Trends in Brucellosis Control

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BRUCELLOSIS is a world health problem ranking near the top of the ever-lengthening list of infections transmitted from animals to man in its effect on human and animal health and in its economic importance (*1–4*). Unfortunately the extent of its prevalence in large areas of the world is virtually unknown. The number of reported cases in human beings undoubtedly does not represent the number of cases that occur. This infection directly affects large segments of the agricultural population and other exposed groups through prolonged illness and reduced capacity for work. These burdens fall particularly heavily on Latin American and Mediterranean areas where the prevalence of *Brucella melitensis* in goats is high.

Epidemiology

Most of the infections result from direct contact with post birth discharges, fetal membranes, and fetuses from infected animals when they give birth or abort, or from contact during slaughter. A significant number of patients also acquire it through ingestion of dairy products from infected cattle and goats.

The three species of *Brucella* known to infect man—*abortus*, *melitensis* and *suis*—resemble each other so closely that they can be distinguished only by special tests. Probably these three species descended from a common bacterial ancestor and acquired special characteristics on adaptation to a new host. The fairly balanced relationship between *B. abortus* and cattle suggests that this species may have been the first. In general, swine and goats react more strongly to infection by *B. suis* and *B. melitensis*, respectively, than cattle do to *B. abortus*. There may be further support for the idea that *B. abortus* is the oldest species in the conjectured absence, or at least infrequency, of natural *Brucella* infection among wild animals. It does, however, occur in the American bison, the water buffalo, and the European hare. The docile ox was among the first animals to be domesticated by man; swine, sheep, and goats entered his environment later. The reactions of all animals, including man, that acquire *B. melitensis* or *B. suis* infections speak of the more recent appearance of these two species.

In areas where numerous herds of cattle are infected by *B. abortus*, persons who drink unpasteurized milk are exposed to it. Although the tissues of these persons are invaded, clinical signs and symptoms arise in remarkably fewer persons than the number actually infected. In a rural population exposed to bovine brucellosis through consumption of raw milk, the intradermal skin test using brucellergen may yield a high percentage of positive results but very few clinical cases of brucellosis.

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In addition to the relatively low virulence of *B. abortus*, other factors tend to lessen the danger of drinking milk contaminated with that organism. Infected milk may be diluted with large amounts of uninfected milk at the dairy; but more important, children, the consumers of the largest quantities of milk, are naturally resistant to clinical illness after exposure to *Brucella*. These conditions account for the fact that most human brucellosis due to *B. abortus* results from direct contact with farm animals or their carcasses, but they do not minimize the necessity for pasteurization.

The organisms usually enter through intact skin or mucous membrane. Most of the people who contract clinical cases of brucellosis due to *B. abortus* have handled infected cattle, occasionally infected hogs. Hogs in close association with infected cattle in feed lots may acquire *B. abortus*. Recently sheep in California have also been found to be infected with *B. abortus*.

In some States, such as Iowa, where swine breeding is carried on extensively, swine brucellosis constitutes a threat to human welfare. It may be transmitted to man in two ways: by direct contact with infected hogs or by indirect spread to dairy cattle sharing premises with infected hogs. Under the latter circumstance, the potentialities are worth considering because *B. suis*, in general more pathogenic for man than *B. abortus*, may, in rare instances, reach consumers of raw milk. Usually, however, it is sporadic, appearing in persons who suffer individual exposure by direct contact with single infected animals on the farm or in the slaughterhouse.

Brucellosis in goats is widespread and is the chief source of human brucellosis in Mexico, France, Italy, Spain, Yugoslavia, Turkey, Israel, and Egypt. About 30 years ago the goat was the source of a limited number of cases in southwestern United States. The mortality rate ranges from 4 to 11 percent. *B. melitensis* may infect cattle too, and the situation mentioned with regard to *B. suis* may arise—consumers of raw milk may become infected. In the United States, human infection with *B. melitensis* has recently been traced to hogs. In other countries, for example, in France and recently Germany (*5*), sheep have been proved important sources of severe, even fatal, human infections.

The portal of entry of *B. melitensis* is by ingestion, contact, or inhalation of infected dust in the environment of the livestock. Both goats and sheep are the source of dairy products, particularly fresh cheese, and these may be teeming with *Brucella*. If the cheese is sticky, it is likely to remain in the mouth for an appreciable time, thus giving opportunity for the organisms to enter by way of the mucous membrane of the mouth and to bypass the potentially destructive gastric juices. Usually, in human brucellosis of caprine origin, several members of a family are infected simultaneously; in brucellosis of bovine origin, only single cases occur in households.

The soil in goat corrals and stables may be heavily contaminated with *Brucella* that continuously pass in the feces. Being resistant to desiccation, the viable organisms are readily disbursed in the dust stirred up by moving animals. Ingestion of stagnant water from watering places in goat corrals is also a potential source of infection for the goats.

Economic Aspects

Brucellosis, in contrast to many other zoonoses, is responsible not only for widespread illness and misery among human beings, but also for serious economic losses to those who deal in livestock. These losses are of such appalling size in some areas that they are the subject of more concern to many groups than the burdens of disease carried by the people who contract it. In general, the losses consist of (*a*) decrease in milk supply—an average of 22 percent; (*b*) loss in offspring because of destruction of fertility—an average of 40 percent—or abortion; and (*c*) decrease in value of infected cows, goats, sheep, swine, and horses.

According to figures of the National Research Council (*6*), at least 1,300,000 dairy cows and 800,000 beef cattle in the United States had brucellosis, and the resultant financial loss was estimated at $100 million. According to a more recent estimate by the Agriculture Research Service, the annual loss has been reduced to $45 million. The damage caused by *B. abortus* to agriculture in Switzerland during 1 year has

been estimated at between $3 million and $25 million. Unfortunately, similar estimates are not available for other countries.

Bovine brucellosis, wherever it occurs, is the cause of very considerable economic loss to the dairyman, with effects probably greater than he generally has realized. Norway has estimated the cost of eradication at less than one year's economic loss due to the disease.

Equally incomplete are the estimates on the losses sustained as the result of brucellosis in goat and sheep raising areas. In southern France, 15 to 40 percent of the flocks or herds are infected; 10 to 40 percent of the infected goats or sheep abort; and thus, annually 100,000 ewes and 50,000 goat kids are lost (*7*). No one has yet appraised the impact of these losses, in combination with the ravages of disease, on health and well-being in many countries. This negligence is in great part attributable to the lack of recognition by many that this disease is a specific entity because it is so insidious and difficult to diagnose.

Control

In improving the welfare of countries throughout the world, brucellosis must obviously be considered. The World Health Organization and the Food and Agricultural Organization, advised by an expert panel comprised of medical and veterinary experts from all parts of the world, closely collaborating with the International Office of Epizootics and several inter-American congresses, are attacking the problems on a worldwide basis. Human brucellosis can be prevented only if the disease is eliminated from animals. The present goal—complete eradication of bovine brucellosis, rather than adaptation to it—guides the measures taken by these groups.

This is a difficult task, but progress made in the United States, Puerto Rico, and Scandinavian countries (*8*) gives cause for hope. That infection in cows can be recognized with considerable accuracy by a simple test for agglutinins in their serum was proved nearly 50 years ago. To eradicate bovine brucellosis, early efforts were directed toward destruction of reactors in this test, just as they were in the campaign against tuberculosis. Regrettably, the test-and-slaughter type of control, when conscientiously followed, is so costly in some parts of the world that it has not been practical to adopt it on a worldwide basis.

Cooperation of the livestock owner cannot depend on legislation alone. An effective educational program, fortified with accurate information, must leave no doubt in the minds of all concerned that living without brucellosis is a desirable necessity.

Efforts to devise an effective, workable, and acceptable control program brought to light several shortcomings in the original planning. Possibly one of the most troublesome is the nature of the agglutination test itself. It has the advantage of being comparatively inexpensive and may be repeated as often as necessary, but it has some disadvantages. For one, the results may be misleading. The serum from some uninfected cows may cause nonspecific agglutination, or early in the infection agglutinins may not be detectable. It has happened that herds considered to be free from the infection on the basis of the test and subsequent slaughter of the reactors later became infected. This disappointing and sometimes disastrous failure occurred usually when the slaughtered animals had been replaced with stock from herds not included in the testing program. Another shortcoming was lack of proper and comprehensive education of all groups concerned and consequently difficulties in the organization of cooperative efforts between livestock owners and livestock sanitary officials.

At a disappointing stage of the control program, progress took a significant and encouraging turn. Through research by Buck (*9*), Buck and Cotton (*10*), and Traum (*11*), a *B. abortus* strain, strain 19, proved effective in preventing abortion in heifers vaccinated when they were 4 to 8 months old. In experimental studies, heifers vaccinated as calves have maintained, during their first gestation, a serviceable protection against brucellosis when exposed by contact with infected cattle. Strain 19, living but attenuated, provides protection, usually without causing disease, because it is of stabilized virulence and produces a low-grade, temporary infection from which the vaccinated ani-

mals recover completely. There is absolutely no transmission from vaccinated to susceptible animals. Abortion has occurred infrequently, and only when the heifer was vaccinated during pregnancy.

The vaccine prepared with strain 19 suspended in saline proved to be a highly perishable product, subject to deterioration by handling under adverse conditions. Many so-called vaccine failures, doubtless due to vaccination with dead or inactive organisms, led to another series of disappointments. Now a process of lyophilization or dehydration of the organisms from the frozen state under vacuum (*12*) seems to offer more promise. The vaccine so produced, which often unfortunately loses 50 percent of the original viable cells in its preparation, is now the one of choice.

To insure proper supervision of production of the standard vaccine, the California Legislature has empowered the State health department to test biological products and enforce proper transport and storage methods. In California, which has adopted a compulsory vaccination program, and in other States with semicompulsory programs, the overall protection has been remarkably good in most cases. Losses from abortion have been sharply reduced.

The degree of immunity provoked by single or multiple vaccinations has been studied, and in no instance has revaccination enhanced immunity. It is fully recognized that ultimate eradication will be achieved only by using the vaccination of calves as a supplement to blood testing and elimination of the reactors.

Until a thoroughly satisfactory practical field test is devised to distinguish between vaccinal agglutination reactions and reactions due to virulent infection, an animal whose serum reacts at certain levels in this test, whether vaccinated or not, particularly after it attains breeding age, must be considered a dangerous animal. The titer now regarded as positive is 1:200 for vaccinated animals. From the standpoint of the public's health, it is absolutely imperative that these animals, particularly if they are shedding *B. abortus* in milk, be eliminated.

Recent observations on 30 herds, consisting of 2,958 animals in which calfhood vaccination had been practiced for 5 years, revealed 89 shedders. Only 4 herds were entirely free from infection. Since the percentage of reactors, and in particular of shedders, is low, it is reasonable to postulate that the next step is to identify the shedders by means of a simple method. From the standpoint of the beef herd, it is relevant only to remove the shedders from its midst; from the standpoint of spread of the infection, it is essential to take whatever preventive measures are possible, preferably slaughter.

The application of new methods of culturing milk and cream on special media has the advantage that it furnishes health agencies with valuable information about the progress of an eradication program in milk herds. The methods outlined by Hess and Sackmann (*13*) or by Goode and his associates (*14*) should be tried.

The agglutination test was modified to detect *Brucella* agglutinins in milk by Fleischhauer (*15*); the modified test is called the milk or cream ring test, Abortus-Bang-Ring (ABR), or lacto-agglutination test. Its success depends on a suitable antigen prepared from a heavy suspension of *Brucella* stained with hematoxylin. It is carried out by adding antigen to milk in the proportion of 1 drop per 1 ml. After incubation at 37° C. for 1 hour, the sample is centrifuged and read. Agglutinated stained organisms adhere to the fat globules and rise to the surface; the fat containing the stained organisms causes the layer of milk or cream to be purple.

This test has been used extensively in the United States and in Denmark. Three consecutive negative ring tests on a composite herd sample, 4 to 6 months apart, followed by one negative agglutination test on blood from all animals in the herd, are the criteria for freedom from infection. In Grade A herds the three negative ring tests alone are considered adequate criteria. In the United States a number of States have surveyed milk sheds in cooperation with livestock disease control agencies. This test was proved valuable as a field test, particularly in detecting *Brucella*-positive herds (*14*).

In an extensive, long-range, thorough study of udder infections, Goode, Amerault, and

Manthei (*14*) used culture of milk to determine the infection status of vaccinated and unvaccinated herds, and by this means were able to evaluate the shedder status, seroagglutination test, and the relationship of these to vaccination with strain 19.

There is no need to emphasize again that the final determination of the infection status, of either an individual or herd, cannot be made safely on the basis of the ring test or culture of milk alone. These procedures have certain inherent limitations, especially when applied to vaccinated herds, but they are exceedingly valuable when used as supplements to the blood test. When proper use is made of all these procedures, each in its proper place, together with prompt identification and removal or segregation of diseased animals and with application of sound sanitary practice, satisfactory progress towards eradication can be expected to continue.

When undue weight is given to a negative ring test, when blood test reactions shown by vaccinated animals are ignored or unduly discounted, or when seroreactors, untested animals, or seronegative animals from herds of unknown origin are permitted to move in the channel of trade other than to immediate slaughter, progress towards eradication is impeded. It is, of course, wasteful to introduce infection-free animals into infected herds, as experience in Latin America has regrettably shown.

Certainly the interstate regulations pertaining to brucellosis within the United States, as recommended in 1953 (*16*), might very well be applied to the regulation of importation of cattle into the Latin American countries.

Caprine Brucellosis

Observations made by Dr. G. Renoux, acting director at the Pasteur Institute in Tunis, described by him during a visit in May 1954, may serve to introduce a discussion of control of caprine brucellosis.

A program is being carried out there with the help of grants from FAO and WHO. Susceptible goats, imported into Tunis from Sweden, were artificially infected by the conjunctival route, and the LD_{50} infective dose for this animal was determined. The mean LD_{50} infective dose of the most pathogenic strain, *B. melitensis* 53H38 of Mexican origin, was 20,000 organisms (range: 8,300–48,000). Probably the goats that received small infective doses recovered spontaneously. Mutton breeds of sheep were resistant; the LD_{50} was 400,000 *B. melitensis* of the same strain. This does not necessarily apply to milking sheep. Age, sex, or pregnancy had no influence on the susceptibility of goats. Kids were susceptible to infection, and some remained infected for many weeks. Subclinically infected goats that acquired the infection as kids doubtlessly play a part in the epidemiology of caprine brucellosis.

With the aid of the inhibiting medium developed by Kuzdas and Morse (*17*), *B. melitensis* was readily isolated from the feces of heavily infected goats, and from the vagina, quite independent of pregnancy and parturition. Milk of nonparturient goats contained *B. melitensis* as often as did the milk from dams. The centrifuge deposits from the milk, more often than cream, contained *B. melitensis*; evidence of *B. abortus* is more often found in the cream.

The rapid plate or test tube agglutination test remained consistently negative in a number of infected goats excreting *Brucella*. The agglutination titer varied widely from test to test in the same goat. The prozone phenomenon occurred frequently in goat serum. Blocking antibodies appeared to be specific. They were found in more than 90 percent of negative serums from infected goats. The injection of melitine, a skin test antigen prepared from *B. melitensis*, had no apparent influence on the agglutination titer.

The allergic state appeared slowly after infection, and the intradermal tests were strongly positive in heavily infected goats.

Until the tests in Tunis are more complete, recommendations for control rest on former experiences.

Caprine or ovine brucellosis is of tremendous social importance in rural life; this was strikingly illustrated when it was recently introduced into Yugoslavia. It has been effectively eradicated by slaughter of entire herds as soon as the infection is discovered. This public health action is justified because the disease in these animals assumes a chronic form which goes undetected unless special tests are made

and then continues to persist in the newly infected country.

In Latin America, the disease has existed for centuries and is chronic. The problems to be met are these:

1. The diagnostic tests needed to guide the recognition and elimination of infected excreters of *B. melitensis* frequently cannot be made on the scale required, and, if it can, only with great difficulties. The usual agglutination test has limitations. It must be interpreted on a herd basis, not on an individual animal basis. In a personal communication, Renoux wrote that the ring test modified for study of goat's milk appears to be highly specific if the test tubes are incubated for 12 hours at 37° C. Work on this test is also being carried out by Alivisatos and Edipides (*18*). The test may offer a solution to some of the present diagnostic problems. Twenty years ago, it was pointed out that the intradermal allergy test detects the infected individual goat probably more accurately than the most refined serologic tests (*19*). Suitable antigens are now available, but the effort required to apply the test is apparently a deterrent factor to its use.

2. Removal of goats with positive reactions to the various tests must be accompanied by strict sanitary herd management, particularly in areas where goats and man live in close association. Those familiar with goat-breeding practices carried out by poor settlers and farmers who own small herds that feed on natural ranges of sandy semidesert and rough and broken terrain fully appreciate the difficulties of accomplishing much in this respect. An educational program—carefully adapted to local needs and skillfully executed until its goals are achieved—is required to familiarize the people with the economic and social consequences of the loss of kids and lambs and the spread of the infection to man. Furthermore, it is never appreciated, and therefore rarely adequately emphasized, that the environment of human habitations where goats infected with *B. melitensis* have been housed for decades is thoroughly impregnated with the infective agent. How to remove the *Brucella* from this contaminated environment is not known, and little effort has been spent in studying the conditions required to accomplish the desired results.

3. Even if all of these requirements could be met and a dependable test-and-slaughter program could be contemplated, the thoughtful health official would still need to look ahead. A primitive people dependent solely on the goat for milk and milk products must receive financial aid from their government because they can meet brucellosis only by replacing stock through importation of clean animals. This is not compatible with the economy of the countries most heavily burdened by caprine brucellosis. Temporary losses will be more than compensated for in the future. Fewer animals will be lost by abortion and productivity will be increased.

The usefulness of calfhood vaccination with strain 19 has already been touched on. This suggests the necessity of further work to find a strain of *B. melitensis* suitable for vaccination of goats and sheep. It seems apparent that no effort should be spared to discover such a vaccine. Meanwhile, one might reflect on some of the problems that lie ahead in this effort. A limited series of tests have shown that goats that have recovered from *B. melitensis* infection have an immunity that rapidly and effectively frees their tissues from the infective agent when they are given relatively large doses of virulent *B. melitensis*. They are not immune to infection with *B. abortus*. Thus, acquired immunity in goats is a proved biological state.

Little present evidence encourages the hope that a killed vaccine or strain 19 can confer an adequate immunity against the continuous chances of infection prevailing in goat-raising areas. On the other hand, an overwhelming amount of data from France, England, Australia, and the United States leave little doubt that at the moment the only effective immunity against *Brucella* abortion is induced by in vivo proliferation of the vaccinating or infecting agent. It must be concluded for the time being that the in vivo synthesis of immunizing antigens is of superior immunogenicity—either in quality or quantity—to that produced by organisms grown on artificial medium. Finding a *B. melitensis* strain with these properties is a difficult task.

Elberg, in a personal communication, offered the hypothesis that a population of *B. melitensis* which has nutritional requirements not satis-

fied by the normal host could be isolated, and this has been done with *B. abortus* and *B. suis* by Berman at Wisconsin University. By supplying a preformed nutritional factor to the host one could propagate the organism at will until an effective immunity is induced. At this point, withdrawal of the required growth substrate would stop growth of the organism, and the host's normal and actively acquired immunity would clear the host's tissue of the organism.

To this end, Elberg and his associates developed a largely streptomycin-dependent mutant of *B. melitensis*. This mutant protected 50 percent of animals vaccinated with it against fairly heavy challenge—the ID_{50} (infectious dose) for mice, 10 to 20 percent; for guinea pigs, 5 to 10; for goats, 1; and for monkeys, 5. Challenge consisted of two injections of 10^{10} cells administered subcutaneously.

Unfortunately, streptomycin could not reach the intracellularly located organisms and therefore could not stimulate their growth. They were able to multiply threefold to fourfold, using the streptomycin they had accumulated in their cytoplasm during growth on the agar containing streptomycin. The strain was not pathogenic according to gross and microscopic histopathologic criteria.

A more proliferative immunizing agent was then isolated from the drug-dependent population on drug-free medium. This mutant was studied carefully and found not to be drug dependent. It was not pathogenic for goats nor for some other animals, but it was less attenuated than the streptomycin-dependent strains in that it multiplied more profusely in mice and guinea pigs. As a result of this ability to multiply, revertant strain 1, among others, was able, in a dose of 10^3 cells, to immunize 70 percent of mice against 220 ID_{50} and 60 percent of guinea pigs against 35 to 50 ID_{50}. Goats and monkeys have not yet been tested.

The in vivo proliferation of revertant strain 1 is such that it multiplies and persists in the spleen of the vaccinated for 11 weeks before it is cleared from the organs. Challenge is conducted at least 6 weeks after the strain is cleared. It is believed that the actual imprint of the immunity depots is more intense quantitatively, and hypothetically it may be taking place with antigens only slightly, if at all, produced in vitro.

These observations can be interpreted as follows: A living attenuated *B. melitensis* that persists in the tissues after inoculation for only a limited time and produces a "native" *Brucella* antigen does protect highly susceptible small laboratory animals against a moderately severe infection. Apparently a *B. melitensis* variant may be obtained through adaptation of the organisms in vitro. The protective value of such a variant in active immunization of goats has yet to be determined.

Why has this apparently complicated road of experimentation been chosen? The chance of selecting from a *B. melitensis* population growing on a culture plate, a variant of low pathogenicity and high immunogenicity, is so low and the work entailed so time consuming that forcing the development of an immunogenic strain by gradual adaptation to streptomycin seems more promising. Actual experimentation justified the taking of this road, and the progress being made brings the development of an effective method of immunization against caprine brucellosis much nearer.

Rather unfortunately, only two laboratories—the Brucellosis Center at the Pasteur Institute at Tunis, supported by FAO and in cooperation with WHO, and the department of bacteriology at the University of California, assisted by a small grant from the Public Health Service National Institutes of Health—are devoting their energies to the solution of this problem of such importance to the welfare of populations who can ill afford the constant direct and indirect injuries inflicted by caprine brucellosis.

General Recommendations

1. A control program against brucellosis should be undertaken with full, friendly cooperation of the United States Department of Agriculture, Agriculture Research Service, State and local health departments, and livestock owners.

2. A systematic survey of the extent and distribution of brucellosis in different animals where this is not known should precede formulation of a control program.

3. Well-equipped and adequately staffed central and field laboratories are essential.

4. Control of bovine brucellosis should be undertaken with aids recognized and available.

5. Measures should be taken to insure pasteurization of milk. The importance of pasteurization cannot be overstated; it kills *Brucella*. In areas where cow's milk is adulterated with goat's milk, this procedure must be carefully supervised.

6. Reliance on pasteurization alone, without regard for other routes of transmission, is unwise because most infections in many regions are spread by direct contact with infected animals.

7. Diagnostic reagents must be standardized and supplied by a central laboratory.

8. The manufacture and distribution of vaccine made with *B. abortus* strain 19 must be under constant control, preferably by a State or national health or agricultural agency.

9. The official brucellosis eradication program should, whenever and wherever practical, be supervised by full-time State, preferably public health, veterinarians, cooperating with livestock sanitary officials.

10. Serious consideration should be given to adoption of regulations that permit importation only of official calfhood-vaccinated animals over 30 months of age and under 36 months of age, provided the blood test within 30 days of shipment does not disclose a reaction exceeding incomplete in a dilution of 1:200.

11. A brucellosis center, devoting all its efforts to the development of an effective vaccine against caprine brucellosis, should be established in one of the Latin American countries. A committee of experts studying the immunology of this infection should be invited to serve as advisers.

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