Eradication of Brucellosis from Cattle in the Territory of Papua and New Guinea—1956-1963.

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Introduction.

It has been the policy of the Administration to establish a cattle industry in the Territory of Papua and New Guinea, free, as far as possible, from serious infectious and parasitic diseases (Anderson 1962). Of the bacterial diseases of cattle, brucellosis (*Brucella abortus* infection, contagious bovine abortion, Bang's disease) is one of the most important because of the economic loss in cattle herds (Technical Committee Report, 1960) and also because the causative organism causes a serious disease in man (Undulant fever).

Brucellosis in cattle causes abortion of the foetus usually at about six months pregnancy. The premature expulsion of the foetus follows localization of the organism in the uterus and foetal membranes. Infection of other animals follows ingestion of contaminated pastures and water. Apart from the loss of the calf, infertility and lowered production in aborting cows is common. Bulls may become infected and develop lesions in the genital organs, resulting in decreased fertility. In the non-pregnant cow, *Br. abortus* tends to localize in the udder and/or associated lymph nodes. The organism is secreted in the milk of infected cows and brucellosis becomes a public health problem.

The diagnosis of brucellosis in cattle is confirmed either by the culture of the causative organism from morbid material, milk or semen, or the application of a serological test to the affected animal. A positive serum reaction to a tube agglutination test is recognized as evidence of infection.

Control schemes for brucellosis have been based on several methods. That most commonly used is the use of a vaccine to control its clinical effects (i.e., abortion and infertility). The vaccine used most widely has been Strain 19—an attenuated strain of *Br. abortus*. Strain 19 vaccination markedly reduces the economic loss due to brucellosis, but it does not eradicate the disease. In areas where the incidence of brucellosis is high, Strain 19 vaccination of calves has been used to reduce the incidence of the disease to a point at which a test and slaughter compaign becomes economically feasible. Any breakdown in a vaccination programme will result in an increase in the incidence of the disease.

Eradication of brucellosis can only be achieved by identification of infected animals and their removal from the herd. In some countries management standards have permitted the running of two herds on one property—an infected one and a non-infected. A clean herd is gradually built up by replacing animals, as they are culled, by livestock shown to be non-infected.

Where the conditions of management do not allow the "two herd" system to operate, eradication must be carried out by a "test and slaughter" policy. Under this policy animals are slaughtered as soon as practicable after their identification as positive reactors. To ensure the co-operation of stock owners, some form of compensation is paid by the governmental authority administering the eradication. (In areas where incidence of brucellosis is high the economic shock of such a policy would otherwise be insupportable).

When it became apparent in 1956 that bovine brucellosis was present in the Territory of Papua and New Guinea, it was decided to undertake a test and slaughter eradication scheme. The scheme was initiated in areas where staff of the Animal Industry Division were available to

collect samples for test and to control the application of the scheme. Since its initiation, the scheme has been extended to the majority of cattle herds in the Territory. This paper records progress in the eradication of brucellosis from the cattle herds in the Territory of Papua and New Guinea.

Laboratory Materials and Methods.

The tube serum agglutination test has been used throughout the campaign. Antigen has been obtained from one supplier in Australia(†). Two techniques have been used according to the type of antigen supplied.

In the first year of the scheme (1956-57) the antigen used was prepared in accordance with the Minnesota technique and was diluted before use 1 in 100 with carbol saline solution. After 1957, *Brucella abortus* antigen, prepared according to methods recommended by the FAO/WHO expert committee on brucellosis, was used.

The test using Minnesota technique.

Serum samples for the test were diluted 1 in 10 with physiological saline. 0.2cc, 0.1cc and 0.03 cc of this diluted serum was placed in one of the three tubes in the test. To each tube was then added 1cc of the diluted antigen suspension. The tubes in racks were shaken and incubated at 37 degrees centigrade for 48 hours.

Interpretation.

Tubes were read at bench temperature after incubation. Agglutination reactions in tubes were classified as complete (+), incomplete (I) and negative (—).

Tubes with complete sedimentation in either the first two or all three tubes were classified as positive.

The following tests were classified suspicious :—

Tube 1	Tube 2	Tube 3
+	—	—
I	—	—
I	I	—

Negative reactions showed no sedimentation in any tube.

(†) Commonwealth Serum Laboratories, Parkville N2, Victoria.

The Test using the Joint FAO/WHO expert committee on Brucellosis technique.

The test used employed a final volume of 1cc. Four tubes (3in. x $\frac{3}{8}$in. diameter) were used for each serum sample. O.8cc. carbol saline was placed in tube 1. Tubes 2, 3 and 4 received 0.5cc. carbol saline. To Tube 1 was added 0.2cc. undiluted serum. After mixing, 0.5cc. from Tube 1 was transferred to Tube 2, and the process repeated through to Tube 4 after which 0.5cc. was discarded. To each tube was added 0.5cc. standard suspension antigen. Shaken tubes were maintained at 37 degrees centigrade for 20 to 24 hours in an incubator. Final dilutions in this test were 1/10, 1/20, 1/40 and 1/80.

Interpretation of the FAO/WHO test.

Tubes in each test were classified as follows :—

++ ++	100 per cent. clearing—agglutination + sedimentation.
+++	75 per cent. clearing.
++	50 per cent. clearing.
+	25 per cent. clearing.

Sera showing a ++ (50 per cent.) reading in the third tube (1/40) or higher titre were classed as positive reactors.

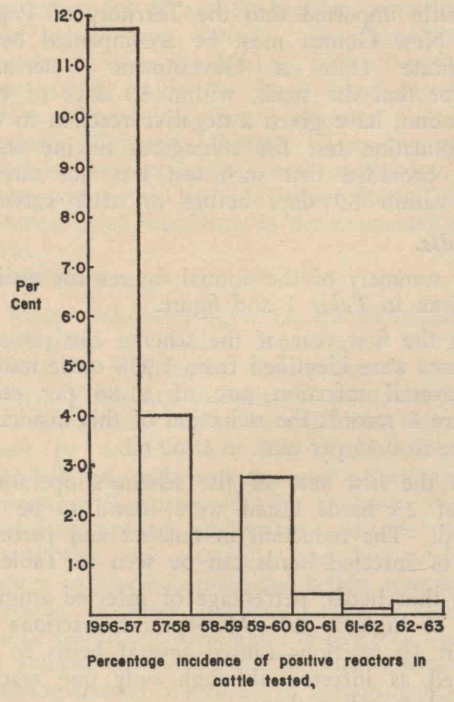
Suspicious reacations were those with a ++ (50 per cent.) reaction in the second tube (1/20) but less than 50 per cent. clearing in the third tube. Negative reactions included all those with less than ++ reading in the second tube (1/20).

Procedure with suspicious reactors.

Titres of suspicious reactors were recorded and at subsequent sampling rises or falls in titre noted. A rise in titre to a ++ (50 per cent.) reading in the third tube (1/40) or higher resulted in a classification of the animal as positive. Three consecutive samples at monthly intervals from suspicious reactors in which no change in titre could be shown, or in which the titre fell, resulted in a classification of the animal as negative.

Procedure with Strain 19 reactions.

Reactions in animals which could be proven to have had Strain 19 vaccination and whose serum did not cause 50 per cent. agglutination at a dilution of greater than 1/40 were classified as negative.



Percentage incidence of positive reactors in cattle tested.

Procedure with haemolysed serum samples.

In the first two years of the campaign, haemolysed serum samples were not tested. Later these samples were tested and noted on the racks as haemolysed. Positive reactions arising from these samples were reported as suspicious and further samples requested. Negative reactions were passed as negative.

Field Methods.

All entire cattle over six months of age were considered eligible for testing.

Blood was collected from the jugular vein using a 13 or 14 gauge 3in. hypodermic needle into 6in. x $\frac{1}{2}$in. diameter test tubes. Tubes were numbered in series from 1 to 100 and the ear tag numbers corresponding to each tube recorded. These tubes were carried in the field and transported in wooden boxes containing 100 tubes.

Blood samples were kept at room temperature for at least four hours and then refrigerated at least overnight prior to being airfreighted to the Central Veterinary Laboratory at Kila Kila, Port Moresby. If prolonged delays in shipment were expected, the serum was poured off into sterile bottles and refrigerated until transhipment to Kila Kila could be arranged.

The Laboratory was notified by radio of the departure of the specimens and generally received them within six hours of removal from refrigerator at point of despatch.

Field Organization.

Until 1962-63, all herds were tested at 12 monthly intervals. If a reactor was found in a herd, monthly testing was introduced until three clean tests at monthly intervals, another three months later, and a fifth six months later, were obtained. The disease was then considered eradicated from the herd and annual testing begun.

In 1962-63, to reduce the burden of repeated testing of clean herds and thus allow extension of testing to previously untested herds, a different field organization was developed. For the purpose of the Brucellosis Eradication Programme, cattle herds in the Territory were grouped into " areas ". These areas consisted of a herd or groups of herds, separated by natural boundaries from surrounding herds.

Areas were classified according to their brucellosis status as :—

A. Brucellosis Free Areas.

B. Brucellosis Eradication Areas.

C. Brucellosis Unknown Areas.

A. Brucellosis Free Areas : To qualify as a Free area all herds in the area must have given two consecutive complete clean tests at twelve monthly intervals. The test carried out on imported cattle prior to importation, was accepted as one of these twelve monthly tests. In Brucellosis Free Areas, testing will be carried out at three yearly intervals.

B. Brucellosis Eradication Areas : Individual herds were classified as :—

(*a*) *Brucellosis clean herds* are those which have been completely tested but never produced a positive reactor, or herds from which the disease has been eradicated. Clean herds are to be tested at annual intervals.

(*b*) *Modified clean herds* are defined as beef herds of over 1,000 head in which at least 50 per cent. of the adult cattle (except steers) when tested annually are negative to the test.

(*c*) *Brucellosis infected herds* were those in which brucellosis has been diagnosed but not eradicated. Herds in an eradication area which did not qualify as clean or modified clean herds were classified as infected until testing showed they qualified as clean or modified clean herds. Testing in herds in which brucellosis has been diagnosed was carried out at one monthly intervals until three clean tests were obtained. If after a further six months a clean test was obtained, the disease was considered eradicated from the herd.

C. Brucellosis Unknown Areas are those in which testing has not been introduced.

Testing prior to Movement.

Negative brucellosis tests are required before movement of breeding cattle from a property in the Territory is permitted, except for cattle from brucellosis free areas. Movement from brucellosis infected herds has been prohibited since 1962-1963.

Compensation.

Compensation for cattle slaughtered during the brucellosis eradication campaign has been either replacement with a similar animal from an Administration owned herd or, at the Administrator's discretion, in cash.

Legislative Powers.

The legislative powers for the brucellosis eradication campaign are embodied in the Animal Disease and Control Ordinance of 1952-1957 of the Territory of Papua and New Guinea.

Quarantine Regulations.

Cattle imported into the Territory of Papua and New Guinea must be accompanied by a certificate from a Government Veterinary Officer that the stock, within 30 days of embarkation, have given a negative reaction to the agglutination test for contagious bovine abortion, provided that such test was not carried out within 30 days before or after calving.

Results.

A summary of the annual figures for testing is given in *Table* 1 and figure.

In the first year of the scheme 230 positive reactors were identified from 1,938 cattle tested, an overall infection rate of 11.86 per cent. Figure 1 records the reduction of this incidence figure to 0.25 per cent. in 1962-63.

In the first year of the scheme's operation, 10 of 23 herds tested were found to be infected. The reduction in number and percentage of infected herds can be seen in Table I.

Within herds, percentage of infected animals was as high as 20. Non-specific reactions or Strain 19 reactions caused several herds to be classed as infected although only one reactor was identified in them.

The increase in incidence in 1959-60 was due to concentration on eradication in two large beef herds, in both of which positive reactors were not destroyed immediately after identification and in which unnecessary infection of clean animals occurred.

The small increase in incidence in 1962-63 was due to the extension of testing to the Madang District. A herd of about 1,000

Table I.

Summary of Brucellosis Testing 1956-63.

Year.	Sera tested.	Cattle under test.	Herds under test.	Infected herds.		Positive reactors.	
				No.	Per cent.	No.	Per cent.
1956-57	3,497	1,938	23	10	43.4	230	11.86
1957-58	8,848	3,427	39	8	20.5	137	3.9
1958-59	5,704	2,800	31	5	16.3	43	1.5
1959-60	7,068	2,500	38	4	10.5	68	2.7
1960-61	8,881	4,724	51	2	3.9	20	0.4
1961-62	5,098	4,598	60	1	1.6	1	0.02
1962-63	13,759	9,557	167	3	1.8	24	0.25

breeders was found to be infected and 22 reactors were identified in it. On two other properties (of 60 and 24 breeders respectively), one reactor was found. The herd of 24 breeders was in the Madang District and there was contact with the large herd mentioned above. The third affected property was in the Eastern Highlands District. The one reactor identified was probably a non-specific one, since there was no evidence of either Strain 19 vaccination or of brucellosis in the remainder of the herd.

Discussion.

Many authorities agree on the desirability of eradication of bovine brucellosis. A report (1960) of a Technical Committee of the Australian Veterinary Association stated *inter alia* that the ultimate aim in Australia with brucellosis must be eradication. The Committee commended the cattle industry and veterinary profession in Tasmania for the progress made in that State towards eradication. Mingle (1959) stated that the economic and human health factors involved justified the fullest co-operation possible between livestock, sanitary officials, public health agencies, and the livestock industry in combating brucellosis. Bothwell (1960) reviewing human brucellosis in Britain, considered that all available preventive measures should be taken to reduce the economic loss in cattle and the incidence of the disease in man.

Eradication of bovine brucellosis is complete in Norway, Sweden and Denmark, and is well advanced in the United States, where a direct loss of $60,000,000 annually is attributed to the disease. Stableforth (1960) reported that twenty entire states of the United States had been declared as modified, certified brucellosis free areas, and that large areas of 28 others were free. In many other countries schemes have been evolved aimed at controlling and ultimately eradicating brucellosis.

In Tasmania, the control and eradication of brucellosis was commenced in 1954. In eleven of thirteen veterinary districts in the State, eradication was commenced with a test and slaughter programme. In the remaining two districts initial high levels of infection led to the use of Strain 19 vaccination of calves as an initial step in reducing the incidence of the disease to a level where test and slaughter was economically feasible. Strain 19 vaccine has successfully reduced the incidence of brucellosis in countries where it has been widely used —New Zealand, South Africa, Australia and the United States of America. Unfortunately, it will not eradicate the disease.

The use of Strain 19 vaccine has the disadvantage that it complicates any test and slaughter campaign that might follow its use. A small percentage of calves vaccinated with Strain 19 at six to eight months of age give a positive reaction to the serum test for brucellosis when they become adult. Furthermore, animals vaccinated with Strain 19 can carry the virulent organism. Consequently the interpretation of a positive serum test in a vaccinated animal is difficult. If it can be avoided economically Strain 19 vaccination is better not carried out in areas where a test and slaughter eradication scheme is operative.

The majority of countries with large livestock numbers had a high incidence of brucellosis in their herds by the time means of controlling the disease were developed. Cattle herds in the Territory of Papua and New Guinea were virtually wiped out during the second world war. After the war, the desirability of re-establishing herds free from serious diseases was realized by the Administration of the Territory. Consequently, quarantine restrictions were applied to provide for the importation of brucellosis free stock. When it was realized that brucellosis was, in fact, present in Territory herds, it was decided to adopt, as soon as possible, a test and slaughter scheme for its eradication. The successful application of this scheme to the Territory's herds is seen as some contribution towards the establishment of a successful dairying and grazing industry in Papua and New Guinea.

The successful eradication of brucellosis from beef herds with 200 to 600 breeders poses several difficulties not likely to be met in dairy herds. In the first instance, musters must be complete and the bleeding programme carried out on a whole herd basis in as short a time as possible. Identification of cattle under test must be permanent and accurate. Handling facilities were found to be inadequate in many instances and could have contributed to the breakdown on several properties. The most essential aspects of an eradication campaign are considered to be the rapid notification of field staff of positive reactors and their immediate removal from the herd. Unnecessarily high losses on two pro-

perties were attributed to the failure to remove positive reactors from the herd. It is also essential that, an infected herd having been identified, a concentrated programme should continue until the disease is eradicated. In our scheme we have found that three complete clean herd tests at intervals of 28 days were evidence of freedom from brucellosis.

Suspicious reactors to the tube agglutination test have not been found to be a serious problem. The titre of suspicious reactors in positive herds was most likely to rise to a positive level. It was found that in some herds a number of animals consistently give suspicious tests. These animals were passed if there was no evidence of rise in titre in three tests at intervals of 28 days and if there was no other evidence of infection in the herd. There has been no recurrence of infection in any herd considered to be freed of the disease and in which this policy toward suspicious reactors has been applied.

Gregory (1960) was of the opinion that suspicious or positive reactions in animals known to have been vaccinated with Strain 19 should be regarded as infected animals. This policy has been followed in Tasmania (Clark 1960). Strain 19 has not been used in the Territory of Papua and New Guinea so that this problem has rarely been met. Animals imported from Australia where Strain 19 is widely used are required to have negative serum agglutination tests before importation.

A possible Strain 19 reaction was met in one herd of 60 head. The herd in question had had no introduction more recent than when the animal which reacted had been imported with 15 other heifers five years previously. The heifers had been vaccinated in Australia. Since there was no other evidence of brucellosis in this closed herd and the titre was within the limits allowed for a reaction to Strain 19 by FAO/WHO the animal was not slaughtered. In other cases where single positive reactions have occurred in herds but in which there has been no evidence of vaccination, there has been no alternative than to class the animal as positive and slaughter it.

The test and slaughter method for the eradication of brucellosis is considered to have been successfully applied in Papua and New Guinea. The scheme will be extended to the few cattle not at present under test. After eradication is complete, the brucellosis free status will be maintained by regular testing of clean herds and the importation of brucellosis free cattle.

Summary.

Progress in the eradication of brucellosis from cattle in the Territory of Papua and New Guinea is described. Incidence was reduced from 11.86 per cent. in the first year of testing to 0.25 per cent. in 1962-63. Cattle under test increased from 1,938 in 1956-57 to 9,557 in 1962-63. Herds under test increased from 23 to 167 in the same period.

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