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LABORATORY TECHNIQUES IN BRUCELLOSIS

SECOND EDITION

G. G. ALTON, LOIS M. JONES & D. E. PIETZ

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World Health Organization
Geneva

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G. G. ALTON

Animal Health Division, CSIRO, Parkville, Victoria, Australia

LOIS M. JONES

Department of Veterinary Science, University of Wisconsin, Madison, Wisc. USA

D. E. PIETZ

United States Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services Laboratories, Ames, Iowa, USA

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PREFACE

Brucellosis remains a zoonosis of worldwide public health and economic importance. A few countries have succeeded in eradicating bovine brucellosis by campaigns based on the elimination of infected animals; others are actively pursuing eradication programmes. Brucellosis of sheep and goats, a more frequent source of human infection, is still widespread especially in developing countries and successful campaigns for its control have been few. Swine brucellosis, though much less prevalent than the other two forms, is very pathogenic for man. Some measure of control in cattle, sheep, and goats can be achieved through vaccination; the more desirable goal of eradication depends, however, on the identification and elimination of infected animals. In either case, soundly based laboratory techniques are required if there is to be any hope of success.

The first edition of this monograph, itself based on an earlier FAO working document, attempted to bring together descriptions of certain well-known and established techniques. It had two main objectives. One was to facilitate the standardization of methods in use in the brucellosis laboratories in different countries so that the results from each laboratory would be more widely intelligible. The other was to provide information and methods to enable workers to expand the scope of their brucellosis laboratory activities; it was hoped that this would improve the prospects for the successful development of eradication programmes, especially for goats, sheep, and pigs.

In 1970 the Joint FAO/WHO Expert Committee on Brucellosis recommended that a second edition of the monograph should be prepared. All the chapters have been thoroughly revised and brought up to date and two new chapters on Brucella ovis *and* Brucella canis *have been added. In particular, the chapter on serological methods has been greatly expanded to include the United States Department of Agriculture methods, since these methods are widely used, especially in North and South America. Several of the other improved serological methods discussed by the Joint Expert Committee are also included for the first time. The Director of the Central Veterinary Laboratory, Weybridge, England, and his staff kindly reviewed those parts of the monograph that describe methods that originated in their laboratory.*

FAO and WHO are pleased to express their gratitude and appreciation to Dr G. G. Alton, Dr Lois M. Jones, and Dr D. E. Pietz for the time and trouble they have expended on the preparation of the second edition of the monograph.

INTRODUCTION

The genus *Brucella* is considered to contain 6 species of bacteria: *Brucella melitensis*, *Brucella abortus*, *Brucella suis*, *Brucella neotomae*, *Brucella ovis*, and *Brucella canis*.

B. melitensis typically causes brucellosis of sheep and goats but also causes active disease in cattle and is a most important zoonosis in man. There are 3 biotypes of *B. melitensis*, but they differ from one another only in their behaviour with monospecific sera.

B. abortus, the cause of contagious abortion in cattle, has 9 biotypes differing from one another in biochemical and serological reactions; all, however, are lysed by *B. abortus* phage and show a characteristic behaviour in oxidative metabolic tests. Infection of animal species other than bovines with *B. abortus* is rare, but troublesome human infections occur quite frequently.

B. suis has 4 biotypes, the first 3 being mainly pathogens of pigs, though in the case of *B. suis*, biotype 2, the European hare (*Lepus europaeus*) is involved in the epidemiology. Biotype 4 causes brucellosis in reindeer (*Rangifer tarandi*). *B. suis* is highly pathogenic for man.

B. neotomae was isolated from the desert wood rat (*Neotoma lepida*), an animal inhabiting western regions of the USA. The importance of *B. neotomae* as a pathogen is unknown as only 27 cultures have been isolated, none of them from domestic animals or man.

B. ovis causes the widespread disease known as ram epididymitis, which is of great economic importance in most of the major sheep-raising areas of the world. Although the male is chiefly affected, transient infections in females also occur. *B. ovis* is not known to cause disease in humans.

B. canis causes a highly infectious form of brucellosis in dogs, both sexes being affected. Infections in other species have not yet been reported, except for a few cases in man. Sporadic infections with *B. abortus*, *B. melitensis*, or *B. suis* also occur in dogs.

The first 4 species of *Brucella* described above occur normally in the smooth form, whereas *B. ovis* and *B. canis* have only been encountered in the rough form. Bacteria in the rough form need a number of special methods for their manipulation, and for this reason all the methods, whether bacteriological or serological, applying only to *B. ovis* and *B. canis* have been placed in 2 separate chapters. All the other parts of this monograph, except where speciation procedures are given, refer to the 3 important *Brucella* species that normally occur in the smooth form.

CHAPTER 1

BACTERIOLOGICAL METHODS

Precautions to be taken in handling infectious or potentially infectious materials

Workers handling materials containing brucellae or working in an environment that may occasionally become contaminated by these organisms run a serious risk of contracting brucellosis or of suffering hypersensitivity reactions unless effective preventive measures are taken. Some precautions that can be taken to minimize the risk will be described. For further information see Shapton & Board (1972).

In addition, it should be emphasized that the production of vaccines and antigens should be in a laboratory completely separated from the laboratory engaged in the isolation and typing of *Brucella*. This is necessary in order to avoid contaminating the vaccine and antigens with virulent strains of *Brucella* or with *Brucella* phage.

Collecting specimens in the field

1. Gloves should be worn and care taken not to contaminate the outside of containers; if such contamination occurs accidentally it must be removed by disinfection.

2. Containers for transporting specimens should be strong and leak-proof and the nature of the contents should be clearly indicated.

3. The material from which specimens were taken should be properly disposed of; transport to the nearest incinerator in stout plastic bags is suggested. All contaminated surfaces and instruments used should be disinfected.

General precautions to be taken in the laboratory

1. When possible, infected materials should be handled in a safety cabinet. This is particularly important in the case of material that could contain *B. melitensis* or *B. suis*.

2. If a safety cabinet is not available, protective clothing, including gloves and face-masks, should be worn.

3. After the work has been completed, materials should be disposed of either by transport in leak-proof containers to an incinerator or by autoclaving in tightly covered metal containers. Re-usable glassware containing milk or blood is difficult to clean after autoclaving; instead the

contents may be discarded into disinfectant or into suitable containers for autoclaving, after which the bottles themselves may be placed in separate containers of disinfectant and soaked before cleaning, or they may be autoclaved while immersed in disinfectant.

4. Surfaces that may have become contaminated should be washed down with disinfectant.

Measures to be taken in specific laboratory processes

It must be recognized that processes that produce aerosols of brucellae provide a great hazard to the laboratory worker, e.g., blowing out the last drop from pipettes, allowing drops from pipettes to fall on the bench, centrifugation, and any process that causes frothing, such as shaking bacterial suspensions. The use of an efficient safety cabinet will protect the operator against all these hazards.

1. In the production of antigens it is preferable to use strains of low virulence, such as *B. abortus* strain 1119-3 [1] or *B. abortus* strain 99,[2] which have the additional advantage that they do not require added carbon dioxide for growth. If a *B. melitensis* strain is required, Rev. 1 [3] is suitable. Even when strains of low virulence are being used for inoculation purposes, care must be exercised to prevent accidental inoculation of personnel or spraying of the suspension into the eyes of anyone present. It is possible that non-virulent and even killed brucellae may cause hypersensitivity reactions.

2. Virulent brucellae should not be freeze-dried and sealed under vacuum in single glass-sealed containers without plugs because of the aerosol that occurs on opening. A suitable method for drying cultures in small tubes contained in larger tubes is described on page 58.

3. Centrifuges may cause dangerous aerosols; if they must be used for virulent materials, they should be operated within safety cabinets. The continuous flow type centrifuge should not be used for virulent strains.

4. Mouth pipetting when making dilutions, e.g., for viable counts, may be avoided by using special rubber bulbs known as pipette fillers or propipettes (see Fig. 1). Open bench work with pipettes should be carried out over a sheet of lint or similar material moistened but not soaked with a disinfectant. Dilutions of suspensions of virulent organisms should be made in safety cabinets.

5. Where large quantities of brucellae are handled, e.g., in the production of allergens, vaccines, etc., the work may be carried out in booths specially constructed to ensure that the air leaving them is properly treated. Such

[1] Available from Veterinary Services Laboratories, P.O. Box 70, Ames, Iowa, 50010, USA.

[2] Available from FAO/WHO Collaborative Centre for Brucellosis, Central Veterinary Laboratory, New Haw, Weybridge, Surrey, England.

[3] Available from Dr S. S. Elberg, School of Public Health, University of California, Berkeley, Calif., 94720, USA.

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