THE USE OF BRUCELLA ABORTUS 45/20 ADJUVANT VACCINE AS A DIAGNOSTIC AID IN THE BRUCELLOSIS ERADICATION CAMPAIGN IN PAPUA NEW GUINEA

MARJORIE A. REID, M.R.C.V.S., and P. R. HARVEY, B.V.Sc., M.R.C.V.S.

Department of Agriculture, Stock and Fisheries, Konedobu, Papua New Guinea

Introduction

It is the policy of the Administration to eradicate bovine brucellosis from the Territory of Papua New Guinea. The program from its commencement in 1956 up to 1963 has been described by Egerton and Rothwell (1963). It is pertinent to note that the use of Strain 19 vaccine was and still is prohibited.

Following the introduction of brucellosis from Australia into two of the largest cattle herds in the country and the existence of a large infected herd in the Madang District, which had been unsuccessfully under eradication measures since 1962, it was decided in 1967 to review the program.

In the early stages of the campaign brucellosis eradication was relatively successful following conventional lines of serum tube agglutination testing and the slaughter of positive reactors. Of 17 herds in which there was serological plus epidemiological or cultural evidence of the disease, eradication was achieved in 16 within one to five years. In the 17th herd, which will be described, a test and slaughter program was enforced for six years with little success.

It is widely accepted that brucellosis eradication will not be successful in the absence of sound management and hygienic practices. The isolation of animals in late pregnancy with separate calving followed by testing prior to rejoining the herd and the application of strict zoosanitary measures have all been recommended in national control and eradication campaigns (Van Waveren 1960; Simpson 1968; Christie et al 1968). These measures may be feasible in dairy or small stud beef herds, but where large numbers of cattle are kept under extensive conditions, it is impracticable to keep a check on individual animals.

In New Guinea this problem is accentuated by relatively high stocking rates. Several thousand cattle may be grazed on one property with the stocking rate ranging from one beast to 4-6 acres on unimproved land to one beast per acre on improved pasture. With pasture rotation, stocking densities may exceed this, thus increasing the opportunity for contact. The situation may be further complicated in beef herds where calves are not weaned until about nine months of age, by which time heifers may already be showing signs of oestrus and have lost their calfhood resistance. The widespread practice of all the year round mating further hampers the use of husbandry techniques in the control of infection.

Together with internal problems of brucellosis control, there is the need to import cattle to expand the national herd as rapidly as possible. Queensland is our regular source of supply due to its proximity and the availability of tropically-adapted cattle. Unfortunately brucellosis occurs in Queensland, and therefore a reliable means of detecting infected animals before importation is important.

Tacken (1964) recorded that a common cause of breakdown in recently cleared herds was the homebred heifer which for control purposes must be regarded as a possible latent carrier. This view has been supported by Cunningham (1971). Furthermore, it may be only after abortion or a full-term infected calving that heifers and recently infected cows will show up as positive reactors in serological tests (Kerr 1938, 1955).

It is the authors' opinion that the early diagnosis and removal of these latent carriers of brucella infection is vital for the ultimate success of an eradication program. This applies both to infected herds under test, and to groups of cattle prior to importation.

Killed 45/20 brucella vaccine was first used for detecting latent carriers by Tacken (1964). His technique was based on an anamnestic response to the complement fixation (CF) test three months after vaccination and was successful. He estimated that he was able to retain on infected farms 519 out of 554 young heifers that would otherwise have been slaughtered. Cunningham (1968) had shown in controlled experiments that the use of killed 45/20 vaccine could be a valuable diagnostic aid in all age groups using the CF test and also an adaptation of the Coombs test. The use of this diagnostic aid will be described both in problem herds and in screening cattle prior to importation.
**Herd A**

Herd A is a mission-owned herd in the Madang District. About 2,150 cattle are grazed under coconut palms on 4,000 acres. The majority are beef cattle, although a few head are used to produce milk for the mission. At the time this herd came under surveillance, there was little evidence of abortion.

Brucellosis was first diagnosed in 1959, but it was not until 1962 that eradication started. The herd was divided into two sections, and eradication was achieved in one in 1964. In 1968 bi-monthly testing was still continuing in the remaining section with about 500 animals under test giving a monthly reactor rate of approximately 0.4%. During the six years of the campaign 259 cattle had been slaughtered.

Under conditions existing in New Guinea management could be described only as fair. Inspection of cattle in the paddock was infrequent, and it was not possible to put into practice any management techniques that would reduce exposure or pasture contamination. Despite this, co-operation was received from the mission during the program.

It was clear that a prolonged test and slaughter policy using the serum agglutination test was unlikely to lead to a solution of the problem. As an alternative to slaughtering the whole herd, it was decided to use killed 45/20 vaccine as a diagnostic aid.

In November 1968 vaccine was given to 522 females and entire males over the age of six months. The animals were bled four and eight weeks afterwards and the serum subjected to the serum agglutination test, the complement fixation test and the modified Coombs test. Testing continued every six weeks for a further eight months followed by two three-month tests and one twelve-month test.

**Herd B**

Herd B is a privately owned beef herd consisting of 5,100 Brahman-cross cattle on 19,000 acres and of these all 2,835 breeding cattle are now under test. Brucellosis was diagnosed in 1967 in the routine testing of heifers after movement to other properties. Between 1961 and 1965 the company had imported 1,001 cattle from Queensland. It is likely that the disease was introduced in these importations. Although a few abortions had been reported, there was no abortion storm.

On diagnosis of brucellosis full co-operation was given by the owners in reducing the internal movement of cattle. Breeding groups were kept separate and tested to determine the incidence and distribution of brucellosis within the herd. Apart from the occasional reactor, the disease was confined to four breeding groups containing 1,646 cattle. Three of these groups consistently gave negative tests after 12 months and have remained negative since. Up to the use of 45/20 brucella vaccine 500 cattle were slaughtered as reactors to the serum agglutination test.

In one group a small number of positive reactions continued to occur. In June 1970 45/20 vaccine was given to 354 cattle. Testing was carried out at one month, four months and eight months.

Because of the risk of latent carriers, the use of 45/20 will be extended to cover all heifers and entire males born in this group when they reach nine months of age. This policy will be continued until one year has elapsed after the last positive reactor is removed.

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**Herd C**

Herd C is a beef herd owned by the Department of Agriculture, Stock and Fisheries and consisting of 4,133 Brahman-cross cattle on 12,000 acres. Approximately 3,400 cattle are currently under test.

Brucellosis was first diagnosed in 1958 and was eradicated by 1960. In 1968 brucellosis was again diagnosed in eight cows imported from Australia and in two station-bred cows. As other introductions into the herd since 1960 came from Territory properties known to be free from brucellosis, the Australian cattle seem the only possible source of the infection. A number of abortions have been recorded, but there was no abortion storm.

As with herd B, breeding groups were kept separate and tested to determine the incidence and distribution of brucellosis within the herd. The disease was originally confined to four groups, but later spread to others. This spread was probably due to a breakdown in internal movement restrictions. Up to the use of 45/20 vaccine 218 positive reactors had been slaughtered.

In July 1970 brucellosis appeared to be confined to one group, to which 45/20 vaccine was given to 125 cattle. Because of the risk of latent carriers, vaccine was also administered to 1,444 unmailed heifers. The use of vaccine will be extended to cover two other breeding groups in which sporadic positive reactors have started to recur.

**Imported Cattle**

Four brucellosis outbreaks have recently been associated with the importation of cattle from Australia; two of these have been described in herds B and C. The other two outbreaks were in dairy cattle run in small herds where the conventional program of test and slaughter has been successful. Between 1960 and September 1968, 6,123 cattle were imported into 45 herds.

These cattle were subjected to a single serum agglutination test within 30 days prior to importation. In practice, however, two tests at 30 day intervals were often given. Tests were not accepted within 30 days before or after calving. With this testing regimen, there was ample scope for the introduction of latent carriers, and it is a wonder that the disease was not introduced into more herds.

In September 1968, Territory import regulations were amended. Cattle are now tested prior to importation:

1. 30-60 days prior to shipment when samples are collected for the serum agglutination test. Cattle showing an agglutination titre of 40 iu/ml or more are not accepted and are removed from contact with cattle to be imported. At the time of collection of samples the cattle are given 45/20 killed brucella vaccine by deep intramuscular injection.
2. 25-35 days after inoculation of 45/20 vaccine and within 30 days of shipment when samples are collected for complement fixation testing. Cattle which showed a titre of 50% haemolysis at the 1:5 dilution and above are not accepted for importation and are removed from contact with cattle to be imported. Cattle which calve or abort during the testing period are also removed from contact and are not accepted for importation.

Since the change in regulations 3,423 cattle have been imported.

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Materials and Methods

Vaccination of Cattle

All animals over six months of age in herds A, B and C and those for importation were given a single dose of vaccine. In herd A, 2 ml of 'Bortin' 45/20 vaccine was inoculated subcutaneously. Herds B and C had 2 ml of 'Duphavac' 45/20 vaccine intramuscularly into the neck. All imports bought between 1968 and 1970 were given 2 ml Bortin intramuscularly and from July 1970 2 ml of Duphavac was injected by the same route before importation.

Serological Techniques

Serum tube agglutination (SA) test — The antigen used was that prepared by the Commonwealth Serum Laboratories, Parkville, Victoria, in accordance with the recommendations made by the FAO/WHO Committee on Brucellosis (1964) Commonwealth Serum Laboratories also supplied internationally standardised positive serum. The test is carried out in small tubes as recommended by Alton and Jones (1967).

Complement fixation (CF) test — The method used was based on that described by Mackinnon (1963) with the following modifications. Complement fixation diluent tablets were used, the antigen described above, and except with herd A, serum standardised by the Commonwealth Serum Laboratories. When testing herd A, a local positive serum had to be used as a standard as no other was then available.

Modified Coombs (ABG) test — A description of the Coombs modified (antibovine globulin) test is given by Brinley Morgan (1967). The technique used in this laboratory is that described by Cunningham (1968) except that sera were centrifuged at 3,000 rpm for 20 minutes and the antigen resuspended in saline with a vortex mixer.

Interpretation of Serological Results

The serum tube agglutination test was carried out on all sera. A titre of 100 IU and above was interpreted as positive, from 30 IU to 100 IU as a suspicious or doubtful reaction and below 30 IU as negative (Anon 1967).

The complement fixation test was carried out on all sera taken from cattle in herds A, B and C and on some from imported cattle. In herd A 50% haemolysis at the 1:10 serum dilution was taken as positive. In herds B and C and the imported cattle a positive titre was regarded as 50% haemolysis at 1:4 serum dilution, which is the Weybridge standard (Anon 1967).

The modified Coombs test was used in two CF the herds but not at every complete test. Details are given with the results. In herd A a 50% agglutination at the 1:80 serum dilution was considered positive, in herd B 50% agglutination at 1:160 serum dilution was considered positive. The interpretation for this test is arbitrary but reference was made to information given by Roerink (1966) and Cunningham (1968) regarding reactions of uninfected cattle to 45/20 brucella vaccine.

Results

Herd A — The serum of one animal gave a positive reaction in the SA test and the sera of 67 cattle gave a positive reaction to the CF test and/or the ABG test in two monthly tests following the inoculation of vaccine. Twenty-eight cattle giving reactions at the higher titres, including the SA testing positive reactor, were slaughtered immediately. The others were isolated and killed over the ensuing months. The rest of the herd continued to be tested by the SA test at intervals of 6 weeks for eight months, followed by two three-monthly tests and one twelve months after that. No positive titres have been recorded since the second monthly test in January, 1969.

Herd B — There were no positive SA test reactors, but the sera of 36 of 354 animals vaccinated were positive to the CF test at the one-month test and two more at a four-month test. The ABG test was performed at four months and two sera were positive, although they gave no reaction in the CF test. All positive reactors were slaughtered soon after identification. Eight months after inoculation with vaccine the group had a negative test.

Herd C — The infected group of 125 cattle was tested monthly for four months after inoculation with vaccine. During this time 56 positive CF test reactors were slaughtered, mainly on the results of the first two tests. There were six animals also positive to the SA test at the first test, and another became positive at the third test. Serum of the latter was consistently anti-complementary. Seven months after inoculation with vaccine the group gave a negative test.

The heifers were tested one, two and four months after inoculation. There were no SA test reactors. One hundred and forty-three animals gave a positive CF test result, most showing up at the first and second test. Many of the positive reactors quickly returned to a negative status. As there was no urgency to slaughter, all reactor animals were retained in the first instance until it could be shown whether the titre was merely a transitory one or likely to be permanent. Twenty-nine heifers showing a stable positive titre have been removed from the group and slaughtered.

Imported Cattle

The records of the Department of Primary Industries in Queensland show that since 1968, when the new regulations came into effect, approximately 108 cattle selected for Territory importation were positive to the SA test before inoculation with 45/20 brucella vaccine and 272 were positive to the CF test one month after inoculation. The number of positive reactors in both tests has fallen sharply between 1968 and 1970, possibly indicating a tendency to avoid heavily infected herds by buying agencies.
Between November 1968, when the first cattle arrived in the Territory after complying with the new import requirements, and June 1970, 3,162 cattle were imported. These were tested in quarantine with the SA test and also at three months, six months and twelve months after arrival. There were 16 positive reactors, few of them reaching above 100 iu level in the SA test. These were slaughtered. From July to December 1970, 261 animals were imported. Of these, to date, six have been positive to the CF test and slaughtered, none to the SA test.

Discussion

In herd A, following removal of the reacting cattle, there have been no further positive reactors for 27 months. The herd is now classified as brucellosis-free. In the infected groups of herds B and C, to which 45/20 vaccine was given, the results of the last test at 8 and 7 months respectively indicate that infection may have been controlled.

It is especially important due to the relatively large numbers of imported cattle that the latent carriers in this category should be identified early. No new outbreaks of brucellosis in the Territory have been attributable to imported animals since the new import procedures were adopted.

The number of cattle slaughtered in herds A, B and C are high, but when related to the total number eliminated previously and the expense of repeated bleeding and testing over years, results would seem to justify the use of 45/20 vaccine if brucellosis can be eradicated speedily. It is suggested that this procedure could be used early in herds under brucellosis eradication schemes.

Both the CF test and ABG test were used in the interpretation of the anamnestic response. In our experience in uninfected cattle inoculated with 45/20, there is little difference in results obtained with either test. In the infected herds, animals will give positive reactions to the ABG test when negative to the CF test. The ABG test can also be used on haemolysed and contaminated sera, which are often anti-complementary. Its main disadvantage is that it is laborious and time consuming to perform, especially where large numbers of sera are involved. Despite this it is now the practice in the Territory to use this test both on sera which are anti-complementary and also three or four months after using 45/20 vaccine in order to identify any possible latent carriers that have been missed by the CF test.

In the diagnostic use of 45/20 vaccine it is not considered possible to differentiate between Strain 19 vaccinated animals and those which have been exposed to field infection. This would present considerable problems in the use of the technique in a population where Strain 19 vaccination has been widely practised. It is fortunate that the use of this vaccine was prohibited in the Territory and consequently the question does not arise. It is intended that the Territory of New Guinea will continue to import cattle from Australia, but if Strain 19 vaccine is brought into common use in Queensland beef herds it could seriously curtail our source of supply.

Apart from the diagnostic value of 45/20 vaccine, it is also possible that a single dose of 45/20 adjuvant vaccine will provide at least some partial immunity. This should assist in limiting the spread of infection within a herd, particularly in the immediate danger period before all positive reactors have been culled. The use of two doses of 45/20 vaccine to provide a more durable immunity was considered by the authors, but it was decided that indeterminate titres might result from a second antigenic stimulus and so confuse diagnosis.

The control of brucellosis in New Guinea can be complicated by large herds mating all year round, high stocking densities, the difficulty in obtaining full musters, and the inability to isolate parturient animals. Cunningham (1971) has recommended that where possible 45/20 vaccine should only be given six weeks after the last known source of spread. Under local conditions this is not possible and could conceivably be a factor against its successful use.

The proper identification of animals, the correct recording of numbers and identification of samples along with efficient herd records to enable the individual animal to be traced and located are of the utmost importance in successful eradication. For identification of cattle several types of metal and plastic ear-tags have proved inefficient due to smallness of numbers or excessive loss from ears. Currently a flexible single piece plastic tag is being tried*. Numbers at least 2.5 cm are painted on to them with a special paint.

The division of large herds into breeding groups is considered particularly important, as internal movement must be restricted and the groups kept as isolated as possible. For infected groups it is worthwhile using separate yards to further reduce both movement within the property and the risks that the sharing of common facilities entails. Calves from infected groups can likewise be kept separated until they have been tested following the administration of 45/20 vaccine.

Because of the risk of latent carriers and the problems associated with diagnosis even after the

*Ritchie Manufacturing Company of Colorado, U.S.A.
use of the vaccine, herds are kept in quarantine until at least one calving interval after the detection of the last positive reactor. Thereafter the herd is kept under surveillance and, for the next two years, the movement of stock for breeding purposes is allowed only after testing.

Summary

During brucellosis eradication campaigns, early latent infection in cattle, not detectable by serological testing, are a major problem. Brucella 45/20 killed adjuvant vaccine has been used in the Territory of Papua New Guinea to induce serologically detectable anamnestic response in these animals. This vaccine has also been used to find latent carriers in groups of cattle being selected for importation into the Territory from brucellosis infected herds in Australia.

References


BOOK REVIEW

AN AUSTRALIAN BIBLIOGRAPHY ON THE BIOLOGY OF THE SHEEP AND THE SHEEP INDUSTRY 1925-1967

Veterinarians and others interested in the sheep industry of Australia will find this a most valuable publication to have on hand. It contains literally every reference of any importance for the period 1925 to 1967 concerned with such important topics as breeds, management, anatomy, physiology, reproduction, nutrition, diseases, parasites, lamb, mutton and wool production. There are approximately 12,000 references with no duplication.

The references are divided into 18 major groups based on subject matter. Those of particular interest to veterinarians are

Group B — anatomy, physiology, breeding;
Group C — reproduction and reproductive disorders;
Group D — nutrition, nutritional and metabolic disorders;
Group E — poisons and poisoning;
Group F — diseases and their control;
Group G — parasites and parasitic diseases.

Within each of these classifications there are further divisions and sub-divisions. For example, the section on parasites is subdivided into arthropods and helminths, which are then further subdivided into appropriate subsections.

If there is any doubt about where references are listed, there is both the authors' index and an alphabetical subject index to help in locating them. There is a list of journals and other publications from which references have been drawn, a list of publications from which statistical information is available and a list of Commonwealth and State laws relating to the sheep industry.

In the preparation of the bibliography a computer was used which enabled both subjects and individual references to be numbered and cross-indexed. The system of numbering is easy to follow and free from errors.

It was compiled by Miss Alma Culey, who was Librarian at the McMaster Laboratory until her retirement. She compiled it at the request of the Australian Meat Board with financial support also from the Australian Wool Board. The result shows her great skill in performing work of this nature. She has produced an extremely dependable publication, which is difficult to fault in terms of accuracy and completeness.

Veterinarians who do not have ready access to a library will find that this publication is an adequate substitute. In fact it is a "must" for those who are interested in sheep.

V. G. Cole