Current Advances in Brucellosis Research

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RESEARCH on brucellosis has been extensive since the discovery of the causative micro-organism, *Brucella melitensis*, by Bruce (*1*) in 1887. Much of the information that has been developed from that date to 1957 has been compiled and summarized in the First, Second, and Third Joint Reports of the FAO/WHO Expert Committee on Brucellosis in 1951, 1953, and 1958 (*2–4*).

To avoid repetition, this report concerns only some of the contributions to our knowledge about brucellosis from 1957 through 1963. The broad areas discussed are characterization and classification of brucellae, characterization of *Brucella* agglutinins, developments in immunology, and advances in diagnostic capabilities.

Brucellae

Development of methods to improve our capabilities of characterizing and classifying members of the genus *Brucella* is one of the most important areas of brucellosis research. Accurate characterization and classification of brucellae are important for the intelligent interpretation of epidemiologic data. Moreover, an efficient brucellosis program of prevention, control, or eradication depends on reliable epidemiologic information.

The conventional methods used for characterization and classification of brucellae have been principally quantitative rather than qualitative but, when standard procedures are used, the majority of brucellae can be classified in one of the three classic species of the genus *Brucella*. However, a minority of brucellae vary sufficiently in their characteristics to make classification difficult. In 1957 Huddleson (*5*) proposed the classification of brucellae into three species, with the species *Brucella abortus* and *Brucella suis* consisting of three biotypes each and *Brucella melitensis* having only one biotype. Some other investigators have proposed different classifications for members of the genus *Brucella*.

Oxidative metabolic tests. Recently Meyer and associates (*6–13*) reported that oxidative metabolic tests, using a series of amino acids and carbohydrates, quantitatively classified all brucellae, except *Brucella neotoma*, into three species (*B. abortus*, *B. suis*, and *B. melitensis*). They showed that each species of *Brucella* exhibited a characteristic metabolic pattern and that biotypes within each species also exhibited the same characteristic metabolic pattern. Wundt (*14*) used the oxidative metabolic tests with similar results. The oxidative metabolic tests appear to have the advantage of clearly separating all strains of *Brucella* into three distinct species, regardless of their colonial, antigenic, or other biochemical characteristics. These tests also have disadvantages. They do not lend themselves for routine use in most laboratories because of the time required to conduct the tests and the high cost of equipment. Consequently, cultures that cannot be typed by conventional methods will have to be referred to laboratories equipped to conduct the oxidative metabolic tests.

Brucella phage. Within the past 5 years,

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Russian (*15*) and Polish (*16–18*) investigators have reported the isolation of phages that were capable of lysing brucellae, particularly *B. abortus*. Other investigators (*8*, *19–22*) generally agree with their findings. The phage most frequently used to determine susceptibility of *Brucella* cultures to lysing is Tbilisi (Tb), although others are available. Morgan's results (*23*) strongly indicate that lysing activity of Tb phage and four phages from Poland are the same. Meyer (*7*) showed that only those cultures with oxidative metabolic patterns of *B. abortus* were susceptible to lysing by phage. Results of Morgan (*23*, *24*) generally agree with those of Meyer. Although the phage susceptibility test compares favorably in some respects with the oxidative metabolic tests and is much more easily applied, its usefulness as a taxonomical test appears to be limited to the identification of *B. abortus*.

The report (*25*) of a 4-year study of speciation within the genus *Brucella* by the Subcommittee on Taxonomy of the genus *Brucella* of the International Committee on Bacterial Nomenclature concluded that brucellae can be classified by available test into three fairly well-defined groups: *B. melitensis*, *B. abortus*, and *B. suis*. Furthermore, that the species *B. melitensis* and *B. suis* can be divided into three biotypes each and *B. abortus* into nine biotypes.

Brucella Agglutinins

The problem concerned with differentiating between agglutinins produced by *B. abortus* strain 19, and by virulent *B. abortus* has been recognized since strain 19 vaccine was first used as a biologic for immunizing cattle against brucellosis. Another problem, which becomes more apparent as eradication of bovine brucellosis progresses, is the presence of seroagglutinins in some cattle that have had no history of exposure to brucellae. Within the past 5 years, three distinct *Brucella* agglutinins have been characterized.

Physical and chemical properties. Lambert and associates (*26*) demonstrated that cattle developed both heat labile and heat stable seroagglutinins after subcutaneous inoculation with strain 19 vaccine or after conjunctival instillation with virulent *B. abortus*. In cooperative research with the preceding authors, Rose and associates (*27*, *28*) demonstrated that the same two seroagglutinins sedimented at different rates by the method of density-gradient ultracentrifugation. The high molecular weight (16–19S) or fast sedimenting agglutinins are the same or are closely related to the heat labile agglutinins; whereas the low molecular weight (7S) or slow sedimenting agglutinins are the same or are closely related to the heat stable agglutinins. There also was a close correlation between the percentage of the fast sedimenting agglutinins and the percentage of agglutinins inactivated with mercaptoethanol, a chemical that inactivates macroglobulins.

Heat labile or high molecular-weight seroagglutinins were first detected in the blood serums of calves 5 to 7 days after vaccination with strain 19 and reached their maximum concentration 13 to 21 days after vaccination. Heat stable or low molecular-weight seroagglutinins were first detected in the same calves 14 to 21 days after vaccination and reached their maximum concentration 28 to 42 days after vaccination. During the stage of receding vaccinal titers the heat stable seroagglutinins usually disappeared before those that were heat labile.

The time that heat labile and heat stable seroagglutinins appeared in the blood serum of cattle after exposure to virulent *B. abortus* was similar to that in the blood serum of calves after vaccination with strain 19. The major difference between infected cows and vaccinated calves was that the heat stable seroagglutinins reached a higher maximum level and persisted for a much longer time in the blood serums of infected animals.

The evidence presented strongly suggests that low molecular weight seroagglutinins were specifically associated with virulent *B. abortus* infection. Although high molecular weight seroagglutinins usually were associated with exposure to both strain 19 and virulent *B. abortus*, occasionally they also have been found in the blood serum of cattle that have no history of association with brucellae. This seroagglutinin frequently has been referred to as nonspecific.

Kenyon and associates (*29*) isolated from the milk of two cows a 12S macroglobulin that agglutinated with *B. abortus* antigen. The heat stability of 12S macroglobulin was comparable

to 7S specific *Brucella* agglutinin, but its agglutinating power was inhibited at pH 4.0 and below as was that of 16–19S nonspecific *Brucella* agglutinin.

Immunological Developments

In vitro interaction between monocytes and brucellae. Several investigators have demonstrated that the intracellular growth of virulent brucellae takes place in monocytes maintained in tissue culture. Stinebring and Kessel (*30*) further demonstrated that the continuous intracellular growth of virulent *B. abortus* within monocytes of normal guinea pigs could be accomplished by periodic transfer from tissue culture to tissue culture. This phenomenon was not demonstrated within monocytes from normal rats, which are relatively resistant to brucellae.

Since degree of invasion and the rate of multiplication of brucellae within monocytes appeared to be related to the susceptibility of the host from which they were obtained, investigations were conducted to determine the interaction between virulent brucellae and monocytes from guinea pigs that had been inoculated previously with attenuated or killed brucellae. Braun and associates (*31*) and Pomales-Lebron and Stinebring (*32*) demonstrated that the intracellular multiplication of virulent brucellae was markedly depressed in monocytes from guinea pigs that had been inoculated previously with living brucellae. Braun and associates (*33*) also reported that intracellular multiplication of virulent brucellae was slightly depressed in monocytes from guinea pigs that received killed brucellae, and that the degree of multiplication was greatest in monocytes from normal susceptible donors. These results indicate that the ability of monocytes to significantly depress intracellular growth of brucellae in vitro is related to a previous experience of intracellular multiplication of brucellae in vivo. The significance of this phenomenon in immunity to brucellosis of naturally susceptible hosts (cattle, swine, goats, and sheep) has yet to be determined.

Relation of vaccination age to immunity of cattle. Lambert and associates (*34*) have recently demonstrated that calves inoculated subcutaneously with strain 19 vaccine at 4, 6, or 8 months of age had relatively the same degree of immunity against brucellosis when they were exposed to virulent *B. abortus* during their first pregnancy. Similar results were reported by Gilman and Wagner (*35*) with calves vaccinated at 4 or 8 months of age and by King and Frank (*36*) with small numbers of calves vaccinated at 3, 6, or 9 months of age.

Lambert and associates (*34*) also demonstrated that the degree of postvaccinal bacteremia was greater in the group of calves vaccinated at 4 months of age than in those at 6 months or at 8 months of age. The significance of this finding is unknown since the degree of immunity was relatively the same in animals of each vaccination age group. All of the animals in these three age groups developed both heat labile or high molecular weight and heat stable or low molecular weight *Brucella* agglutinins after being vaccinated with *B. abortus* strain 19. Neither the intensity nor the persistence of either of these seroagglutinins was indicative of the degree of subsequent immunity of each animal at the time of exposure to virulent *B. abortus.*

Vaccination of heifer calves at 4 months of age markedly reduced the disadvantage of persistent postvaccinal seroagglutinin titers, which frequently have interferred with the accurate diagnosis of brucellosis during the sexually mature life of animals vaccinated at more advanced ages.

Immunization of goats and sheep against Brucella melitensis. Elberg and associates (*37–39*) developed a nondependent mutant, Rev. 1, from a streptomycin-dependent strain of *B. melitensis*, which was reported to have properties of low pathogenicity and high immunogenicity. These authors demonstrated that 13 goats vaccinated with Rev. 1 were completely protected against an exposure of 33 ID_{50} of virulent *B. melitensis.* Subsequent studies in Spain also showed that a high degree of immunity was induced in goats with Rev. 1 vaccine. Research by several other investigators (*40–42*) also has confirmed the effectiveness of this vaccine in goats. Limited trials suggest that the length of time that immunity may be expected to be effective in goats is 15 months.

The length of time required for Rev. 1 to dis-

appear from tissues of vaccinated goats appeared to be associated to some degree with age of the goats at the time of vaccination. Rev. 1 persisted longer in the lymph nodes of female goats vaccinated at 4 to 5 years of age than in those vaccinated at 3 to 8 months. To avoid causing abortions or udder infection with Rev. 1 vaccine, vaccination of goats is recommended at least 2 months before mating.

Although the immunizing qualities of Rev. 1 vaccine have been well established, the stability of virulence of the Rev. 1 strain of *B. melitensis* has not been thoroughly investigated. This should be done before this vaccine is recommended for universal use.

Another vaccine that has shown immunizing qualities similar to those of Rev. 1 is that developed by Renoux and associates (*43*). This vaccine (Killed Smooth) was prepared from a formalin-killed smooth strain of *B. melitensis*, which was mixed with an adjuvant of mannide monooleate and mineral oil.

In comparing other characteristics of Rev. 1 and Killed Smooth vaccines, the latter produces a considerably more persistent tissue reaction at the site of inoculation as well as more prolonged serologic reactions which would interfere with diagnostic interpretations. Advantages of Killed Smooth vaccine, however, are its superior keeping qualities and decreased risk of abortions when used in pregnant goats.

Information about the efficacy of vaccines for immunizing sheep against brucellosis is limited and incomplete. Studies, however, are in progress in several countries.

Immunization of swine against B. suis. Information on immunization of swine against *B. suis* infection also is limited; however, several recent reports of research have been made by Cedro and co-workers (*44–46*). Biological materials used for preparing the vaccine are identified as an attenuated strain of *B. abortus* and a gluco-lipid *B. suis* antigen. Results indicate that the vaccine was innocuous, and it reduced abortions and increased the average number of live births per animal. Confirmatory as well as additional controlled experimentation are needed to prove the value of this vaccine.

Vaccination of humans with viable Brucella organisms. A number of reports have been made by Russian investigators on the use of 19-BA vaccine for the protection of human beings against brucellosis. Vershilova (*47*) reported a reduction in the incidence of brucellosis of 60 percent among 3 million persons, who had been vaccinated with strain 19-BA, over a period of 6 years (1952–58). Although the author claims 19-BA vaccine is innocuous, the data presented indicate that a significant percentage of people showed evidence of brucellosis after vaccination.

Recently Spink and associates (*48*) compared the safety of two viable *Brucella* vaccine preparations in human volunteers. Sixteen were vaccinated with 19-BA (strain 19 vaccine) and 16 with Rev. 1 vaccine. Two of 16 men who received strain 19 vaccine and 11 of 16 who received Rev. 1 vaccine developed acute brucellosis. *Brucella* agglutinins developed in all of the vaccinated persons, but titers were higher in the Rev. 1 group. Dermal hypersensitivity occurred in all of the Rev. 1 group and in 9 of the strain 19 group. All responded satisfactorily to tetracycline treatment. This report demonstrated that the Rev. 1 strain is considerably more virulent for humans than strain 19, but both are capable of producing acute brucellosis.

On the basis of these reports, the practicability of vaccinating people against brucellosis with either 19-BA or Rev. 1 vaccine is questionable.

Advances in Diagnostic Capabilities

Most of the diagnostic tests discussed were designed to supplement and not to replace the standard seroagglutination tube and plate tests for brucellosis. Their greatest significance is associated with clarification of the brucellosis status of animals, which have doubtful reactions to the standard seroagglutination tests, and of the occasional animal that is located in an infected herd but has an insignificant serologic reaction. Supplemental tests are most applicable in herds from which brucellosis has not been eliminated with standard test procedures.

Heat inactivation test. Amerault and associates (*49, 50*) demonstrated that most of the so-called nonspecific seroagglutinins encountered with the standard seroagglutination tests for brucellosis were inactivated at 65° C. for 15 minutes. Moreover, most of the seroagglutinins which were not inactivated at 65° C. for 15

minutes could be identified as specific for *Brucella* infection.

The heat inactivation test was particularly efficient in diagnosing infection in cattle during the first 60 days after exposure to virulent *B. abortus*. It also identified all of the artificially and naturally exposed cattle which were classified as suspect or reactors to the standard seroagglutination tube test and from which *Brucella* was isolated. Negative serologic reactions were obtained with the heat inactivation test from 75 percent of the artificially and naturally exposed suspect cattle that were bacteriologically negative for brucellae.

Rivanol precipitation test: The rivanol precipitation test, like the heat inactivation test, was developed to detect high molecular weight agglutinins which are frequently termed nonspecific for *Brucella*. Results reported by R. K. Anderson and associates (personal communication) indicate that the rivanol precipitation test has considerable promise as another supplemental test for the diagnosis of brucellosis.

Complement fixation test. The complement fixation test is not a new procedure for diagnosing brucellosis but recent research has suggested its possible application in brucellosis under defined conditions. Alton (*40*) stated that the complement fixation and the standard seroagglutinin reactions paralleled each other closely in goats vaccinated with Rev. 1, except when the seroagglutinins receded to a low level the complement fixation titers had disappeared completely. Lambert and Amerault (*51*) also demonstrated that the postvaccinal complement fixation titers receded more completely than the seroagglutinin titers of heifer calves vaccinated with strain 19. Moreover, 95 percent of the animals that resisted exposure to virulent *B. abortus*, but showed a temporary rise in postexposure agglutinins, remained negative to the complement fixation test. Cedro and associates (*52*) reported that the complement-fixing antibodies were specific in muscles of infected swine.

Whey agglutination test (ring test antigen). The value of this test has been proved in detecting some serologically negative or suspect animals with udder infection. These usually are chronically or recently infected animals in herds from which it has been difficult to eradicate brucellosis (*53–57*).

Experience with all supplemental tests emphasizes that they must be conducted exactly as described by the authors if the user expects to attain results which are reliable.

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Anticancer Research

The National Cancer Institute, Public Health Service, and the National Aeronautics and Space Administration are cooperating in a 1-year medical research project to study the anticancer, carcinogenic, and antiradiation potentials of a group of chemicals closely related to plant growth regulators.

The study extends earlier NASA research that showed that certain plant growth regulators which prolong the life of cancer cells in a test tube can produce a lethal effect when altered. Mixtures of the regulators and their related compounds were even more lethal.

The effects of a variety of these compounds on tumor cells in test tubes and in laboratory animals and on the survival of irradiated normal and tumor-bearing mice are being investigated.

The research is being conducted in the Space and Information Systems Division of North American Aviation, Downey, Calif., under a $198,185 contract with the Public Health Service. The National Cancer Institute is providing technical direction for the project, which is being financed through a transfer of funds to the Public Health Service by NASA under its technology utilization program.