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PRESENT KNOWLEDGE OF BRUCELLOSIS

A Summary

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This summary will attempt to highlight, rather than to detail, certain problems and recent developments concerning brucellosis in man and animals, particularly as they relate to international needs in this field. More complete information and background on particular phases of the subject can be found in the cited references, which for the most part are reviews. Recent books on brucellosis worthy of note are Harris's *Brucellosis (undulant fever)* ;[26] Signorelli's *L'Infezione brucellare nell'uomo* ;[76] *Brucellosis, a symposium* ;[2] and Otero's *Studies of brucella infection in Puerto Rico*,[63] an unusual epidemiological study of the disease from its inception in a geographically segregated community. Huddleson's book, *Brucellosis in man and animals*,[30] although last revised in 1943, provides much basic information on the subject.

I. Prevalence of Infection

A cursory glance at the available statistics on this disease, particularly when they are compared with those of other communicable diseases like tuberculosis and malaria, might give the impression that one need not feel undue concern about this problem. Unlike tuberculosis and malaria, however, which patently afflict large sections of the world's population, the ravages of brucellosis are in great part still not recognized as originating from a specific entity, because of the insidious nature of the disease and the difficulties involved in its diagnosis.[17] Thus, brucellosis might be compared to an iceberg, since only its peak is visible to the casual observer.

Even our limited knowledge of this infection, however, reveals that the prolonged physical suffering and reduced capacity for work caused by chronic brucellosis affect large numbers of agricultural workers and other exposed groups, and that huge economic and nutritional losses are resulting from decline in milk production and breeding efficiency in affected livestock. In France, for example, it is estimated that the economic losses from brucellosis infection based on work-days missed, care of the sick, and decline in meat and milk production exceed 100 million US dollars annu-

ally ; [a] in the USA the same figure is estimated for annual losses to the agricultural economy alone, apart from the human element involved.[60] These facts urge the conclusion that, in restoring and improving the health and economic well-being of countries throughout the world, brucellosis must be given primary consideration.

The following statistics concerning human infection in both large and small countries are illuminating. In 1949 the number of cases of brucellosis in human beings officially reported was : [92] France 1,400, Italy 9,426, Malta 910, Mexico 1,369, Peru 491, Spain 5,484, USA 4,124. The number of undiagnosed and unreported cases was undoubtedly far greater than these official figures. Thus, despite the fact that only 4,000 to 6,000 cases per year for the past several years have been officially reported in the USA, it has been reliably estimated that the number of cases of brucellosis actually occurring each year in that country is probably between 40,000 and 100,000. [21, 37] Comparable figures for France are : 1,400 reported, and a minimum of 9,000 cases as having actually occurred last year.[a] When one considers the chronic nature of this disease, which often lasts over a period of years, and sometimes for the lifetime of the individual, the implications of these figures become more apparent.

A recent survey by the World Health Organization on the prevalence of brucellosis infection in animals in various countries [40] has yielded only very general information. The disease is known to exist in almost all parts of the world where there are susceptible livestock. The existence of widespread infection in cattle, sheep, and goats of the Mediterranean countries and Latin America is well established. In the United Kingdom and in northern and central Europe, except for the Scandinavian countries, between 15% and 50% of the cattle herds are infected with brucellosis.[40] For the USA, the figure is approximately 16%.[77] As far as is known from the meagre information available, the disease is apparently of little importance among animals in the economically retarded countries of Asia and Africa, except in areas where European breeds of livestock have been introduced. It should be pointed out, however, that where careful local studies have been pursued in some of these countries as, for example, in certain parts of India, on native village cattle,[67] indigenous infection has been shown to be surprisingly prevalent (10%-50%).

2. Epidemiology

New developments in our knowledge of brucellosis have suggested little to change the conception that this disease occurs primarily among domestic ruminants and pigs, and is transmitted to man (the accidental intruder in the biological cycle of the micro-organism) by contact with infected animals

[a] Carrère, L., Directeur, Centre de Recherches sur la Fièvre Ondulante, Montpellier, France. Personal communication to the author.

or by the ingestion of their food products. Certain observations have been made recently, however, which throw new light on the epidemiological picture.

The discovery of naturally infected hares [13] and camels [93] adds these species to the known group of possible reservoirs of infection among domestic and wild animals.[26] Contrary to the opinion previously held in many quarters, the bull has been shown to be capable of disseminating the disease in cattle both by means of artificial insemination and natural conception.[3, 58] Natural cross-infection of susceptible domestic animals with any of the three types of brucella organisms must now be considered almost a certainty where conditions for such transfers exist.[33, 58]

As regards human infection with brucellosis, interhuman transmission is so infrequent as to be a medical curiosity.[26] No longer rare, however, are the reports of brucellosis as a disease affecting infants and children,[26] (also personal communication from M. Janbon) and as a cause of abortion in women.[26, 80]

3. Diagnosis

3.1 *Sero-agglutination test*

Brucellosis authorities disagree as to the limitations of the sero-agglutination test (both rapid and tube techniques) as an aid to diagnosis in human beings and animals, although it is widely accepted as the most practical method. Most workers using this test have observed the apparent absence of serum agglutinins in certain cases of bacteriologically proved disease. A recent study in human beings indicates, however, that where a careful and standard technique is used, significant levels of agglutinins can be demonstrated in practically all such cases.[57] Perhaps further clarification of the problem of agglutinin-blocking antibodies, which are found in both human and animal sera,[16, 24] would increase the usefulness of the test. It would also be of interest to find an explanation for the contradictory results which have been reported concerning the appearance of agglutinins in human blood serum following the ingestion of killed brucella.[7, 55]

In addition to the cross reactions due to tularaemia or cholera antibodies,[53] encountered in the brucellosis sero-agglutination test, increased consideration must now be given to the possibility of false positive reactions caused by Q fever [59] and other rickettsioses, because of the demonstrated prevalence of these diseases.

Surveys have clearly demonstrated that there are wide variations in the results obtained from, and the techniques of performing, the sero-agglutination test in different veterinary and medical laboratories.[20, 75, 79] These variations include antigens of unequal sensitivity, the use of different serum dilutions, and divergent interpretations as to what constitutes a significant positive titre. In recent years efforts have been made, parti-

cularly in the veterinary field, to standardize the sero-agglutination test. In 1937 the International Office of Epizootics adopted an international standard serum and recommended its use to member countries with the hope that a degree of uniformity would be achieved with the test in veterinary laboratories. This project was interrupted by the war and was resumed in 1948. At the last session of the International Office of Epizootics in May 1950, it was resolved to advance the standardization of the sero-agglutination test by the use of standardized antigens.

Since the same test is used in most human and veterinary diagnostic laboratories, it is felt that international agreement on standardized biological products and techniques for this test would be a great help in encouraging uniformity of procedure and interpretation of results.

3.2 *Complement-fixation test*

The complement-fixation test is not used as a routine procedure in most laboratories as it is more troublesome than, and shows no distinct superiority over, the sero-agglutination test. Some work indicates, however, that the complement-fixation test might prove useful in helping to differentiate the type of brucella organism causing the infection,[47] or in indicating acute infections rather earlier in the disease than does the sero-agglutination test.[90]

3.3. *Bacteriological culture*

A definite diagnosis of brucellosis in human beings is achieved by the isolation of the organism from the patient, usually from the blood-stream. The difficulties of this procedure when undertaken as routine practice by hospitals and clinics are well known. The typing of brucella strains is also difficult for the average laboratory.

In the past few years successful attempts have been made, notably by Castaneda,[14] to simplify haemoculture. With the introduction of improved media (bacto-tryptose plus vitamin B_1, trypticase-soy) and with increased knowledge concerning the physiology of the brucella organism [29] there is hope that the bacteriological diagnosis of brucellosis will be facilitated in the smaller hospitals and clinics.

3.4 *Intradermic test in human beings*

There is considerable difference of opinion about the application and interpretation of the intradermic test for brucellosis,[26, 30, 78] although many workers believe it to be almost as valuable as the tuberculin-test for tuberculosis. A serious disadvantage, however, of many antigens used at present for this test (killed suspensions or extracts of brucella organisms) is their allergenic properties. The indiscriminate use of these antigens in epidemiological surveys causes positive reactions to the sero-agglutination test in many individuals, and this increases the already

appreciable difficulties of diagnosis of brucellosis in these people. It would be highly desirable, therefore, to reach agreement as to the significance of the intradermic test, its place in diagnostic and epidemiological studies, and the standardization of the antigen used.

3.5 *Opsonocytophagic test*

This test involves the determination of the extent of phagocytic action on brucella organisms by leucocytes from the blood of individuals suspected of having the disease. The opsonocytophagic test is applicable only in the more advanced laboratories, and the significance of the test is still under dispute.[26, 30, 54, 78]

3.6 *Smooth-selecting factor in blood-serum*

Interest has been aroused by Braun's observation[9] that a factor associated with the gamma- and beta-globulin fractions in blood-serum was capable of suppressing non-smooth variants of *Brucella abortus*. This factor has been found in the blood-sera of animal species susceptible to brucellosis, but it has not been detected in the sera of relatively insusceptible species. Braun[9] has suggested that diagnostic tests based upon the activity of the smooth-selecting (SS) factor might be developed. It remains to be seen what practical application these tests will have.

3.7 *Bactericidins in sera*

There are some indications that the level of brucella bactericidins in the whole blood or sera of man and animals can be used as an index of resistance. These tests are laborious to perform and more work on this problem is needed before their use in the serology of brucellosis can be evaluated.[36]

3.8 *Clinical diagnosis in human beings*

The protean nature of symptoms of brucellosis is too well known to require more than a passing reference. It is generally conceded that a diagnosis of brucellosis cannot be made with any degree of certainty without the assistance of laboratory equipment.

3.9 *Intradermic test in livestock*

Despite certain early hopes, intradermic agents for diagnostic purposes have not been very successfully employed in domestic animals.[52] There is some evidence, however, that a non-allergenic intradermic agent for cattle prepared by Danish workers,[64] and showing some promise for diagnosis in bovine animals,[65] might be useful in the case of sheep and goats.

3.10 *Milk ring test*

This test is based on the fact that when a few drops of concentrated suspension of stained brucella organisms are added to a small quantity

of mixed herd milk containing brucella antibodies, the antigen is agglutinated and adheres to the fat globules. When the milk is allowed to stand for a short period of time, the globules rise to form a cream layer, and the agglutinated stained antigen gives this layer a ring-like appearance. The test affords a reasonably accurate and inexpensive screening procedure for determining the presence of infection in herds, but not in individual animals.

The successful application of the milk ring test during the past decade has been a notable advance in the control of brucellosis in dairy cattle. The work has been carried out principally in Denmark, and its usefulness has been corroborated in other countries.[11, 32, 41, 70, 71] Despite extensive work in these countries on the value and limitations of the milk ring test, many problems remain to be solved concerning its application in countries of different economic levels.[42]

The development of a tetrazolium-chloride-stained antigen [4, 91] is encouraging in that its preparation is very much simpler than that of the haematoxylin-stained antigen now commonly used. Further research work is in progress concerning the comparative merits of the haematoxylin and tetrazolium-chloride-stained antigens, and their standardization, utilizing the international standard serum adopted by the International Office of Epizootics.

4. Treatment

Until the development of antibiotics such as streptomycin, aureomycin, and chloramphenicol, chemotherapy of brucellosis in human beings had yielded unsatisfactory results. Insufficient data are as yet available to evaluate del Vecchio's "antibrucellina" (a naphthoquinone derivative) [66, 87] and Huddleson's sulfadiazine-blood transfusion treatments.[19] A distinct step forward was made by the development of the streptomycin-sulfadiazine treatment, but relapses and evidences of toxic reactions are not infrequently encountered, which limit somewhat the usefulness of this combination.[19, 38, 61] Striking results in the treatment of acute brucellosis have recently been obtained with aureomycin and chloramphenicol.[8, 23, 27, 39, 44, 47, 48, 62, 68, 72] Reports to date have not included an adequate number of cases with a sufficient period of observation to permit conclusive statements to be made on these antibiotics. Further information is also needed on their effectiveness in chronic brucellosis. It is to be hoped, however, that with the continued observations in progress in clinics and hospitals in different parts of the world a clear-cut picture of the efficacy of these drugs will be obtained in the not too-distant future.

Eisele [19] cautions against premature acceptance of any apparently effective therapy in the treatment of brucellosis. He stresses as a case in point the sulfonamides, whose early claim to effectiveness was later shown to be highly exaggerated. In view of the spontaneous remissions encountered

in this disease and the fact that many relapses after antibiotic treatment have already been observed, this warning is timely with respect to the new antibiotics. It would appear essential to establish careful criteria of " cures " in brucellosis to permit better evaluation and comparison of the results reported by different workers. Furthermore, new combinations of these drugs, such as sulfadiazine-streptomycin-aureomycin, require careful study in order to assess their value in relation to other combinations now in use. With new antibiotics continually being developed—such as terramycin—which show promise against brucella,[45] the task becomes even more complicated.

It should be borne in mind that, for many countries throughout the world, the new antibiotic drugs are not available or are too expensive. Thus, for sufferers in these countries cheaper methods of treatment must still be used until the new drugs are available to them. Vaccine therapy still occupies a fundamental place in the treatment of brucellosis in many countries, but the preparation of the vaccine, dosage, and method of application vary widely with different clinicians.[23, 26]

The treatment of cattle and pigs with combinations of sulfonamides, antibiotics, and blood-transfusions has apparently not been successful.[58, 89] (Also L. M. Hutchings, cited by Eisele.[19]) It is questionable whether the application of therapeutic measures in domestic livestock is either feasible or desirable.

5. Prevention and Control

The prevention of brucellosis in human beings depends essentially upon minimizing the contact of individuals with infected animals, and upon adequate processing of milk and milk products in order to destroy any brucella organisms which may be present in them. Further information would be desirable on the persistence of *B. abortus* and *B. melitensis* in meat and dairy products under the usual conditions of preparation and consumption of these products in various localities. Reports in this field are incomplete and conflicting,[1, 17, 25, 26, 34, 81, 83, 85] and further work is warranted.

5.1 *Cattle*

The principal measures used in combating brucellosis in cattle are a combination of sanitary procedures, testing and elimination of infected animals, and vaccination. A vast fund of experience is available regarding the effective use of these measures.[22, 77, 84, 86] Many unsolved questions still exist, however, with respect to the application of certain of the diagnostic methods, as pointed out previously, and the use of vaccines and vaccination procedures.

Numerous vaccines and antigenic fractions made from the brucella organism have been investigated for their potential usefulness in cattle,

and of these agents Strain 19 vaccine has thus far proved to be the best from the standpoint of safety and efficiency.[28, 46, 51, 58, 69, 74, 84] Strain 19 vaccine is far from a perfect product, however, and advances in this field should be sought for and encouraged. In this connexion, the results of large-scale trials with Huddleson's M vaccine are being awaited with interest.[15, 18, 31, 43]

The difficulties of properly producing Strain 19 vaccine are very great in countries where veterinary facilities and trained personnel are lacking. Desirable goals would be : simplification of procedure in vaccine production, possibly by means of the new cultural techniques described by Sanders Huddleson,[73] provided death-rates and dissociation of the organism are kept minimized ; effective preservation of the vaccine through cheaper and improved drying methods ; reduction of dosage in cattle, perhaps through the use of intradermal and intracaudal inoculations ; [12, 56] and, if possible, the development of a relatively simple biological test for antigenic potency in a small laboratory animal, either as a control test on successive batches of vaccine or as a periodical check on the Strain 19 itself. This latter test would be in addition to the morphological studies and acriflavine test now used for the determination of dissociation.[10] Also, further work is indicated on the duration of immunity conferred by Strain 19,[5, 6] the differentiation of infected and vaccinated animals by means of serological tests,[9, 88] and other aspects of vaccination (persistence of agglutination titres, vaccination of bulls and pregnant animals).

5.2 *Sheep and goats*

An effective vaccine for sheep and goats is lacking, although some promise is offered in a recent study on a small group of animals.[69] It is to be hoped that increased attention will be paid to this problem, particularly in the advanced laboratories, since an effective vaccine is a much needed weapon in the fight against the disease in the smaller ruminants. Control measures in these animals are difficult to apply in most countries where melitensis infection is prevalent. Periodic application of the sero-agglutination tests and the removal of positive reactors are not very practicable procedures in these countries.

5.3 *Pigs*

The control of swine brucellosis is analogous to that of sheep, in that no effective vaccinal agent has as yet been developed.[33, 50] Segregation and, if possible, elimination of infected animals are indicated.

It is surprising to note the apparent rarity at the present time of swine brucellosis in parts of the world other than North and South America. This is particularly true with regard to European countries where swine husbandry is carried out on an extensive scale, and where swine brucellosis has been troublesome in the past.[35, 82] It is possible that a more careful

search for this disease would reveal its presence in countries at present believed to be free of infection.

The deficiencies of the sero-agglutination test as applied to individual pigs have been amply demonstrated,[33] and a more reliable diagnostic technique which could be used without too much difficulty would be welcome.

6. International Studies and Research

The World Health Organization and the Food and Agricultural Organization (FAO) have attempted to meet some of the problems discussed previously by organizing WHO/FAO brucellosis centres in countries throughout the world to serve the needs of various regions. These centres are occupied in studies of the bacteriology, transmission, diagnosis, and therapy of the disease. They also serve as teaching centres for clinical and laboratory workers of their own and nearby countries, and in their work emphasis is placed on the aspects of brucellosis of most importance to their particular regions. Thus, the centres in France and Yugoslavia are investigating the use of intrapalpebral inoculations of a non-antigenic intradermal agent for the diagnosis of brucellosis in sheep and goats, and the possible application of the milk ring test for these animals ; in Copenhagen, studies are in progress on the comparative efficacy and standardization of milk ring test antigens ; and consideration is being given in other centres to the problem of agglutinin-blocking antibodies in the sero-agglutination test, and the simplification of strain 19 production. Fellowships have been awarded to workers in various centres to study brucellosis techniques in countries other than their own, and financial assistance has been provided, where necessary, for the purchase of apparatus and materials. In addition, literature on the latest advances in brucellosis is circulated to these centres by WHO and FAO.

A WHO/FAO Expert Panel on Brucellosis comprising medical and veterinary experts from all parts of the world has been organized to serve as an advisory body to WHO and FAO on this disease. Moreover, these organizations are collaborating with the International Office of Epizootics and the Inter-American Congresses on Brucellosis in this work.

By these means, WHO and FAO are endeavouring to bring about closer relationships between brucellosis workers everywhere, and thereby to achieve a concerted attack on this problem on a world basis.

7. Problems and Proposals

The problems and needs discussed in this paper can be summarized as follows :

1. Surveys in various countries on the prevalence of the disease in man and animals ; the improved reporting of statistics.

2. Further epidemiological studies, particularly concerning transmission of the disease by the different animal reservoirs ; the importance of brucellosis as a cause of abortion in women, and of infection in infants and children.

3. The international standardization of the sero-agglutination test ; clarification of the limitations of this test and of the complement-fixation test, and their role in the diagnosis of brucellosis.

4. Simplified bacteriological techniques for cultural and typing purposes.

5. The improvement and standardization of intradermic diagnostic agents for man and animals.

6. Studies with reference to the opsonocytophagic test, and the smooth-selecting (SS) factor and bactericidins in sera.

7. The use of milk ring tests in the control of brucellosis in livestock.

8. Antibiotic-sulfonamide and vaccine therapy in human brucellosis.

9. The persistence of brucella in meat and dairy products under the various local conditions of production and consumption of these products.

10. The use and improvement of Strain 19 and other vaccines in the control of bovine brucellosis.

11. Improved diagnostic and control techniques with reference to the disease in pigs, sheep, and goats.

SUMMARY

Throughout the world large numbers of agricultural and other workers exposed to the infection suffer from chronic brucellosis, and immense losses in milk and meat products are caused by infection of livestock. In the USA the estimated number of annual cases of human brucellosis is 40,000 to 100,000 and in France and Italy a minimum of 9,000 ; the figures for reported cases in various countries are much lower. Only general information is available on the prevalence of the disease among cattle.

Various diagnostic tests are discussed. International standardization of the sero-agglutination test is required and the limitations of this and the complement-fixation test, and their role in the diagnosis of brucellosis, should be clarified. Simplification of haemoculture and introduction of improved media should facilitate bacteriological diagnosis. The significance of the intradermic tests for man and animals should be evaluated and the antigens standardized. Further study is

RÉSUMÉ

Dans l'ensemble du monde, de nombreux travailleurs, agricoles ou autres, qui sont exposés à l'infection, sont atteints de brucellose chronique ; d'autre part, l'infection du bétail est cause de pertes considérables de produits laitiers et carnés. On estime qu'aux Etats-Unis le nombre de cas de brucellose humaine varie entre 40.000 et 100.000 par an, et qu'en France et en Italie il s'élève à 9.000 au minimum ; dans divers pays, le nombre des cas déclarés est beaucoup plus bas. On ne dispose que de renseignements peu précis sur la fréquence de la maladie chez les bestiaux.

Plusieurs méthodes de diagnostic sont examinées dans cet article. Il est nécessaire de parvenir à une standardisation internationale de l'épreuve de séro-agglutination ; il s'agit aussi de déterminer de façon plus précise les limites de cette épreuve et de l'épreuve de fixation du complément, et de définir le rôle que chacune d'elles peut jouer dans le diagnostic de la brucellose. Le diagnostic bactériologique pourra être facilité par la simplification de l'hémoculture et l'amélioration des milieux.

required on the opsonocytophagic test and those based on the smooth-selecting factor and the bactericidins in sera. The milk ring test has been successfully used in cattle ; further research work is in progress concerning the comparative merits of the haematoxylin and tetrazolium-chloride-stained antigens, and their standardization.

Il faudrait évaluer la portée des épreuves intradermiques chez l'homme et chez l'animal, et standardiser les antigènes. Il est nécessaire de poursuivre l'étude du test opsonocytophagique et des épreuves basées sur le facteur de sélection des micro-organismes lisses et sur la présence de bactéricidines dans le sérum. Pour les animaux l'épreuve de l'anneau sur le lait (milk ring test) a été employée avec succès ; de nouvelles recherches sont en cours, qui portent sur les avantages respectifs de l'antigène coloré par l'hématoxyline et de l'antigène coloré par le chlorure de tétrazole, ainsi que sur la standardisation de ces antigènes.

The treatment of brucellosis with single antibiotics and combinations of these drugs has given striking results which should not, however, be prematurely accepted. Vaccine therapy is widely used but techniques vary.

Le traitement de la brucellose par divers antibiotiques, employés isolément ou associés, a permis d'obtenir des résultats impressionnants qu'il est cependant prématuré de tenir pour acquis. La vaccinothérapie est d'un usage très répandu, mais les techniques sont diverses.

The prevention and control of the infection in animals is outlined. Further information is required on the persistence of *Brucella abortus* and *B. melitensis* in meat and dairy products. Other points discussed in the paper include : vaccines and antigens for the control of brucellosis in domestic animals ; the need for improved reporting of statistics and for further epidemiological studies, particularly concerning the animal reservoirs ; and the importance of brucellosis as a cause of abortion in women, and of infection in infants and children.

Les méthodes prophylactiques et les mesures de lutte contre l'infection chex les animaux sont succinctement exposées. Il importe de réunir des données plus complètes sur la persistance de *Brucella abortus* et de *B. melitensis* dans la viande et les produits laitiers. Les questions suivantes sont également examinées dans l'article : les vaccins et antigènes pour la lutte contre la brucellose chez les animaux domestiques ; la nécessité de meilleures statistiques et d'études épidémiologiques plus poussées, notamment en ce qui concerne les animaux qui constituent le réservoir de l'infection ; l'importance de la brucellose en tant que cause d'avortement chez les femmes enceintes et source d'infection des nourrissons et des enfants.

In order to achieve a concerted attack on the problem on a world basis WHO/FAO brucellosis centres have been organized for laboratory and field studies, and fellowships have been awarded. An advisory body, the WHO/FAO Expert Panel on Brucellosis, has been set up and collaboration established with the International Office of Epizootics and the Inter-American Congresses on Brucellosis.

En vue de permettre une action concertée contre la maladie sur le plan mondial, des centres mixtes OMS/OAA d'études sur la brucellose ont été créés pour procéder à des recherches en laboratoire et sur le terrain, et des bourses d'études ont été accordées. Un organe consultatif, le groupe OMS/OAA d'experts de la Brucellose, a été constitué, et une collaboration a été établie avec l'Office international des Epizooties et les Congrès interaméricains de la Brucellose.

REFERENCES

1. Alivasatos, G. P. (1949) *Report of Proceedings of the 4th International Congress for Microbiology, Copenhagen, 1947*, Copenhagen, p. 242
2. American Association for the Advancement of Science (1950) *Brucellosis, a symposium* ... Washington, D.C.
3. Bendixen, H. C. & Blom, E. (1947) *Vet. J.* **103**, 337
4. Bendtsen, H. (1949) *Nord. Vet.* **1**, 915
5. Berman, D. T. & Beach, B. A. (1949) *Amer. J. vet. Res.* **10**, 208
6. Berman, D. T., Irwin, M. R. & Beach, B. A. (1949) *Amer. J. vet. Res.* **10**, 130
7. Braude, A. I., Gold, D. & Anderson, D. (1949) *J. Lab. clin. Med.* **34**, 744
8. Braude, A. I., Hall, W. H. & Spink, W. W. (1949) *J. Amer. med. Ass.* **141**, 831
9. Braun, W. (1950) *Variation in the genus* Brucella. In : American Association for the Advancement of Science, *Brucellosis, a symposium*... Washington, D.C., p. 33
10. Braun, W. & Bonestell, A. (1947) *Amer. J. vet. Res.* **8**, 386
11. Bruhn, P. A. (1948) *Amer. J. vet. Res.* **9**, 360
12. Buddle, M. B. (1949) *Vaccination against bovine brucellosis in New Zealand* (Presented at the 14th International Veterinary Congress, London)
13. Bürgisser, H. (1949) *Schweiz. Arch. f. Tierheilk.* **91**, 273
14. Castaneda, M. R. (1947) *Proc. Soc. exp. Biol., N.Y.* **64**, 114
15. Clark, C. F. & Phelps, L. (1949) *Proceedings of the 53rd Annual Meeting of the United States Livestock Sanitary Association*, Columbus, Ohio, p. 29
16. Cox, C. D. & Kutner, L. J. (1950) *Science*, **111**, 545
17. Dalrymple-Champneys, W. (1950) *Lancet*, **1**, 429, 477
18. Edginton, B. H. & King, N. B. (1949) *Proceedings of the 53rd Annual Meeting of the United States Livestock Sanitary Association*, Columbus, Ohio, p. 36
19. Eisele, C. W. (1950) *Therapy of brucellosis in man.* In : American Association for the Advancement of Science, *Brucellosis, a symposium*... Washington, D.C., pp. 148, 150, 151
20. Eisele, C. W., McCullough, N. B. & Beal, G. A. (1947) *J. Lab. clin. Med.* **32**, 847
21. Evans, A. C. (1947) *Amer. J. publ. Hlth*, **37**, 139
22. Gilman, H. L. (1950) *The control of brucellosis in animals employing test and slaughter methods.* In : American Association for the Advancement of Science, *Brucellosis, a symposium* ... Washington, D.C., p. 236
23. Greppi, E. & Dotto, M. del (1949) *G. Malatt. infett. parassit.* **1**, 352, 370
24. Griffitts, J. J. (1947) *Publ. Hlth Rep., Wash.* **62**, 865
25. Hammer, B. W. (1948) *Dairy bacteriology*, 3rd ed. New York
26. Harris, H. J. (1950) *Brucellosis (undulant fever)*, 2nd ed. New York, pp. 38, 51, 61, 84, 194, 278, 311, 331, 454
27. Harris, H. J. (1950) *J. Amer. med. Ass.* **142** 161
28. Hausmann, A., Flatken, A. & Ruge, K. (1949) *M. prakt. Tierheilk*, **1**, 111
29. Hoyer, B. H. (1950) *Some aspects of the physiology of* Brucella *organisms.* In : American Association for the Advancement of Science, *Brucellosis, a symposium*... Washington, D.C., p. 9
30. Huddleson, I. F. (1943) *Brucellosis in man and animals*, rev. ed. New York, pp. 239, 255
31. Huddleson, I. F. (1950) *J. Amer. vet. med. Ass.* **116**, 50
32. Huddleson, I. F. & Carillo, C. C. (1949) *Vet. Med.* **44**, 240
33. Hutchings, L. M. (1950) *Swine brucellosis.* In : American Association for the Advancement of Science, *Brucellosis, a symposium*... Washington, D.C., pp. 188, 192, 196
34. Hutchings, L. M., Brunnel, D.E., Donham, C. R. & Bay, W. W. (1950) *J. Amer. vet. med. Ass.* **117**, 108
35. Hutyra, F., Marek, J. & Manninger, R. (1946) *Special pathology and therapeutics of the diseases of domestic animals*, 5th ed. London, **1**, 818

36. Irwin, M. R. & Berman, D. T. (1950) *Bactericidal tests in brucellosis.* In : American Association for the Advancement of Science, *Brucellosis, a symposium* . . . Washington, D.C., p. 85
37. *J. Amer. med. Ass.* 1947, **134**, 876
38. Janbon, M. & Bertrand, L. (1949) *Pr. méd.* **57**, 753
39. Janbon, M. & Bertrand, L. (1950) *Pr. méd. (In press)*
40. Kaplan, M. M. (1950) *Proceedings of the 3rd Inter-American Congress on Brucellosis,* Washington, D.C. (In press)
41. Kaplan, M. M. (1950) *The preparation, standardization and use of the hemotoxylin stained antigen for the* Brucella abortus *ring test (ABR)* (Unpublished working document WHO Brucellosis Information Series No. 4)
42. Kaplan, M. M. (1950) *The use of the* Brucella abortus *ring test (ABR) in the control of brucellosis in dairy cattle* (Unpublished working document WHO Brucellosis Information Series No. 2)
43. Killham, B. J., Reed, G. W. & Clark, C. F. (1949) *Proceedings of the 53rd Annual Meeting of the United States Livestock Sanitary Association,* Columbus, Ohio, p. 25
44. Knight, V., Ruiz-Sanchez, F. & McDermott, W. (1950) *Amer. J. med. Sci.* **219**, 627
45. *Lancet*, 1950, **1**, 1080
46. Larson, C. L. (1949) *J. Immunol.* **63**, 471
47. León, A. P. & Bernal, E. (1948) *Rev. Inst. Salub. Enferm. trop.* **9**, 199
48. León, A. P., Cano, C. & Bernal, E. (1949) *Rev. Inst. Salub. Enferm. trop.* **10**, 155
49. Lindeck, E. W. (1950) *Brit. med. J.* **1**, 985
50. Lindley, D. C. & Lander, J. R. (1949) *J. Amer. vet. med. Ass.* **115**, 359
51. Live, I. (1949) *Amer. J. vet. Res.* **10**, 347
52. Live, I. & Stubbs, E. L. (1947) *Amer. J. vet. Res.* **8**, 380
53. McCullough, N. B. (1949) *Amer. J. publ. Hlth*, **39**, 866
54. McCullough, N. B. (1950) *Laboratory tests in brucellosis.* In : American Association for the Advancement of Science, *Brucellosis, a symposium* . . . Washington, D.C., p. 120
55. McCullough, N. B., Eisele, C. W. & Beal, G. A. (1949) *Publ. Hlth Rep., Wash.* **64**, 1613
56. McDiarmid, A. (1950) *Vet. Rec.* **62**, 361
57. Magoffin, R. L., Kabler, P., Spink, W. W. & Fleming, D. (1949) *Publ. Hlth Rep., Wash.* **64**, 1021
58. Manthei, C. A. (1950) *Brucellosis in cattle.* In : American Association for the Advancement of Science, *Brucellosis, a symposium* . . . Washington, D.C., pp. 172, 174, 175, 177
59. Meyer, K. F. & Eddie, B. (1949) *Calif. Med.* **70**, 292
60. Mingle, C. K. (1948) *Vet. Med.* **43**, 255
61. Molinelli, E., Ithurralde, D., Basso, G., Miyara, S., Speroni, A., Vital, V. C. Cabassi, E., Pandolfo, G. P., Pirosky, I., de Gambino, R., Matera, A., Tamaroff, L. & Cricco, J. (1949) *Dia méd.* **21**, 2812
62. Molinelli, E. A., Pandolfo, G. P., Schulte, O., Ithurralde, D., Repetto, O. M., Basso, G., Miyara, S., Speroni, A., Thompson, V. E. R. & Vital, V. C. (1950) *Recent advances in the therapy of human brucellosis* (Unpublished working document WHO Brucellosis Information Series No. 6)
63. Morales Otero, P. (1948) *Studies of* Brucella *infection in Puerto Rico,* San Juan, University of Puerto Rico
64. Ottosen, H. E. & Plum, N. (1949) *Amer. J. vet. Res.* **10**, 5
65. Ottosen, H. E. & Plum, N. (1949) *Intradermal brucellosis tests in cattle with a non-antigenic agent* (Presented at the 14th International Veterinary Congress, London)
66. Panebianco, F., Napoli, A. & Aniello, L. d' (1948) *Igiene Sanit. pubbl.* **4**, 230
67. Polding, J. B. (1947) *Indian J. vet. Sci.* **17**, 147

68. Ralston, R. J. & Payne, E. H. (1950) *J. Amer. med. Ass.* **142**, 159
69. Renoux, G. (1950) *Rev. Immunol.* **14**, 279
70. Roepke, M. H. & Clausen, L. B. (1948) *Proceedings of the 52nd Annual Meeting of the United States Livestock Sanitary Association*, Columbus, Ohio, p. 147
71. Roepke, M. H., Paterson, K. G., Driver, F. C., Clausen, L. B., Olsen, L. & Wentworth, J. E. (1950) *Amer. J. vet. Res.* **11**, 199
72. Ruiz Castaneda, M., Carrillo, C. C. & Guerrero Ibarra, G. (1949) *Antibioticos en la brucelosis* (Presented in the symposium on antibiotics, Academy of Medicine, Mexico)
73. Sanders, E. & Huddleson, I. F. (1950) *Amer. J. vet. Res.* **11**, 70
74. Schlingman, A. S. & Manning, M. C. (1949) *Proceedings of the 53rd Annual Meeting of the United States Livestock Sanitary Association*, Columbus, Ohio, p. 48
75. Schubert, J. H. & Herndon, J. F. (1950) *Publ. Hlth Rep., Wash.* **65**, 883
76. Signorelli, S. (1949) *L'Infezione brucellare nell'uomo*, ed. 2, Napoli
77. Simms, B. T. (1950) *Federal aspects of the control of brucellosis.* In : American Association for the Advancement of Science, *Brucellosis, a symposium* . . . Washington, D.C., pp. 241, 244
78. Spink, W. W. (1950) *Clinical aspects of human brucellosis.* In : American Association for the Advancement of Science, *Brucellosis, a symposium* ... Washington, D.C., p. 143
79. Stableforth, A. W. (1950) *Standardization of the diagnosis of bovine brucellosis by the sero-agglutination test* (Report presented at the 18th session of the International Office of Epizootics, Paris)
80. Tallo, G. B. (1948) *G. Ostet. Ginec.* **12**, 449
81. Tesi, A. (1949) *G. Malatt. infett. parassit.* **1**, 342
82. Thomsen, A. (1934) *Acta path. microbiol. scand.* Suppl. 21
83. Topley, W. W. C. & Wilson, G. S. (1946) *Principles of bacteriology and immunity*, 3rd ed. London, p. 821
84. Traum, J. (1950) *The control of brucellosis in animals by the use of vaccine.* In : American Association for the Advancement of Science, *Brucellosis, a symposium* ... Washington, D.C., p. 225
85. Van Drimmelen, G. C. (1948) *J. S. Afr. vet. med. Ass.* **19**, 58
86. Vaysse, J. & Placidi, L. (1950) *Note sur la prophylaxie vaccinale et la chimio-thérapie des brucelloses animales* (Report presented at the 18th session of the International Office of Epizootics, Paris)
87. Vecchio, G. del (1948) *Igiene Sanit. pubbl.* **4**, 209
88. Venzke, W. G. (1948) *N. Amer. Vet.* **29**, 484
89. Watts, R. E., Boley, L. E. & Grieg, W. A. (1950) *Amer. J. vet. Res.* **11**, 304
90. Wise, B. & Craig, H. W. (1942) *J. infect. Dis.* **70**, 147
91. Wood, R. M. (1950) *Science*, **112**, 86
92. World Health Organization (1950) *Epidem. vital Stat. Rep.* **3**, 110
93. Zaki, R. (1948) *J. comp. Path. Therap.* **58**, 145