ANIMAL BRUCELLOSIS IN PENINSULAR MALAYSIA

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Introduction

Brucellosis is an important zoonotic disease in many parts of the world. With the exception of laboratory acquired infections all cases of human brucellosis have an animal origin. As such the presence and spread of the disease in the animal population of a country pose a serious hazard to human health. The disease itself is of considerable economic importance in farm animals. Loss of livestock, and hence of animal protein, occurs from abortion, premature birth, infertility, and decreased milk yield. (Joint FAO/WHO Fifth Report, 1971).

Brucellosis is a disease of antiquity. We are told that a disease syndrome consistent with brucellosis in man was mentioned by Hippocrates around 450 B.C. In the Mediterranean area the disease has probably been present for the last 2,000 years. Likewise, contagious abortion is recorded as having plagued farmers' flocks for centuries (Spink, 1956).

In Malaysia the disease was first detected in cattle in 1950 in a government farm now called Institute Haiwan (I.H.), Kluang in the State of Johor. Porcine brucellosis was first detected in 1963 in pig farms around Ipoh in the State of Perak. The disease is known to occur in the countries surrounding Malaysia.

Brucella species and hosts affected

The genus *Brucella* includes 6 species: *Br. melitensis, Br. abortus, Br. suis, Br. neotomae, Br. ovis* and *Br. canis*. Each species has a preferred host but occasionally infections occur in other hosts, notably in man. The animals that are commonly known to serve as sources of human infection are goats, sheep, cattle, water buffalo, and swine. Infection of reindeer, caribou, camels, and yaks is of epidemiological importance in some parts of the world. Dogs have long been known as carriers oof *Brucellae,* and the newly recognized species *Br. canis* may be transmitted from dogs to man. The modes of transmission to man are ingestion, contact, inhalation, and accidental inoculation (Joint FAO/WHO Fifth Report, 1971).

In Malaysia only *Br. abortus* and *Br. suis* have so far been isolated from cattle and pigs respectively. No cross infections have been noted and brucellosis has not been detected in buffaloes, goats or sheep. Canine brucellosis has not been investigated in Malaysia. Similarly foci of infection in wild animals have not been investigated so far. The prevalence of brucellosis in man in Malaysia is not known.

Prevalence of the disease in animals

1 Cattle

The first case was confirmed in 1950 during an outbreak of contagious abortion at the I.H., Kluang. This followed the detection of the disease among cattle in Singapore in 1949 (Lancaster, 1951). The source of the disease at I.H., Kluang was not established. The assumption of the absence of the disease prior to 1950 was based mainly on the lack of clinical evidence. It is probable that the disease was in existence for some years prior to that without being suspected (Lancaster, 1951). A serological survey of representative cattle herds in the country in 1951 revealed that the disease was widespread. Serological reactors were detected in the States of Johor, Malacca, Selangor, Perak, Penang and Kelantan (Wells, 1953). However, isolation of *Br. abortus* was made only from I.H., Kluang and it was not until 1964 when isolations were also made from cattle farms in the Ipoh area of Perak State. With the control of the disease at I.H., Kluang by 1953 interest in

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the disease died down. In 1969 there was a resurgence of the disease in I.H., Kluang because of the following factors:

(1) Cessation of vaccination in 1959.
(2) Introduction of imports of unknown *Brucella* status from Singapore, Australia and Pakistan.
(3) Introduction of untested cattle from other parts of the country.
(4) Lack of adequate number of calving boxes.
(5) Improper attention to sanitation and hygienic measures at calving.
(6) Increased stocking rate.

By the early seventies brucellosis had become the major disease and management problem at I.H., Kluang. The veterinary Research Institute (V.R.I.), Ipoh undertook surveys of cattle herds in I.H., Kluang and elsewhere in the country. The Regional Veterinary Diagnostic Laboratories (R.V.D.Ls.) also assisted in these surveys. Tables 1 and 2 give the serological and bacteriological findings for the 10-year period 1969 - 1978. The two main serological tests used during the period were the Serum Agglutination Test (SAT) by the tube method and the Complement Fixation Test (CFT) by the tube method and later by the micro-titre method.

Up to December 1977 the SAT was the official test and the CFT was used only on SAT inconclusive or doubtful samples. From 1978 onwards the CFT has become the official test and the SAT is only carried out on animals with a history of vaccination with strain 45/20 killed adjuvant vaccine.

Table 1 Percentage reactors to brucellosis serological tests on cattle sera, 1969 to 1979

State/Station	1969	1970	1971	1972	1973	1974	1975	1976	1977	1978
Perak			15.2	16.4	10.8	8.5	9.4	6.2	6.4	9.0
Kelantan							0.0	0.5	0.9	
Trengganu						6.9	5.8	12.7	11.9	
Pahang									3.3	0.0
Selangor							6.2		4.5	
Negeri Sembilan				7.5	13.0	8.3	1.1	14.7	2.9	
Malacca							0.5		0.5	0.3
Johor (excluding IH, Kluang)						27.3			8.0	3.5
I. H., Kluang	21.3	9.5	14.5	18.7	32.0	17.5	15.1	1.5	0.8	2.6

Table 2 Number of *Brucella abortus* isolations from cattle, 1969 to 1978

State/Station	1969	1970	1971	1972	1973	1974	1975	1976	1977	1978
Perak				1			2	2		
Selangor				1						
Negeri Sembilan								4		
Johor (excluding IH, Kluang)					1	1	1			
I. H., Kluang	1	2	8	9	54	56	33	2		

2 Pigs

Although the first case of porcine brucellosis was confirmed in 1963 in Perak State, its presence was suspected much earlier. In 1953 a number of abortions in pigs had been reported but the sows were all serologically negative. In 1955 - 56 a number of pig sera tested had low titres to the SAT. During the period 1969 - 1978 *Br.suis* was isolated from pig farms in Penang, Perak, Selangor and Johor (Table 3).

Table 3 Number of *Brucella suis* isolations from pigs, 1969 to 1978

State	1969	1970	1971	1972	1973	1974	1975	1976	1977	1978
Penang						1				
Perak	1		10						1	
Selangor		1	4	1						
Johor		3								

Serological tests for the detection of porcine brucellosis have been carried out on farm basis in the above States where pig-rearing is on an intensive scale. The SAT and CFT are carried out. The interpretation of the results is much more difficult as all the serological tests have limitations in determining the status of individual animals. Many herds in the States of Penang, Perak, Selangor and Johor were found to be infected.

3 Other animals

Goats at I.H., Kluang, where bovine brucellosis was present, were serologically tested a number of times over the 10-year period 1969 - 1978. No serological positives were detected. Similarly all abortion cases in goats at I.H., Kluang were also examined bacteriologically and none were infected with *Brucellae*. Goat farms in other parts of the country were also tested, with negative results. Between 1964 and 1966 large numbers of sera from goats, sheep and buffaloes collected at slaughter were subjected to the SAT, with negative results. A similar survey was conducted in 1971 with the same results. Up to now there has been no evidence of brucellosis in sheep, goats or buffaloes.

Diagnostic procedures

1 Serological tests

Until 1973 the SAT was the only test available. The antigen for the test is standardised against the Second International Standard anti *Br. abortus* Serum so that fifty percent (2+) agglutination occurs with 2 I.U. of antibody per ml. The antigen[1] used is a commercially available one and prepared from strain 99 *Br. abortus*. In cattle the SAT detects both IgM and IgG and therefore cannot differentiate reactions due to infection from those due to recent vaccination with strain 19 and, in a proportion of chronically infected animals, the test may be inconclusive or negative. The test is also less effective in detecting early infections (Brinley Morgan *et al.*, 1971).

In I.H., Kluang where brucellosis had become a major problem difficulties were encountered in detecting all infected animals because of adult strain 19 vaccinations. Many of the strain 19 vaccinated animals were not identifiable as such and these posed difficulties in interpretation of test results. In 1973, therefore, the CFT using the WHO recommended method of cold fixation was introduced (Alton and Jones, 1967). The CFT has been shown to be an accurate and sensitive

1) Commonwealth Serum Laboratories, Melbourne, Australia.

diagnostic test and is of particular value in distinguishing serological reactions resulting from vaccination from those produced by natural infection. The CFT carried out at the VRI was later modified according to the Weybridge technique using warm fixation (Brinley Morgan *et al.*, 1971) and this was subsequently carried out by the micro-titre method. The micro CFT is carried out using semi-automatic minipipetter[2], minidiluter[3], 96-well U-shaped permanent lucite plates[4], a micro-shaker[5] and a specially adapted centrifuge[6]. The test antigen is the same agglutinating antigen used for the SAT but standardised to give 50% fixation at 1/220 final dilution of the Second International Standard anti *Br. abortus* serum.

The CFT was initially employed to retest all sera giving doubtful or inconclusive titres to the SAT. However, with the introduction of the CFT as the official brucellosis test in 1978 all cattle sera are subjected to the CFT. In the case of animals with a history of strain 45/20 vaccination the SAT is also carried out in addition to the CFT.

In addition to the above two tests the Rose-Bengal Plate Agglutination Test (RBPT) is also carried out in the country as a field screening test with a buffered antigen[7]. The test is also carried out at the RVDL and samples that are positive are submitted to the VRI for the CFT.

In testing pig sera the SAT and CFT are both carried out. The RBPT is being used on an experimental basis in conjunction with the other two tests. All the serological tests are effective in detecting the presence of brucellosis in a pig herd, but have limitations in determining the status of individual animals. There are some swine from which *Brucellae* may be isolated that do not react in serological tests (Joint FAO/WHO Fifth Report, 1971). These tests are therefore used as herd diagnostic procedures. In any sizeable herd of pigs, a few may show low titres to the SAT even when no infection is present in the herd. Similarly low titres may be seen in farms where infection is present. As a practical rule if there are reactors in the herd with titres of 100 I.U./ml and above, then low titres in the herd are considered significant. In the absence of titres of 100 I.U./ml and above the low titres do not confirm the presence of the disease.

In the interpretation of the SAT and CFT allowances are made for strain 19 vaccinated cattle. The guidelines adopted in Malaysia are given in Table 4.

Table 4 Interpretation guidelines for the Brucellosis SAT & CFT

Status	SAT*			CFT**		
	Negative	Doubtful	Positive	Negative	Doubtful	Positive
Strain 19 calfhood vaccinated cattle and over 30 months of age	≤100	100–200	≥200	≤1/4	2/4–1/10 (positive at 3rd test)	≥2/10
Unvaccinated cattle or cattle of unknown vaccination status	≤50	50–100	≥100	≤1/2	2/2–1/4 (positive at 3rd test)	≥2/4
Bulls and bull-calves	≤27	34–53	≥67	≤1/2	2/2–1/4 (positive at 3rd test)	≥2/4
Pigs+	≤50	50–100	≥100	≤1/2		≥2/2
Goats	≤50	50–100	≥100			

* SAT titres are in I.U./ml.

** CFT titres refer to the degree of fixation (numerator) over the dilution of the serum (denominator).

\+ In pig the tests are used to identify infected herds rather than infected individuals.

2), 3) Cooke Laboratory Products, Dynatech Singapore (Pte) Ltd.

4), 5) Dynatech Singapore (Pte) Ltd.

6) International Centrifuge Model V with adapter to take plate carriers (Dynatech Singapore (Pte) Ltd.)

7) Commonwealth Serum Laboratories, Melbourne, Australia.

2 Smear examinations

The property of *Brucellae* to resist decolourization with weak acids is made use of in the special staining techniques such as the Modified Ziehl-Neelsen Method (Stamp *et al.*, 1950). Although the method is not specific for *Brucellae* the special staining technique is useful in screening samples such as placentas and stomach contents for presumptive evidence of *Brucella* infection.

3 Cultural examination

The isolation of *Brucella* affords proof of infection although, of course, failure to do so is not proof of the absence of infection. It is not always possible to isolate the causative organism from infected animals as *Brucellae* are shed along with the after birth at abortions or at normal calvings or intermittently in the milk. Materials from which isolations of *Brucellae* are best attempted, e.g. placentas, milk samples, are often heavily contaminated, and these often overwhelm the slow growing *Brucella*. The isolation rates at the VRI improved once selective media were used. A selective medium for *Brucellae* must:-

1. allow for growth from very small numbers of cells in the inoculum;
2. allow for growth of the most fastidious strains;
3. give the minimum variation between batches; and
4. inhibit selectively the growth of contaminants.

The basal medium used at the VRI consists of serum dextrose (SD) agar comprising BBL Trypticase soy agar with 1% dextrose (BDH) and 5% *Brucella*-negative bovine seitz-filtered serum. The selective medium used is SD agar with antibiotics (SDA). The antibiotics added are 25,000 units of Bacitracin[8], 6,000 units of Polymyxin B[9] and 100 mg. of Cycloheximide [10] ("Actidione") per litre of basal SD medium, (Alton and Jones, 1967).

Materials received for culture will vary according to the status and circumstances prevailing in the herd. In the case of cattle and pig herds materials submitted would usually be vaginal discharges (swabs), foetus or foetus stomach contents and placentas from the aborting cows or sows and semen from the suspected bulls and boars. In infected herds in addition to abortions all dams producing premature calves, normal calves and retention of placenta would be checked for *Brucella* infection. In these cases vaginal swabs and composite milk samples may be the only specimens submitted. Calves with hygroma would also be subjected to *Brucella* examination.

Cultures are incubated at 37°C in an atmosphere of 10% CO_2 and examined for a minimum period of 7 days. Over the 10-year 1969 - 1978 period a total of 178 *Br. abortus* and 22 *Br. suis* were isolated. They were isolated from field specimens consisting of uterine discharges, placenta/foetal membranes, uterus, foetal organs and stomach contents, lymph nodes, hygroma fluid, milk and semen as well as from guinea pigs inoculated with several of the above field specimens.

Identification of *Brucella* species is made in accordance with methods recommended by Alton and Jones (1967). The cultures are tested for their dissociation (smooth or rough), CO_2 dependence (10% added CO_2), serum dependence (5% bovine serum), production of H_2S (on SD agar slopes with lead acetate papers), urease[11] activity (quantitative estimate), sensitivity to graded (1:25,000; 1:50,000; 1:100,000) concentrations of thionin[12] and basic fuchsin[13] in SD agar and agglutination with both monospecific (A and M) antisera[14]. To differentiate strain 19 *Br. abortus* from CO_2 independent biotypes of *Br. abortus*, sensitivity in 10 I.U. penicillin[15] and erythritol[16] (1 mg/ml of SD agar) as well as virulence studies in guinea-pigs are determined according to procedures described

8) Burroughs Wellcome & Co. (M) Sdn. Bhd., Petaling Jaya, Malaysia.
9) Pfizer (Private) Ltd., Petaling Jaya, Malaysia.
10) Upjohn, J.L. Morison, Son & Jones (M) Sdn. Bhd., Petaling Jaya, Malaysia.
11) Urea agar base (Christensen)—Difco Lab., Detroit, Michigan, U.S.A.
12) BDH, Poole, England.
13) Edward Gurr Ltd., London, England.
14) Central Veterinary Laboratory, Weybridge, England.
15) BBL Antibiotic discs, BBL, Cockeysville, Maryland, U.S.A.
16) Koch-Light Labs. Ltd., Colnbrook, England.

by Brinley Morgan and Gower (1966) and Alton and Jones (1967).

Reference cultures of *Br. abortus* strain 544, *Br. melitensis* strain 16M and *Br. suis* strain 1330, supplied by the Central Veterinary Laboratory, Weybridge, England, are used in testing all media and reagents used.

4 Biological tests

In spite of the use of selective media (SDA) culture plates on many occasions are grossly contaminated especially in the case of placenta and milk samples. To overcome this, from 1973 onwards, in addition to culture techniques, guinea-pigs have also been inoculated with an inoculum prepared from field specimens. The guinea-pigs (preferably males) are bred at the VRI and are tested for *Brucella* antibodies prior to their use in the biological test. The guinea-pigs are bled and euthanised 4 weeks post-inoculation and their spleens cultured onto SD agar. The serum samples collected are subjected to serological tests. A positive finding on culture or on serology constitutes a positive biological test. The biological tests have so far enabled to detect 9 cases of *Br. abortus* infection that went undetected by culture methods.

Control measures

The initial outbreak of bovine brucellosis at I.H., Kluang was soon brought under control by the use of a test and slaughter policy combined with a vaccination scheme for heifers with strain 19 vaccine. The vaccinations stopped in 1959 presumably because no reactors were detected after 1953. We have seen how the disease re-emerged at I.H., Kluang and attained epidemic proportions within a short period. The test and slaughter policy could not be employed as large numbers of animals were already considered serological reactors. To minimise the abortion rate adult vaccinations were resorted to. This, in effect, made it difficult to adopt a test and slaughter policy as the SAT did not differentiate vaccinal titres from infective titres. In 1974 a decision was made to salvage female calves from the infected herd of around 500 animals at I.H., Kluang. This involved the separation of calves three days after birth to a holding area where they are reared till they are yearlings. At 3 to 6 months of age they are vaccinated with strain 19 vaccine provided they do not have high antibody titres prior to vaccination. When they are yearlings (12 - 18 months old) they are transferred to a *Brucella*-free herd. All additions to the *Brucella*-free herd should have been vaccinated in their calfhood and negative to the official serological test. This clean herd has been in existence for the last 5 years and its free-status has so far not been breached.

Elsewhere in the country brucellosis control has been on a voluntary basis as no legislation now exists for mandatory test and slaughter policy. In heavily infected areas, farmers have got rid of their infected animals to unsuspecting farmers. By this means the disease has spread from one farm to another. To complicate further, herds belonging to a number of farmers are taken to graze together or sent to a common grazing area, thus increasing the chances of spread of the disease. Where farmers are aware of the public health danger of the disease and its economic loss to them, they have generally been co-operative in having their animals tested and the reactors sold for slaughter. In the case of small scale cattle rearers culling of one or two animals (and possibly their best producers) is too great a loss for them to bear.

Strain 19 vaccination, however, is well received. Vaccinations are carried out free and the calves so vaccinated are identified by a hole punched in their right ear. But, vaccination alone cannot possibly control the disease especially when there is widespread infection. In 1977 therefore the Veterinary Division adopted a National Programme for the area-wise eradication of bovine brucellosis. The scheme envisages the compulsory testing and culling of reactors and the vaccination of female calves (3-6 months) with strain 19 vaccine. Certain areas are to be designated Brucellosis Eradication Areas under the provisions of the Animals Ordinance, 1953 as revised by Animals (Amendment) Act, 1975. The scheme has a provision to pay compensation over and above the carcass value and compensation rates would depend on the breed and age of the cattle culled. The scheme also has provisions for the restriction of movement of cattle into, out of and within an

eradication area. The National Programme was accepted in principle by the Government but its implementation has been delayed because of the outbreak of foot and mouth disease in the country in 1978.

The control of porcine brucellosis is more readily achieved if the farmer and the laboratory work together. Generally pig farmers are co-operative in this regard and a number of infected farms have been cleared of infection and in many cases restocked with *Brucella*-free animals. No vaccinations are carried out and control is based on a test and slaughter policy. However, because of the limitations of the various serological tests in their ability to identify infected animals, very often a herd that has a large number of reactors would need to be restocked with *Brucella*-free breeders. A few farms have done this.

Research activities

Research activities have so far been directed to solving field problems. Because of the use of different types of vaccine and vaccination schedules in the late sixties and early seventies, it was felt necessary to study the serum antibody response following calfhood vaccination with live strain 19 and killed 45/20 adjuvant vaccines. The studies (Joseph *et al.*, 1976) indicated that the vaccination of calves between the ages of 3 to 6 months should have no influence on the interpretation of routine serological tests for the diagnosis of brucellosis in calves reared in an infected environment. Joseph *et al.* (1977) conducted further studies to examine the serum antibody response in cattle following revaccination with killed strain 45/20 adjuvant vaccine and adult vaccination with live strain 19 vaccine. The studies indicated that revaccinations with strain 45/20 vaccine, especially on former strain 19 vaccinates, caused interference with the CFT. The adult strain 19 vaccinates, if not properly identified, would, because of the persistence of antibody titres, cause problems in the serological diagnosis of brucellosis.

A third study attempted, for the first time, to biotype the *Br. abortus* isolates made over a 5-year period from 1971 to 1975 (Joseph and Ham Thong Yee, unpublished) at the VRI. Of the 130 smooth

Table 5 Comparison of results of Brucellosis SAT, CFT and RBPT on pig sera

Category	Results of:			No. of Sera	Percent
	RBPT	SAT*	CFT*		
1	–	–	–	423	70.5
	–	–	+	40	6.7
2	–	0	–	5	0.8
	–	0	+	2	0.3
3	–	+	–	0	0.0
	–	+	+	1	0.2
4	+	–	–	3	0.5
	+	–	+	33	5.5
5	+	0	–	2	0.3
	+	0	+	29	4.8
6	+	+	–	2	0.3
	+	+	+	60	10.0
		Total		600	99.9

* Titres for negative (–), doubtful (0), and positive (+) as for pig sera given in Table 4 – "Interpretation Guidelines for the Brucellosis SAT and CFT".

Br. abortus cultures biotyped 86.9 percent were of biotype 2, 10.0% of biotype 1, 2.3% of biotype 9 and 0.8% of biotype 6. These 130 isolates originated from 5 farms in 3 States in Peninsular Malaysia with two of the farms having more than one biotype. Biotypes 1 and 2 are known to occur in Australia (Aldrick, 1968) and it is probable that the biotypes originated from cattle imported from Australia. The origin of biotypes 6 and 9 is not known; they are however non-existent in Australia (Dr. G.G. Alton-personal communication).

In the field the use of the RBPT for the screening of cattle sera is being encouraged; besides, in the proposed National Brucellosis Eradication Programme, the RBPT would be used in all herd tests and with the exception of the first or qualifying test, only RBPT positives would be subjected to the CFT. A number of workers have shown that there was a much closer correlation between the results of the RBPT and the CFT than with the SAT (Morgan *et al.*, 1969; Corbel, 1972). The use of the RBPT was started on an experimental basis for the screening of pig sera and in 1976-77, 600 pig sera submitted by the RVDL, Bukit Tengah, Penang were tested by all three tests—RBPT, SAT and CFT. The results are compared in Table 5. It was found that the correlation between the RBPT and SAT for either negative or positive was 87.5% whereas the correlation between the RBPT and CFT was 91.67%. However, if the SAT doubtfuls were included in the SAT positives the correlation between the SAT and RBPT was 92.67%. This compared favourably with the correlation rates for the SAT.

There is a need to screen dogs in Malaysia for *Br. canis* infection and also to look into survival rate of *Brucella* in our environment. There is a need also to assess the status of the disease in the human population of the country.

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Discussion

Gatapia, S.L. (Philippines): From your paper, it appears that the most effective method of control of brucellosis is the application of the "test and slaughter" method. Therefore, the government is prepared to compensate the farmers.

Answer: Yes. The "test and slaughter" method is the best means of control presently. The government would have to compensate the farmers for the animals slaughtered due to infection.

Yugi H. (Japan): Are there other immunological tests for the detection of porcine brucellosis, as the commonly applied methods make the diagnosis difficult?

Answer: The interpretation of the results is particularly difficult as low levels of antibodies may be detected in pigs in a non-infected herd. However, if there are 1 or 2 animals with titres of 100 I.U./ml or above, to the SAT, then low titres in the farm become significant. The tests are "herd-tests" and are not used to detect individual infected animals as in cattle.