serum. The appropriate dilution of a proved negative serum is necessary, however, when they are used for comparison with low dilutions of serum, especially if these are deeply coloured.

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