THE CONTROL AND ERADICATION OF BRUCELLOSIS IN CATTLE FROM THE STANDPOINT OF HUMAN HEALTH

SCRIPT

By
YEO BOON KIAT

FACULTY OF VETERINARY MEDICINE
BOGOR AGRICULTURAL UNIVERSITY
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SUMMARY

YEO BOON KIAT. The Control and Eradication of Brucellosis in Cattle from the Standpoint of Human Health.
(Under the supervision of INDRAWATI RUMAWAS).

_Brucella abortus_ occurs both in man and cattle. The organism may thrive wherever cattle are raised by man for breeding purposes. As such, spread to humans can easily be lessened if the disease is properly controlled and/or eradicated in cattle.

Control of _Brucella abortus_ in cattle can be undertaken by continued vaccination, proper attention to hygiene and the particular care taken in the introduction of only brucellosis-free animals into cattle farms. Vaccination using the strain 19 or strain 45/20 _Brucella abortus_ vaccine is commonly used, though the former is more popular. Each of these vaccine strains has its own advantages and disadvantages. Eradication of _Brucella abortus_ by the test and slaughter policy can also be carried out after proper vaccination programmes have been initiated and a stage reached where only a very small percentage of positive reactors is found.

Antibiotic treatment of affected animals does not prove to be effective because of the intracellular nature of _Brucella abortus_, and as a matter of fact it seldom is undertaken. In man, Streptomycin and Tetracycline have shown their efficacies in improving symptomatic signs only.
THE CONTROL AND ERADICATION OF BRUCELLOSIS IN CATTLE FROM THE STANDPOINT OF HUMAN HEALTH

SCRIPT

By
YEOL BOON KIAT
B.15.0945

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This script has been examined and approved by the adviser

I. Rumawas

Indrawati Rumawas DVM, MPH.

Date: 8th March 1983.
BIOGRAPHY

The author was born in Labuan, Sabah, Malaysia on July 6th, 1957. He is the eldest son of Yeo Lian Ann and Pinlee.

He received his primary and secondary education from the Government English School in Labuan, Sabah and completed the Overseas School Certificate examination in 1974.

Before entering the Bogor Agricultural University he was attached to the Education Department in Sabah for approximately two years. In 1978 the State Government of Sabah awarded him a scholarship to study at the Bogor Agricultural University, Indonesia. He graduated as a Sarjana Kedokteran Veteriner in 1982.
ACKNOWLEDGEMENTS

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He also thankfully acknowledges his gratefulness to the Dean of the Faculty of Veterinary Medicine and all members of his staff for assisting and guiding him during the course of study at the university. His appreciation goes to each and everyone of them.

The author also wishes to express his sincere thanks to the Chief Minister's Department in Sabah for awarding the scholarship and other facilities while studying in Bogor. To all librarians of the university libraries and the Research Institute for Animal Disease library in Bogor, the author expresses his gratitude for their assistance in various ways.

His oceans of thanks are also devoted to his parents, brothers, sisters and beloved one for their prayers and encouragement aimed at inspiring the author during the course of his academic enterprise.

Finally it is the author's hope that this writing will be of advantage to those reading it and may stimulate further interest in this area from appropriate quarters.
LIST OF TABLES

<table>
<thead>
<tr>
<th>Number</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Differential characteristics of the species of the genus Brucella and their biotypes</td>
<td>4</td>
</tr>
<tr>
<td>2.</td>
<td>Weekly serological results for sera collected from a cow experimentally exposed to Brucella abortus</td>
<td>16</td>
</tr>
<tr>
<td>3.</td>
<td>Interpretation of weekly serological results for sera collected from a cow experimentally exposed to Brucella abortus</td>
<td>17</td>
</tr>
<tr>
<td>Number</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>--------</td>
<td>-----------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>1.</td>
<td>Big knee of cattle often associated with <em>Brucella abortus</em></td>
<td>17</td>
</tr>
<tr>
<td>2.</td>
<td>Hygroma, opened, situated on the withers of a bovine carcase</td>
<td>18</td>
</tr>
<tr>
<td>3.</td>
<td>Placental smear showing a group of <em>Brucella abortus</em> organisms</td>
<td>19</td>
</tr>
<tr>
<td>4.</td>
<td>Temperature pattern of a case report (human brucellosis) in Indonesia</td>
<td>23</td>
</tr>
<tr>
<td>5.</td>
<td>Serum agglutination test and complement fixation test titres following S 19</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>vaccination</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Serum agglutination test and complement fixation test titres following 45/20</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>vaccination</td>
<td></td>
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</table>
I. INTRODUCTION

Cow's milk is a very valuable food substance as it contains all the essential food constituents such as proteins, carbohydrate, fats, vitamins and minerals. Thus milk is an excellent food for the growing animals and man.

Several organisms including those of tuberculosis, brucellosis, salmonellosis and Q-fever are transmissible to man through consumption of milk and milk products. With the increasing demand for milk and milk products, these diseases may increase in prevalence if hygienic and control measures are not undertaken to prevent them.

*Brucella abortus* is one of the several organisms which causes brucellosis in animals, and also undulant fever in man. The disease in man is characterised by a fluctuation in body temperature - the so called intermittent fever. The causative agent in cattle is transmissible to man via the milk and infected materials from aborted foetus and discharges from infected animals.

Brucellosis in cattle is gaining in importance as the demand for milk is increasing. In Indonesia, *Brucella abortus* was first isolated in 1925 by Kirschner in Bandung. With the present move by the government to encourage development of the dairy industry through cooperatives, the chances of cattle contacting brucellosis
should not be underestimated.

The control, eradication and prevention of brucellosis in dairy herds is not a very easy task. Even in some developed countries like the United States of America and Canada, complete eradication proved difficult. Besides, the problems encountered in the diagnosis of the disease, control and vaccination programmes, herd management and hygiene, complete eradication usually means the spending of large sums of money especially for compensation of culled animals and also for the replacements and acquisition of new brucellosis-free animals.

A brief description of the method for the diagnosis, and the control and eradication of brucellosis in cattle is presented in this writing.
II. THE BRUCELLOSIS INFECTION IN CATTLE

Etiology

*Brucella abortus* belongs to the genus *Brucellae* which are small, aerobic, Gram negative cocccobacilli or short rods that are non-motile, non-sporeforming and noncapsulated. The *Brucellae* grow best in medium enriched with animal serum and glucose, and especially for *Brucella abortus*, the addition of 5 - 10% carbon dioxide to the atmosphere on primary isolation is required (Buchanan and Gibbons, 1974; Duguid *et al.*, 1978; Jawetz *et al.*, 1978). Their temperature range is 20° - 40°C; 37°C being optimum. Ph range is 6.6 - 7.4 and the colonies take several days to appear and are circular convex with a smooth, glistening surface and an entire edge.

*Brucella abortus* may also produce amounts of hydrogen sulfide when grown on liver extract agar for at least four days on isolation (Stewart and Esrick, 1977). The bacillus which causes contagious abortion includes nine biotypes. The differential characteristics of the species of the genus *Brucellae* and their biotypes can be seen from table 1.
### Table 1. Differential characteristics of the species of the genus *Brucella* and their biotypes (Joseph and Ham, 1979)

<table>
<thead>
<tr>
<th>Species</th>
<th>Growth on days**</th>
<th>Agglutination in mono-specific sera</th>
<th>Lysis by Phage Tb</th>
<th>Oxidative metabolic tests</th>
<th>Most common beast reservoir</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>CO₂ requirement</td>
<td>H₂S production</td>
<td>Thionin</td>
<td>Basic</td>
<td>fuchsia</td>
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<tr>
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<td></td>
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<td>a</td>
<td>b</td>
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<td>B. neotomaes</td>
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<td>+</td>
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<td>B. canis</td>
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<td>+</td>
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</table>

**Dye concentrations a = 1/25,000; b = 1/50,000; c = 1/100,000.
Pathogenesis And Pathology

In the epidemiology of bovine brucellosis, the placenta, foetal fluid and aborted foetus serve as major sources of infection. Bacteriological examinations on two naturally infected cows revealed that the number of organisms isolated from the umbilicus ranged from $2.4 \times 10^8$ to $4.3 \times 10^9$ per gram sample, from the foetal cotyledons $5.2 \times 10^{11}$ to $1.4 \times 10^{13}$ per gram and as high as $9.5 \times 10^{10}$ per millilitre in the foetal fluid (Alexander et al., 1981).

Entrance into the bloodstream and lymphatics may occur through mucous membranes of the oropharynx and upper respiratory tract, the conjunctiva, scarified skin and cervix. The major route in natural infections appears to be oropharyngeal (Berman, in Ristic and McIntyre, 1981). Infection may also occur via the teat from infected milk of another cow and via the vagina from infected semen (Arthur, 1977). Localizations of the organism subsequently occur in the uterus, mammary gland, lymph nodes, testicle and accessory male sex glands, joint capsules, bursae, spleen, liver, bone marrow or in the placental tissues of pregnant animals (Blood et al., 1979). The endometrium and associated chorionic tissues become oedematous, and the intercotyledonary membranes become thickened and leathery and the villi destroyed. As a result circulation to the foetus is decreased and pregnancy
usually terminates in abortion, usually in the last three months of pregnancy (Berman, ____ In Ristic and McIntrye, 1981; Toelihere, 1981). Congenital infection can occur in newborn calves as a result of in-utero infection and the infection may persist in a small proportion of calves which may also be serologically negative until after their first parturition or abortion (Lapraik, 1975). In many cases, pneumonia of fibrinous or cellular type, oedema of the skin, pericardium and umbilical cord, and the haemorrhagic serous exudates in pleural, pericardial and abdominal cavities of foetus usually occur.

Of animals which abort, approximately 80% do so only once and more than two abortions by the same animal is rare. Reinvasions of the uterus occur in subsequent pregnancies but placental damages is limited and full term pregnancies are usual. In this way Brucellae are shed and serve as a source for further spread between herds.

Following abortion, Brucella abortus tends to localize in the udder, and adjacent lymph nodes in the form of a granuloma formation. Following localization in the regional or adjacent lymph nodes, there is a bacteremic phase seeding of organisms within phagocytic cells in lymph nodes and spleen; and microscopic granulomas without necrosis are seen (Berman, ____ In Ristic and McIntrye, 1981). Such persistent infection of the udder and lymph
nodes, with constant or intermittent shedding of organisms therefore serve also as an important source of infection for calves and humans drinking the milk. Other sites of localization include the carpal and suprascapular bursae where hygromas containing large populations of the organism may be found.

In the male, localization in the testis, epididymis, and accessory sex organs is common, and organisms are shed in semen. This may result in infertility especially in intrauterine insemination with infected semen is applied. However, in view of the measures taken to ensure that artificial insemination bulls are free from brucellosis, this eventuality is very improbable under present conditions.

Clinical Signs And Symptoms

In highly susceptible non-vaccinated pregnant cattle, abortion during the last three months of pregnancy is the cardinal feature of the disease in cows. According to Toelihere (1981), the rate of abortion varies from five to 90% in a herd, depending on the number of pregnant cows, the rate of infections and the virulence of the organism.

Retention of the placenta and metritis are common sequelae to abortion. As a result such animals experience delayed involutions of the uterus and are prone to
secondary bacterial invasions which may be acute with septicaemia and death following, or chronic leading to temporary infertility or sterility. In as much as it initiates many cases of puerperal metritis, *Brucella abortus* may predispose to the formation of ovabursal adhesions.

Establishment of the carrier state may also lead to reduction in the milk yield by twenty percent (West, 1977) and the production of dead calves at term and an increased frequency of retained placenta (Siegmund, 1979). Interstitial mastitis and enlarged supramammary lymph nodes are also observed.

Orchitis and epididymitis occur occasionally in the bull. Such bulls are thus potential spreaders of the disease if used for artificial insemination. Agglutinin may be demonstrated in the seminal plasma of such bulls. The *Brucella abortus* organism can also often be isolated from lesions of non-suppurative synovitis and hygromatous swelling of cattle.

**Diagnosis**

Brucellosis has no particular features which allow a clinical diagnosis to be made accurately. Laboratory methods must therefore be used. Laboratory tests used in the diagnosis of brucellosis include isolation of the organism and tests for the presence of antibodies of
Brucella abortus in blood, milk, whey, vaginal mucus and seminal plasma. For abortion cases, this can be done by cultures from the organs and lymph nodes of the foetus, the placenta, milk, vaginal mucus or uterine exudate. Where contamination is probable the suspected material is inoculated into guinea pigs which are later slaughtered and their spleens cultured on blood agar plates and their serum collected for serum agglutination test. Also the organism maybe isolated from the genital tract after abortion or normal calving after periods of up to ten weeks in 50% of infected animals (Siegmund, 1979).

There is no satisfactory diagnostic method to detect cattle in the incubative stages of the disease. However, Cunningham and O'Connor (1971) and Dolan (1980) reported the use of the 45/20 adjuvant vaccine as an anamnestic agent to detect latent infections. The use of an injection of 45/20 vaccine results in high titres of complement fixing antibodies.

Simple agglutination tests will surely remain the basis for examinations of large numbers of sera, and they should often be used for screening. Thus selected supplemental tests are often neccessary to confirm preliminary test findings.

The antibody response following infection depends on the stage of gestation and pregnancy. Agglutinins and
complement fixing antibodies become positive four weeks following experimental infection during the fourth to sixth month of gestation and not until about ten weeks if experimental infection occurs two months before or after insemination. The serological diagnosis is considered to be unreliable when applied during the periods of two to three weeks before and after abortion or calving (Blood et al., 1979).

The Serum Agglutination Test

This test detects non-specific antibodies as well as specific antibodies from Brucella abortus infection and vaccination. Immune serum when added to a uniform suspension of organism causes them to slump together. Serial dilutions of the serum provides an endpoint at which agglutination takes place when a standard amount of antigen is added.

The use of an International Standard Antibrucella abortus Serum (ISAbS) which is obtainable from the Ministry of Agriculture, Fisheries and Food International Laboratory of Biological Standards at Weybridge or from the Statens Serum Institute Copenhagen for standardizing antigens for the serum agglutination tests and a unitage system is widely used for expressing the antibody levels of sera.
Several limitations of the serum agglutination test which include the inaccuracy of this test at periods after abortion, during incubation stages of the disease (Blood et al., 1979) and in the chronic stage of the disease (Arthur, 1977) and the presence of non-specific agglutinins and cross agglutination with other organism like Yersinia enterocolitica, can in certain instances invalidate the test.

False positive animals are also of some concern as it mean the unnecessary slaughter of uninfected animals. False positive or suspicious titres may be due to residual vaccinal titres, heterospecific antigens and specific antigen stimulation without apparent permanent infection (Nicoletti, 1975). Strain 19 vaccination can have a persistent effect upon the serum agglutination test reactions, except when used on young calves (Mylrea, 1972). This test may be used as a screening test, but supplemental procedures should be performed on sera with titres.

The Complement Fixation Test

Many workers have reported on the superiority of the complement fixation test (CFT) over the agglutination tests in specificity and sensitivity. This test rarely exhibits non-specific reactions and is useful in differentiating the titres of calfhood vaccinations from those
due to infection. It is not necessary to use the complement fixation test on all sera but with titres on agglutination tests in screening procedures can be most accurately studied further with the complement fixation test.

The complement fixation test gives one antigen-antibody system a chance to react in the presence of added complement and a second antigen-antibody system is added to act as an indicator that is to see if a first reaction did occur. In the first system a standardized suspension of *Brucella abortus* is mixed with guinea pig complement and the bovine serum being tested. If the tested serum contains antibody to *Brucella abortus* the free complement will be 'fixed'. Thus no complement will be left available for the second system.

The second system consists of sheep red blood cells and antiserum (haemolysin) prepared against sheep red blood cells in rabbits. When the sheep red blood cells are exposed to haemolysin they are referred to as 'sensitized' sheep red blood cell. The complement is required to allow the haemolysin to disrupt the sensitised sheep red blood cells. Haemolysis will occur if the complement is available from the first system. Thus the second or indicator system reveals whether the first system did react.

The complement fixation test titres do not wane as the disease becomes chronic and often the complement fixation
test reaches diagnostic levels sooner than the serum agglutination test following natural infection (Blood et al., 1979).

Recent technical laboratory advances have allowed much greater speed and accuracy in performing the CFT. Te Punya (1971) and Elliott and Pullan (1972) have reported on the automated complement fixation test. In the Northern Territory, Australia, McPherson (1974) succeeded in developing a combined Rose Bengal test with a single dilution plate complement fixation test for the rapid screening of bovine brucellosis. He was able to detect 138 positive reactors out of the 139 positive reactors. However vaccination with 45/20 vaccine can influence the complement fixation test (Cunningham and Connor, 1971).

The Rose Bengal Test / Card

This test is a simple rapid test which detects early infection and can be used as an initial screening test. There are apparently a few false negatives (Mylrea, 1972). The rose bengal plate test was found to have three percent false positives and four percent negatives (Davis, 1971 In Cheah and Arunasalam, 1976). Several workers have suggested that the rose bengal test or card test be limited to a screening procedure.
Corbel (1972) found that the rose bengal test detected antibody to \textit{Brucella abortus} restricted to the IgG class. False negative reactions are due to residual antibody activity from vaccination, colostral antibody in calves, cross reaction with certain bacteria and laboratory error.

The Milk Ring Test

The milk ring test is the most practical and economical method for locating infected dairy herds and for surveillance of brucellosis-free herds. If performed on pooled herd milk in cans or bulk three or four times a year on each herd, it will detect the majority of infected herds. Herds with a positive milk ring test can then be examined by serological tests to identify the infected individuals.

Mylrea (1972) suggested that a combination of the bulk milk ring test, rose bengal and complement fixation tests could provide a good diagnostic cover for a herd of animals.

The major limitations of the milk ring test is the dilution factor which occurs in large dairy herds where large quantities of milk are stored in bulk tanks.

Other tests which may be used to diagnose brucellosis include the mercaptoethanol test, the acridine dye test, the antiglobulin (Coomb's) test, the fluorescent antibody
test, the vaginal mucus agglutination test and the heat test. These tests are not as popular as the serum agglutination, complement fixation, rose bengal, and the milk ring tests.

As can be seen above, serological methods of detecting brucellosis can be difficult with persistent latent Brucella abortus. No serological method is exclusively superior because results of tests on sera from culture positive cows are occasionally negative.

An experiment by Heck et al. (1981) to determine the Brucella abortus serologic profile, which related time since infection to antibody activity in serum, proved that the method used, enzyme-linked immunosorbent assay (ELISA) is capable of identifying Brucella abortus infected cows when results of conventional serologic methods are negative (See tables 2 and 3).
Table 2. Weekly serological results for sera collected from a cow experimentally exposed to *Brucella abortus* (Heck *et al.*, 1981).

<table>
<thead>
<tr>
<th>Week post exposure</th>
<th>Card</th>
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<td>0.22</td>
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<tr>
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Table 3. Interpretation of weekly serological results for sera collected from a cow experimentally exposed to Brucella abortus (Hock et al., 1981)

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*Doubtful

Figure 1. Big knees of cattle often associated with Brucella abortus infection (Hungerford, 1970)
Figure 2. Hygroma, opened, situated on the withers of a bovine carcase. Brucella infection has involved the bursa situated between the two funicular portions of the ligamentum nuchae at their point of attachment to the superior spines of the dorsal vertebrae. Hygromas may contain viable Brucella abortus organisms and 2 pts (1.2 litre) or more of fluid (Thornton and Gracey, 1974)
Figure 3. Placental smear showing a group of *Brucella abortus* organisms X 1500. Stained modified Ziehl-Neelsen. (Doxey, 1971)
III. THE BRUCELLOSIS INFECTION IN MAN

Epidemiology And Pathogenesis

Brucellosis is a typical zoonosis, a disease of animals transmissible to man but not transmissible from man to man in nature. Also called Undulant Fever, Malta Fever or Intermittent Fever, this disease is characterised by an acute septicemic phase followed by a chronic stage that may extend over many years and may involve many tissues. There is also an undulating fluctuations of the body temperature.

The distribution of Brucella abortus is world-wide. It is carried wherever cattle herds are introduced for breeding purposes. Brucellosis is known to exist in 32 of the 49 African countries (Thimm and Wundt, 1976). In Sweden Brucella abortus was the only source of brucellosis (Hansen, 1975).

Human infections arise through contact with infected animals or their discharges, through the handling of infected carcases or through consumption of infected milk or milk products (West, 1977). Accidental self-inoculation with strain 19 vaccine may also be another cause (Rowland, In Scott, 1978).

Swann et al. (1981) reported that some strains of Brucella abortus were able to survive temperatures of
above 65°C and were only killed at 85°C for 75 minutes. Thus Brucella strains in milk may survive in the low temperature pasteurisation process (65°C for 30 minutes) and the high temperature short time pasteurisation method (71.6°C for 15 minutes), and as such brucella will subsequently contaminate milk for human or calves.

Brucella abortus mainly occurs in farming communities and tends to be an occupational disease affecting farmers, butchers and veterinary surgeons. Cayton et al. (1975) who carried out serial brucella agglutination tests on veterinary students between 1962 and 1968 at Bristol University showed a steady rise in the number of those with a significant positive titre. Only seven percent of those with significant rise in titre reported symptoms suggestive of clinical disease. Cayton et al. (1975) attributed this to an increased exposure to farm livestock.

The organisms enter the body through the mucous membranes of the alimentary tract and abraded skin surfaces, through ingestion of infected milk or contact with infected tissues of animals (Duguid et al., 1978; Jawetz et al., 1978). Other routes include through the mucous membranes of the respiratory tract and the conjunctiva (Duguid et al., 1978). The organisms reach the bloodstream by way of the lymphatics. In the lymphatics tissue, liver, spleen, bone marrow and other parts of the RES,
granulomatous nodules form and the brucella organisms are situated intracellular. Osteomyelitis, meningitis or cholecystitis also occasionally occur (Jawetz et al., 1978).

Signs And Symptoms Of Infection

Brucellosis is an infection having no pathognomonic symptoms or signs. The incubation period was found to have a range of one week to seven months. According to Rowland, in Scott (1978) the infection in a non-immune person may give rise to an acute febrile illness after an incubation period of perhaps two to three weeks.

Brucellosis in man can range from a mild to severe acute illness and is frequently recurrent. The most prominent symptom is a fluctuation fever with temperatures rising variably during the day (Alton and Gulasekharam, 1974; Jawetz et al., 1978), and fall during the night accompanied by drenching. The intermittent fluctuations in the body temperature in a case report by Danusantoso et al. (1972) of a 15 month old boy whose blood specimens later proved positive for agglutinating antibodies against Brucella abortus can be seen from figure 4.

The onset is insidious with malaise, weakness, aches, muscular pain especially in the shoulder and neck regions, joint pains, anorexia and loss of weight. Complications accompanying brucellosis may consist of emotional or
Figure 4. Temperature pattern of a case report (human brucellosis) in Indonesia (Danusantoso et al., 1972)
nervous disturbances as well as frank lesions in viscera, bones and joints. Lymph nodes and spleen becomes palpable (Stewart, 1977).

Bone destruction, especially in the lumbar region may result in the narrowing of intervertebral spaces and which are difficult to differentiate radiologically from tuberculosis or pyogenic osteomyelitis. On healing there is deposition of bone from one vertebral body to the next and this is especially characteristic of brucellosis (Rowland, In Scott, 1978).

From the initial infection, a chronic stage may develop with signs of periodic exacerbations and manifestations like sweating, lassitude, and joint pains (Stewart, 1977), and complications such as meningo-myelitis, meningo-encephalitis, endocarditis, spondylitis and weakness, aches and pains and other psychoneurotic symptoms have been reported.

Unlike in the acute stage where there is usually bacteremia, in chronic brucellosis the organism cannot be isolated. Low antibody titres more frequently are indicative of chronic infections while high IgG antibody titre suggests brucella activity.

Abortion and mastitis appears to be a rare event in woman but orchitis which may relapse is not uncommon in man.
Diagnosis

The best way of achieving a positive diagnosis is to isolate the organisms of the patient; which can be cultured with difficulty from blood and urine samples. Liver and lymph node biopsy specimens can also be used. Subcultures should be prepared for at least six weeks before being discarded as negative (Jawetz et al., 1978).

In chronic brucellosis where it is difficult to obtain a positive blood culture, bone marrow culture by sternal puncture is said to increase the chances of a positive culture by 50 - 100% (Parnas, 1972 in Alton and Gulasekharam, 1974). However Stewart (1977) suggested that blood cultures may be carried out using liver extract broth or glucose serum broth or by the Castanedas method where a mixture of blood and broth is used.

The serum agglutination test may also be adequate to detect acute brucellosis where a high or rising titre accompanying appropriate symptoms maybe accepted as confirming the diagnosis. However it should be noted that individuals injected with cholera vaccine may develop agglutinin titres to brucella. Besides, in some cases the patient's serum may show a marked prozone, that is an inhibition of agglutination in the higher serum concentrations.

For chronic cases the anti-human globulin (Coomb's)
and the complement fixation tests are more useful. The skin test which consists of an intradermal injection of killed organisms or extracts (brucellin, brucellergin) as a means of diagnosis is considered unreliable although some consider it as a valuable epidemiological and diagnostic tool.

Treatment And Vaccination

In the recently acquired infection, tetracycline given at a daily dose for adults of one to two grams orally for three to four weeks is probably adequate. Although there may be symptomatic improvement complete sterilization of the patient's tissue by antibiotic therapy is difficult because of the intracellular location of the brucella organisms in the patient's tissues.

A combination of streptomycin and tetracycline has been reported suitable for the treatment of brucellosis (Williams, In Alton and Gulasekharam, 1974). Danus-santoso et al. (1972) reported having treated a patient with 200 milligram streptomycin daily for five consecutive days and was unable to culture Brucella abortus in blood cultures later.

Vaccination is given by skin scarification to those who have been shown to be negative to the skin test. However side effects and hypersensitivity develop and vaccination is not recommended.
IV. THE TRANSMISSION FROM ANIMALS TO MAN

Contact with infected cattle or their tissues or fluids allows Brucella abortus to invade microscopic abrasions of the skin and the mucous membranes. The organisms usually localize in the uterus, placenta and mammary gland. Therefore they commonly leave the host via the vaginal and teat canals. Foetal membranes, vaginal discharges and foetus from infected animals may be heavily contaminated.

Abortion, although a frequent manifestation does not occur in all infected animals, and even following a normal gestation period it is possible for animals to shed large numbers of the organism. Handling newborn animals or foetal membrane is a common direct method for transmission to man.

Ingestion of contaminated milk, cream, cheese or other dairy products made from raw milk containing brucella organism is another source of infection. The incidence of brucella organism in raw milk depends largely upon the level of the disease status in the dairy herd population. In some areas of Great Britain, as many as ten percent of milk samples contains Brucella abortus (West, 1977). During the first month or two after calving, a large number of organism is expected in the milk of the infected cow.
**Brucella abortus** dies out fairly rapidly in raw milk held at room temperature (Smith, 1934, and Pullinger, 1935 *In* Rammell, 1967). Brucella are therefore clearly of importance in dairy products made from milk that has not been adequately pasteurised.

Pickled meats and other uncooked foods contaminated by excretions of infected animals have also been incriminated. However, brucellosis in man is only rarely acquired by eating infected meat as cooking destroys the organisms.

A great hazard of exposure to infection is due mainly to special types of contact such as marketing, castrating and handling of newborn or aborted bovine foetus, placenta or uterine discharge by farmers or veterinarians. Veterinary students, due to increased exposure to farm livestock, showed significant positive titre in brucella agglutination tests (*Cayton et al.* 1975). Another factor which is of importance in brucellosis infection in veterinarians is carelessness which can lead to accidental injection.

Inadequate performance of diagnostic procedures to detect brucellosis may lead to the release of reactors and latent carriers (*Dolan, 1980 and Lapraik, 1982*) into new clean herds. In this way animal infection will propagate and the potential for human infection will therefore increase.
The prevalence of brucellosis in wild life is particularly important because of the ecologic overlap of wild animals with cattle. Swann et al. (1980) detected *Brucella abortus* biotype 1 in opossum (*Didelphis marsupialis*) and raccoon (*Procyon lotor*). Abortions and retained placentas have been observed in bison (*Bison bison*) herds and additional brucella isolates have been made from bulls with orchitis (Gorner and Conell, and Tunnicliff and Marsh in Moore and Schnurrenberger, 1981). Other known animals involve in brucellosis infection as described by Moore and Schnurrenberger (1981) include amongst others the elk (*Cervus canadensis*), white tailed deer (*Odocoileus virginianus*), moose (*Alces alces*), water buck (*Kobus ellipsiprymnus*), eland cow (*Taurotragus oryx*), and in non ruminants like the Norway rats (*Rattus norvegicus*), hyena (*Crocutta crocutta*), striped skunk (*Mephitis mephitis*), black bear (*Ursus americanus*) and others.
V. CONTROL AND ERADICATION IN CATTLE

Control And Eradication

Brucellosis is primarily a disease of animals, with man an inadvertant and end host. Eliminating the disease in man depends ultimately upon eradicating infection from animals. Reducing the incidence of human brucellosis is contingent upon preventing the transmission of the organism from the food-producing animal reservoirs to the consumers.

The principles of minimizing transmission can be done through efforts such as segregation of preparturient animals and keeping them separate for a week or more after parturition, prompt disposal of aborted foetuses and other materials both in intensive husbandry and in traditional systems. Carriers are marked and slaughtered or segregated to remove sources of infection, and vaccination protects the susceptibles and reduces spread by lowering of abortion rate.

The program of eradication may involve any geographical area or political unit depending upon economic and other circumstances such as the variety of systems of husbandry, range of climatic conditions and variability in prevalence of infection, as well as in the availability of financial resources all influence what can be expected in control efforts.
The program of eradication maybe considered a success in a limited area where the program is carried out, but such brucellosis-free herds in high or moderate prevalence areas are at a great risk of reinfection from neighbouring herds. Continued vaccination, attention to hygiene and acquisition of replacement animals from brucellosis-free herds or areas are all essential to maintenance of brucellosis-free status.

Likewise, the effectiveness of immunizing agents in preventing abortion is related to the quantity and virulence of organisms an animal may encounter during exposure. Massive exposure will overcome a previously induced immunity. Thus, the use of immunizing agents helps to control the spread of infection but additional measures are necessary to achieve complete eradication. In the United States of America a retrospective study reveal that only in those states where 40% or more of eligible calves were vaccinated could eradicate brucellosis (Jones and Berman, 1976).

Vaccination With Brucella abortus Strain 19

An attenuated strain for immunization with the following conditions are required:–

a. it should not produce disease in the vaccinated animal
b. it should not give rise to any latent disease which might be transmitted to other animals
c. it should have minimal effect on diagnostic tests
d. it is not pathogenic to man
e. a high level of protection against field infection is offered
f. it should be of an attenuated virulence with such stability that a return to virulence would be extremely unlikely
g. the cost of production is reasonable.

The living attenuated *Brucella abortus* strain 19 is classified as biotype 1 but differs from other biotype 1 strain in that:

1. it does not require added carbon dioxide for growth
2. it is more sensitive to penicillin
3. it is inhibited, rather than stimulated by erythritol
4. it is less virulent for guinea pigs.

Thomas *et al.* (1981) describes in detail a study of the characterisation of the above properties. Strain 19 *Brucella abortus* has a low virulence (Thomas *et al.*, 1981) and is incapable of causing abortion except in a proportion of cows vaccinated in late pregnancy. It can
cause undulant fever in man. Vaccinated animals have a high degree of protection against abortion and 65 - 75% are resistant to most kinds of exposure (Blood et al., 1979). The rest of the vaccinated animals may become infected but usually do not abort.

After infection with virulent strains or vaccination with attenuated live *Brucella abortus* strain 19, antibody to the IgM class is first to be detected in the serum, followed by the IgG class. Using the enzyme-linked antiglobulin test, it was found that a reduced dose of strain 19 vaccine produced the IgM response after nine days (Anon., 1981). The type of serological test used influences the titre reported, as the immunoglobulin classes and subclass differ in activity depending upon physical conditions.

The size and virulence of the inoculum and the stage of pregnancy of the animal determined the first appearance of antibody. Strain 19 vaccinated animals had peak levels of serum agglutinin at four weeks and complement fixing antibody between four to eight weeks (Joseph et al., 1976). On the average antibody reaches diagnostic titres four weeks after exposure during the fourth and sixth month of gestation and at about ten weeks after exposure in non-pregnant animals or animals in the first trimester of gestation (Berman, Ristic, 1981).
Preparturient animals concentrate serum immunoglobulin especially IgG in colostrum, and previously vaccinated cattle with low serum antibody titres have high colostral titres (Corner and Alton, 1981) and early postparturient milk antibody titres.

The magnitude and duration of antibody response following vaccination with Brucella abortus strain 19 is directly related to the age at vaccination and the number of organisms administered. Joseph et al. (1976) studied the serum antibody response following calfhood vaccination with live strain 19 and found that the complement fixing antibodies were discernable after about five months postvaccination. The serum agglutination and complement fixation yield after strain 19 vaccination in his study can be seen from figure 5. As such vaccination of calves between ages of three to six months have no influence on the interpretation of routine serological tests for the diagnosis of brucellosis in cattle. Only a very small proportion of animals (1:100000) inoculated resulted in persistent systemic infection after calfhood vaccination with strain 19 (Thomas et al., 1981).

However revaccination with strain 45/20 on former strain 19 vaccinates interferes with the complement fixation test; and also vaccination of adults with strain 19, if not properly identified would cause problems in the serological diagnosis of brucellosis (Joseph et al., 1977).
Figure 5. Serum agglutination test and complement fixation test titres following S 19 vaccination (Joseph et al., 1976)

Figure 6. Serum agglutination test and complement fixation test titres following 45/20 vaccination (Joseph et al., 1976)
In animals vaccinated at three to eight months of age with $5 \times 10^{10}$ viable organisms, antibody is usually detectable within two weeks and drops to a very low level of four to six months later. For adult cattle, Corner and Alton (1981) recommended a lower dose of $3 \times 10^8$ viable cells for whole herd vaccination.

Vaccination of bulls is not recommended because of possible persistent agglutinins, persistent excretion of the organism via the genital tract, the development of orchitis and lowered fertility.

Vaccination With Strain 45/20

Strain 45/20 in adjuvant maybe use to overcome the disadvantage of persistent agglutination titre which occurs after vaccination with strain 19 vaccine. It is a dead vaccine, and can be used both for the adult vaccination of male and female cows.

Cattle vaccinated with Brucella abortus strain 45/20 adjuvant vaccine developed agglutinins for rough Brucella antigens which are largely of the IgG classes (Corbel, 1976).

The use of strain 45/20 Brucella abortus vaccine reduces the risk of infection in adult animals and lessen the abortifacient effect if administered in the early stages of established disease. Joseph et al. (1976) studied the serum antibody response following
calfhood vaccination with killed strain 45/20 adjuvant vaccine and the serum agglutination and complement fixing antibody titres (figure 6) can be compared to that after strain 19 vaccination. Jospeh et al. (1977) and Ray (1976) also showed that a booster dose of strain 45/20 to previously strain 45/20 vaccinated cattle did not produce any significant rise in agglutinin titres. Thus there appears to be no significant difference in the level of protection for cattle when ten or twelve weeks interval between doses were used in cattle of six months of age or older (Ray, 1976).

One disadvantage of the strain 45/20 vaccine is that severe reactions up to eight centimetres in diameter at the site of injection is not uncommon (Blood et al., 1979). Furthermore this vaccine is expensive and is not completely non-agglutinogenic though titres in serum rarely reach inconclusive levels.

Other Vaccines

In Russia control of brucellosis using strain 19 vaccine was reported to be unsuccessful, and research into other vaccines from strain 82, Neveskiy-12 and Rev-I is being undertaken (Talov and Mikheyev, 1977).

Brucellosis is one of the bacterial infections in which effective antibacterial defence is considered to require participation of cell-mediated immune reactions,
which are presumed to operate through activation of tissue macrophages after recognition of specific antigens. A different pattern for humoral response and the cell-mediated immune response after vaccination with *Brucella abortus* strain 19 was reported by Kaneene et al. (1979). Kaneene et al. (1979) also indicated that the lymphocyte stimulation test (an in vitro measurement for cell-mediated immune response) might be useful in detecting cattle with persistent residual titres due to *Brucella abortus* strain 19 vaccine.

Prevention And Eradication By Test And Slaughter

After a successful calfhood vaccination programme where the level of infection on an area has been reduced to below four percent, eradication by the test and slaughter policy may be undertaken (Blood et al., 1979). Brucellosis control areas must be established and testing and disposal of reactors and their calves are carried out.

Infected herds are quarantined and retested at intervals until negative. In Indonesia the milk ring test is performed three times at intervals of four months and then a final serological test six months after the last milk ring test must prove to be negative in order to declare a herd brucellosis-free (Anon., 1977).

In heavily infected herds, complete depopulation is often necessary, but economic factors sometimes have to
be taken into account. Often where there is an abortion storm, the disposal of reactors may be unsatisfactory because spread is faster than eradication. In these circumstances vaccination of all non- reactors is recommended or if testing is impossible, all cows are vaccinated.

In lightly infected herds where infection from surrounding areas is likely to occur, vaccination of calves may be introduced and immediate culling of positive reactors may be carried out. After a brucellosis-free status herd is achieved only negative animals from brucellosis-free herds may be introduced for any replacements.

In light of the test and slaughter policy an educational program to promote herd owners understanding of the importance and problems of brucellosis is also necessary. Management must be such that it is motivated at eradicating the brucellosis disease permanently. Vaccination histories, proper testing of blood and milk samples regularly and quarantine facilities are also important. The development of a two herd system based on segregation of weaned heifer calves from adult cows and maintenance of testing pressure on the adults will reduce the chance of infection of heifers. The movement of animals from infected herds to uninfected or brucellosis-free herds need also to be considered in eradication.
In artificial insemination of cows, note should be taken that only semen from brucellosis-free herds is used. A modal for marking and culling of cattle is described by Toma (1975).
VI. CONCLUSION

After a detail description of the diagnosis and control methods in cattle brucellosis in the preceding chapters, the following points conclude this writing:

1. Vaccination of calves with strain 19 at the early ages of three to six months have no influence on the interpretation of routine serological tests.

2. No one serological method of diagnosis is superior to the others. For dairy herds the milk ring test might be practical and economical; serological tests like the serum agglutination test should also be performed on positive milk at the milk ring test to identify the infected individuals. A continuance of blood and milk testing for brucellosis is sure worthwhile for prevention.

3. Transmission of brucellosis from cattle to man can be reduced by reducing contact with infected animals and their discharges, and also observing the consumption of only pasteurised milk and the careful handling of infected materials by veterinarians.

4. There is no reported data on the successful treatment of cattle brucellosis. Treatment is not usually undertaken as antibiotics are unable to sterilize the infected host due to the intracellular characteristics of Brucella abortus.
5. Vaccination of calves with strain 19 is a valuable aid in brucellosis control as it offers a fairly good protection, and the 'overkill' in infected herds due to the test and slaughter policy is greatly reduced. The problem of persistent titres can be overcome by early calfhood vaccination.

6. The strain 45/20 vaccine is not encouraged in eradication programmes when testing and culling/slaughter are essential ingredients because a booster vaccination of strain 45/20 on former strain 19 vaccinates produces a high level of agglutinating and complement fixing antibodies.

7. A consistent vaccination policy and proper identification of vaccinated animals is important when the control and eradication is to be implemented.

8. Introduction of new replacements into brucellosis-free herds or the buying of cattle should discriminate between brucellosis-free and infected or latent diseased animals.

9. Movement of animals should be properly checked especially during periods where abortion storms occur.

10. An educational or extension-work programme aimed at providing the knowledge to cattle owners with regard to sanitation, disease diagnosis, control and vaccination programmes and other topics, could be an additional factor required to enhance complete eradication.
REFERENCES


Anonymous. 1981. Diagnosis and control of regulatory diseases : Bovine Brucellosis. CSIRO.


