AGRICULTURAL PRODUCTION - PHASE II

INDONESIA

IMPLEMENTATION OF ELISA FOR BRUCELLOSIS AT DGLS LABORATORIES IN INDONESIA

UNITED NATIONS DEVELOPMENT PROGRAMME

INTERNATIONAL ATOMIC ENERGY AGENCY

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IMPLEMENTATION OF ELISA FOR BRUCELLOSIS AT DGLS LABORATORIES IN INDONESIA

Report prepared for the Government of the Republic of Indonesia

by

the International Atomic Energy Agency
acting as Executing Agency for the United Nations Development Programme

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International Atomic Energy Agency

Vienna 1992
Title: Implementation of ELISA for Brucellosis at DGLS Laboratories in Indonesia.

Project Number: INS/3/021 (INS/88/013) Task 11-67

Project Title: Animal Production Phase II, Disease of Livestock, ELISA in diagnosis of Brucellosis

Expert: Dr. Barry Patten B.V.Sc. Ph.D., Bogor, Indonesia

Terms of reference:

1. Access the current status of activities in regards to Brucellosis testing at the Regional DICs of the DGLS

2. Assist counterpart in establishing and validating the ELISA technique for detecting Brucella antibodies

3. Develop workplans for counterparts at DICs to continue with epidemiological studies on brucellosis

4. Advise IAEA on future requirements for equipment, experts and training

Background Information:

A training course was held in February 1989 in the technique of ELISA with particular reference to Brucellosis. Equipment was provided by the UNDP Project through IAEA to BATAN for distribution to a number of the DIC laboratories of the DGLS. Unfortunately the computers supplied at the time were configured to the German standards whereas the Indonesian country standard is English configuration and the printers were for 110 volt use whereas all other equipment was for 220 volt - 240 volt use. Changes were made to the equipment and printers and the materials were ready for release from BATAN to DGLS in February 1990.
Equipment was reportedly tested at BATAN prior to packing and handing-over to a representative of the laboratories concerned or to a representative from Directorate Kesehatan Hewan (Keswan) Directorate General Livestock Services (DGLS).

Equipment was provided to the following laboratories:

- Plate Reader, Computer, Printer, Voltage Stabiliser - BPPH Denpasar Bali
- Plate Reader, Computer, Printer, Voltage Stabiliser - BPPH Maros South Sulawesi
- Plate Reader, Computer, Printer, Voltage Stabiliser - PusVetma Surabaya Java

Ancillary equipment consisting of reagent basins, 8 channel pipette, 5-50 ul pipette and 50-200 ul pipette were supplied to Denpasar, Maros and Surabaya. A Handiwasher unit was supplied to Maros and Denpasar.

The equipment for Maros was handed-over to Dr B Patten on Wednesday March 7th at BATAN at the time the remaining computer was handed over to Drh Gde Sudana Dir Keswan DGLS. The equipment for Maros was consigned by Dr Patten by ELTEHA (Voltage Stabiliser) and by Garuda airlines as unaccompanied baggage (Printer, Plate Reader, Computer CPU and VDU) to Ujung Pandang.

Equipment for Denpasar and Surabaya was handed over to Drh Alit from Denpasar.

Equipment for Yogyakarta was handed over to Drh Gde Sudana from Dir Keswan DGLS.

Cables to connect the RS232 serial port on the Plate Reader to the computer were missing/not present. Dr Patten at the request of Dr Hendratno BATAN arranged for a local computer shop to fabricate x 4 24 pin connectors cables according to the specifications listed in the Titertek manual. The original manual for the Titertek MKII model supplied could not be located, a manual for a different version of the MKII was provided from BATAN and was copied by Dr Patten for distribution to the laboratories. Subsequently a manual for the correct version of the MKII was located and received by Dr Patten on March 27th. The manual was photocopied by Dr Patten and distributed to the
Faulty Plate Readers: The Titertek Plate Readers sent to DIC Yogyakarta and to Pus Vetma Surabaya were found to be faulty when tested at the laboratories. Both units were returned to Bogor for further testing. Several facsimile messages were sent to IAEA and Labtechnic Germany for advice concerning the warranty on the units and for solutions to the problems. Flow Laboratories Sydney Australia supplied details of the inbuilt self-test routine for the units following a written request from Dr Patten. The provision of the self-test routine was of great benefit in diagnosing and correcting the faults in the units.

Yogyakarta Plate reader: The testing of the machines indicated that one light detector (channel C) on the MACU-02 board was not recording any light. A replacement board was sent by Labtechnic Germany and received at Bogor on May 16th. The board was fitted to the unit by Dr Patten at Bogor. After a final adjustment to the Analog/Digital gain value on the new board the unit was despatched to the DIC laboratory by local carrier (May 28th 1990).

Pusvetma Plate reader: The testing of this unit indicated that no white or black light was being detected by the light detectors on the MACU-02 board. A new board was despatched through Labtechnic and received in Bogor on June 8th. The board was fitted and tested by Dr Patten, however the self test routine indicated that the new board was receiving or recording too much light. The cause of the problem was still being accessed as at June 14th by Labtechnic and Flow Laboratories Germany.

Work Program:

March 7th, 1990: Visit to BATAN Jakarta to discuss the distribution of the equipment with Dr Hendratno. Dr Patten accepted for delivery to DIC Maros the computer and Plate Reader equipment, 2 ELISA kits from IAEA for delivery to DIC Wates-Yogyakarta and DIC Maros and ancillary equipment consisting of Pipettes, 4 band tips and reagent basins accepted for distribution to each of the centers receiving equipment.
Discussions were held with Or Gde Sudana and Or Hendratno concerning the possibility of PusVetma producing reagents for the test locally so that the laboratories would have a sustainable supply of reagents. Dr Patten indicated that some reagents were presently produced at Balitvet however the limitation with staff numbers meant that the supply could not be guaranteed. Dr Sudana agreed that as PusVetma was producing Brucella vaccine then the center should be able to produce an ELISA antigen. Dr Patten agreed to provide any technical assistance required to PusVetma should they request it.

March 9th, 1990

Visit DBLS Jakarta for discussions with Drh Gde Sudana and Drh Hutabarat concerning the visit to the laboratories and PusVetma.

The establishment of a Standardised Brucella Database to record serology results from the laboratories was discussed. It was agreed that a database based on Panacea should be sent to the laboratories. The database would be returned every 3 months to Keswan for tabulation and analysis. Dr Hutabarat agreed to have a letter drawn up by Dr Koswara for the Kepala Balai of each BPPH advising them of the requirements to record and submit the results for Brucella serology.

Dr Patten suggested that if possible the laboratories should also test any cattle serum samples received for Brucella antibodies as it appeared that many samples were not being tested. Dr Sudana agreed that this would be a cost-efficient means of obtaining data on the prevalence of Brucellosis in the country. The problems associated with the reporting of the results to Dinas Peternakan were discussed however Dr Hutabarat indicated that a letter could be issued from Keswan advising that the Brucella serology results were for the purposes of surveillance only and that no action for the present would be taken on the results. Dr Patten indicated that he considered it would be counter-productive if animals were slaughtered without compensation on the basis of the surveillance results when effective control programs were not yet in place.

Visit UNDP and Buana Travel (Travel agent handling UNDP business) to attempt to finalise details concerning the issuing of an airline ticket. UNDP had not received full authorisation for a ticket from IAEA but supplied Dr Patten with a photocopy telex for submission to Buana Travel to issue the ticket.
March 15th, 1990: Visit DGLS to collect letters for the Kepala Balai of the laboratories from the Director Keswan concerning the testing of cattle serum samples for Brucellosis.

Visit UNDP/Buana Travel to try and obtain airline tickets. UNDP maintained that they had not received authorisation from IAEA to issue an airline ticket. Dr Patten suggested that a telephone call advising him of the difficulties would have been of value to advise him of the problem, or alternatively UNDP should have contacted IAEA. An airline ticket was finally issued after Dr Patten had supplied a declaration to the travel office UNDP stating that he had been advised by IAEA that they would provide an airline ticket for the consultant. A letter and duplicates of the documentation from IAEA to Dr Patten was later submitted in support of the declaration.

Travel to Soekarno-Hatta airport to despatch by unaccompanied baggage goods for DIC Maros South Sulawesi. Return to Bogor.
March 16th, 1990: Depart Bogor 5am for Soekarno-Hatta airport for travel to Yogyakarta.

Visit to BPPH Laboratory Wates-Yogyakarta

Date of visit

March 16th arrive from Jakarta 8:00am
March 18th depart for Surabaya, 6:00am
March 26th arrive from Denpasar 7:30am, depart for Jakarta 4:00pm.

Contact Person:

Dr Budi Kepala Balai
Drh Tuti Scientist, serology section

Equipment supplied:

A Titertek Plate Reader and a Voltage Stabiliser had been previously delivered from Jakarta by Dr Tri Virology section. The Plate Reader was attached to a Brother printer through the serial port but was not printing-out properly. The unit was connected to an IBM XT compatible computer through the serial port and was sending Data to the computer. A fault was noted with the read-out from Channel C where all values were recorded as 0.0000. Contact was made through Australia to Flow Laboratories however the service technician was not available to advise on correction to the Unit. The unit was collected by Dr Patten on March 26th and returned to Jakarta for servicing.

ELISA test:

An Immunoassay Kit for Brucellosis supplied by IAEA was delivered by Dr Patten. The Kit was unpacked, the Tween 20 container had leaked but the tween was contained within the outer plastic wrap around the bottle. The positive control serum was poorly labelled only having a code and date on the glass vial. The LPS antigen batch 10 supplied was labelled for dilution at 1:1600 whereas the manual suggested dilution at 1:3200. The conjugate bottle label did not list a batch or dilution and so the method and dilution factors listed in the manual were followed.
laboratory. The plates were coated with the dilution of antigen recommended on the bottle (1:1600). The titration of the positive and negative serums produced a slow colour change in the test, using 100 ul substrate, that was readable after 40-60 minutes rather than the 10 minutes listed in the Manual (stopping reagent was not used). The pH of all solutions were checked and found to be:

- Citric acid buffer pH 4.5
- Coating buffer pH 9.2
- PBS-Tween pH 7.5
- Aqua dest pH 6.9

The substrate and conjugate appeared to function properly when added to a test tube at the rate of 10 ul of 1:320 dilution of conjugate to 1 ml substrate buffer. A test was set up using continuous shaking at room temperature and at 37°C with shaking every 15 minutes. The room temperature test appeared to give much better uniformity of colour reaction than the 37°C test. Both developed colour only after 30-50 minutes and not after the recommended 10 minutes reaction time (using 100 ul substrate and no stopping reagent).

A plan to do a titration of antigen and conjugate using aqua dest from a glass still and sterile PBS from the virology laboratory was left with Dr Tuti. The results of the test were then discussed with Dr Tuti on the return of Dr Patten to the laboratory on March 26th.

**Brucella activities**

A letter concerning the testing of serums for Brucellosis from the Director Keswan was delivered to Dr Budi. A diskette with a database formatted for Panacea and instructions for use were given to Dr Tuti. The database and its use were discussed with Dr L McClure of the CIDA-DIC Yogyakarta project. The reporting of results from the Brucellosis survey were discussed with Dr Budi and Dr McClure. Samples being received by the laboratory include samples from the Model District Project Boyolali, routine samples from Dinas Peternakan and samples from the Quarantine section. It was estimated that up to 1,000 serum samples per month may be received from cattle.

An active service project aimed at sampling animals in each Kabupaten in Java is also proposed which would provide a
selection of serums from Java for testing.

One difficulty evident within the laboratory system at Wates was that whilst the laboratory has a separate serology laboratory this laboratory appeared to perform only a portion of the serology in the laboratory. The serology section was equipped and performed the ELISA for brucellosis however the RBPT and CFT serology was performed in the Bacteriology laboratory by the bacteriologists and the ELISA for New Castle Disease virus (NDV) and some bovine viruses was performed in the virology laboratory whilst the serology laboratory performed the HAI test for NDV. The situation was confused and staff in one laboratory were unaware as to what their colleagues were doing. The organisation and division of responsibilities for serology need to be addressed within the laboratory.

Follow up of visit to DIC Wates-Yogyakarta

(i). The Titertek MKII plate reader was collected on March 26th and returned to Bogor. Contact was made with IAEA Vienna by fax and a follow-up to the previous request for information from Flow Sydney Australia was made. A self-test routine for the unit was supplied by Flow Australia however the technician recommended that the unit be returned to a qualified repair person for a thorough examination. He also mentioned that a good case could be made for the service to be covered by warranty. Subsequent testing of the unit indicated a possible fault with the detector for channel "C" as no light was being registered on that channel. The test results were forwarded by fax to Flow Laboratories Sydney with a request for advice concerning repairs to the unit. Further tests indicated that the fault lay in the MACU-02 board which contains the photocells and associated electronics.

(ii). Diluents and a conjugate used at the DIC in ELISA were supplied to Balitvet and were used in an ELISA for Brucellosis at Balitvet. The results of the test at Balitvet indicated that the diluents were having no adverse effect on the performance of the test.

<table>
<thead>
<tr>
<th>Serum Diluent</th>
<th>Bvet</th>
<th>Bvet</th>
<th>Yogya</th>
<th>Yogya</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pos/Neg ratio 15min</td>
<td>5.1</td>
<td>6.0</td>
<td>5.2</td>
<td>6.4</td>
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<tr>
<td>Pos/Neg ratio 30min</td>
<td>5.2</td>
<td>6.1</td>
<td>5.6</td>
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</table>
Visit to PusVetma Surabaya

Date of travel:
March 18th arrive from Yogyakarta 08:30am
March 20th depart for Ujung Pandang South Sulawesi 02:00pm

Contact Persons:
Dr Soenardi Director Vetma

Equipment Supplied:
A Plate Reader, computer, printer and voltage stabiliser were previously delivered by Drh Alit.

The equipment had been unpacked and assembled. The earthing had not been connected properly and this was corrected. An RS-232 connecting cable was supplied by Dr Patten along with a manual for the Plate Reader. The equipment was checked and all expect the Plate Reader was found to be functioning properly. Whilst the Hard disk had been loaded no systems operating disks were provided with the computer.

The Plate Reader was giving a ++No Light++ signal after measuring. The lamp and optics appeared to be functioning properly. Further repairs could not be effected at the laboratory.

Activities in the Brucellosis area

The Research and Development area was presently working on the standardisation of MRT and CFT reagents for the diagnosis of Brucellosis. A production problem concerning low cfu counts with the Strain 19 vaccine was also being addressed. Problems with the stability of complement were discussed and Dr Patten stressed the need to provide ample green feed/vitamin C to the guinea pigs and to keep the blood/serum chilled once collected.

Field trial evaluations were being conducted on MRT reagent although the results were not being correlated with serology or culture results. A discussion was held concerning the collection of specimens for the confirmation of MRT. The possibility of the
production of a Brucella ELISA antigen at the laboratory was discussed with Dr Soenardi who indicated that he had no objections to Vetma producing the antigen. Dr Patten outlined the steps required in the production of smooth Lipopolysaccharide antigen from a phenol-saline suspension of B. abortus cells. The method was that outlined in the standard methods for the diagnosis of Brucellosis published by the Australian Department of Primary Industry.

A 2 hour discussion was held with the staff on the production of antigen and on the titration of the antigen for the ELISA test. The production of a positive control serum was discussed. The staff indicated that Vetma had a "National Positive Control Serum" for Brucellosis which contained 1000 iu/ml of Brucella antibody. Discussion also involved the differentiation of vaccinated and non-vaccinated animals and the production of anti-species antiserum and conjugation of antiserum. It was apparent that the laboratory had limited facilities for immunochemistry which would hamper the production of conjugates.

Follow-up to visit to Vetma

(i). The faulty Plate Reader was returned to Bogor on April 9th for checking and to organise repairs. The self test routine supplied by Flow laboratories Sydney indicated that the Reader was not registering any white or black light, further testing indicated that the problem lay in the MACU-02 board which contains the photocells and associated electronics.

A replacement MACU-02 board was received by Dr Patten from UNDP via Batan on June 9th. The board was fitted to the unit which was then tested. A fault was still present in that the unit was receiving or recording too much white light. A further request for information was sent to Labtecnic Germany on June 11th.
Visit to BPPH Wil VII Maros, South Sulawesi

Date of Visit:

Tuesday 20th March arrive from Surabaya 4:30 pm
Depart to Denpasar Friday 23rd March 5:00 pm

Contact Person:

Drh Isep Sulaiman deputy Kepala, Bacteriologi/serology laboratory

Equipment supplied:

Equipment comprising a TiterTek MKII, an Amstrad 640K - 20MBHD and an Epson LX-850 printer consigned by unaccompanied baggage air-freight and a voltage stabiliser consigned per ELTEHA to the laboratory were unpacked and assembled. The cable to connect the printer to the computer and a power cable for the Plate Reader were missing from the boxes. Substitute cables were obtained from a local source. Two Sul-50ul pipettes had been supplied instead a 5-50 and a 50-200ul pipette. The manual for the Plate Reader although for a MkII was not for the model supplied. No systems diskettes were supplied with the computer unit although the system had been loaded onto the Hard disk. The equipment was tested and found to be functioning properly.

ELISA test:

A 2 hour seminar was conducted by Dr Patten for the staff of the laboratory on Day 1 in which the fundamentals of the ELISA test were discussed. The ELISA reagents provided by IAEA were unpacked and checked. The Brucella LPS antigen (batch 10) was reconstituted to 1 ml in sterile distilled water and a 500ml volume of coating buffer was formulated. The laboratory had no microtitre plates suitable for the ELISA and so some Nunc round bottom plates which had been supplied courtesy of ACIAR PN8382/8907 Balitvet were coated with a 1:1600 dilution of the LPS antigen. The recommended dilution of 1:1600 was taken from the bottle as the manual indicated a dilution for the antigen of 1:3200.
The antigen coated microtitre plate was processed on Day 2 using 2 fold dilutions of a positive serum and the negative serum supplied. The conjugate was diluted as recommended to 10ml and dispensed in aliquots for storage at -20°C.

A Flow Handiwasher supplied by IAEA was set up and a vacuum pump was connected via a glass reservoir to the washer. Unfortunately the vacuum inlet on the washer was faulty and the vacuum developed by the pump caused the glass reservoir to implode, no injuries were experienced by any of the staff involved. The washer was disassembled and the fault corrected, an attempt was then made to connect a plastic reservoir but this collapsed under the vacuum. The washer was subsequently used without any vacuum attached and the wash solution was discarded by hand from the plates.

The results of the test indicated that the conjugate supplied with the kit (no batch number included on the bottle) when diluted at the recommended dilution of 1:3200 had a high background absorbance to the antigen coated wells (conjugate control wells).

A 2 hour discussion was conducted with the scientific staff at the Laboratory to discuss the results and to work through the possible problems associated with the test.

The ELISA test was repeated on Day 3 using microplates coated with a 1:1600 dilution of antigen, together with a plate with antigen coated at a dilution of 1:1600, 1:3200 and 1:6400 in Coating buffer. The conjugate was diluted in PBS-Tween as control, PBS-Tween with 0.2% skim milk and PBS-Tween with 0.2% casein hydrolysate (Difco Laboratories). The control conjugate again produced a high background absorbance in the conjugate control wells, the absorbance was reduced with the increasing dilution of antigen as was the absorbance of the positive serum control. The casein hydrolysate diluent had no significant effect on the background absorbance. The skim milk powder diluent reduced the background absorbance of the conjugate to the antigen coated plates however the specific activity of the conjugate was also reduced.
The problem of the background absorbance was discussed with Isep and a program to titrate the antigen and conjugate was planned. A sample of conjugate and an antigen coated plate was returned to Bogor for further testing.

Activities in the Brucella area:

During 1989 267,663 serum samples were collected in the field by Dinas Peternakan for testing for Brucella antibodies. The samples were tested in the field by RBPT using an antigen produced by PusVetma Surabaya. Any positive samples were forwarded to the BPPH Maros for confirmatory tests.

The samples were further tested at Maros by RBPT using antigen from Surabaya and from Commonwealth Serum Laboratories (CSL) Melbourne Australia. The RBPT positive samples were tested in the CFT test using antigen and complement from CSL Australia, complement produced locally and haemolysin produced locally.

The laboratory found that 50%-70% of the field RBPT +ve samples were negative by RBPT in the laboratory. From the 267,663 samples collected 2312 were designated positive Brucella infected by the DIC using the CFT as the definitive test.

Serum sampling by Dinas Peternakan has now ceased. Serum samples are presently being received from the Quarantine testing of breeding animals being 'exported' from South Sulawesi, this amounts to approx 1500 samples per month. The samples are tested by RBPT and the result confirmed by CFT.

A vaccination program has been undertaken in association with the serum sampling by Dinas Peternakan. During 1989 animals < 8 months of age were vaccinated, during 1990 it is apparently intended to vaccinate all ages of cattle with a 1:40 dose of PusVetma Strain 19 Brucella vaccine. At present vaccinated animals are not being marked to indicate their vaccination status and no preference or selection for vaccination is being given to the more highly infected Kabupaten.

The laboratory staff were unsure as to whether any further sero-surveillance was to be undertaken. It was suggested to Dr Isep that he enter into discussions with the Kepala Dinas Peternakan to provide a technical input into the problems associated with the vaccination program and to advise people of the difficulties.
encountered in sero-diagnosis for Brucellosis on animals with an uncertain vaccination history.

Visits have been undertaken by staff from the DIC to the local abattoir to collect lymph node samples from cattle. The prescapular, supramammary, retropharangeal and mesenteric lymph nodes were collected. A total of 44 sets of samples from sero-positive animals have been collected and whilst not all tissues have been cultures a total of 17 positive cultures have been isolated. The serology from the 44 animals was that all were positive by RBPT, 2 were negative by CFT, one as AC and the remainder positive by CFT. Dr Isep was asked to test all serums in the ELISA for comparison of the results.

A letter from the Director Keswan was provided to Dr Isep concerning the testing for Brucella antibodies of all submitted cattle serum samples. Dr Isep considered that this would pose no problems for the laboratory. It was agreed that all RBPT positive samples be tested by CFT and ELISA and that where possible all samples be tested by ELISA and any positive be tested by CFT.

A Diskette containing the Brucella Database was given to Dr Isep along with the instruction sheet for entry of Data. Panacea was not available in the laboratory except on an IBM computer with 3.5in disk drives and so the database could not be immediately demonstrated.

Follow up of visit to Maros:

(i) The cable for the printer and Plate reader were located in a computer CPU box which had been delivered to Dit. KeswanJakarta from BATAN. The laboratory at Denpasar had received 2x 50-200ul pipettes, one of these was exchanged for the duplicate 5-50ul pipette from Maros. The cables, pipette, casein, and current Titertek manual were despatched to Maros by Dr Patten using a local carrier.

(ii). The subsequent testing of the conjugate at Balitvet showed that the conjugate if diluted in PBS-Tween has a high background absorbance of > 0.500 (and usually > 1.500) which was reduced to < 0.100 if the conjugate was diluted in PBS-Tween with 0.2% casein (Sigma chemical). The titration of the conjugate using PBS-Tween 0.2% casein as diluent indicated that on the basis of positive/negative ratios the conjugate could be
diluted to 1:5000 to 1:6000 and that using the Balitvet LPS antigen the absorbance of the negative serum was high if the conjugate was diluted at 1:3000 (c.f. recommended dilution 1:3200).  

<table>
<thead>
<tr>
<th>Conjugate Dilution</th>
<th>1:3000</th>
<th>1:4000</th>
<th>1:5000</th>
<th>1:6000</th>
<th>Control Balitvet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pos/Neg ratio</td>
<td>8.2</td>
<td>9.2</td>
<td>11.5</td>
<td>10.1</td>
<td>11.2</td>
</tr>
</tbody>
</table>
Visit to B.P.P.H Wil V Denpasar

Date of visit:

March 23rd arrive from Ujung Pandang 19:00hrs
March 26th depart for Yogyakarta 06:00hrs
April 20th arrive from Jakarta 21:00hrs
April 25th depart for Jakarta 13:30hrs

Contact Persons:

Dr Adat Peranginangin Kepala Balai
Drh Alit Project Leader, Co-ordinator Bacteriology

It was intended to remain at Denpasar for 3 days, steps having been taken prior to departure from Jakarta to determine that Hari Raya Nyepi - a major Hindu holiday - would not cause problems with the visit. However after leaving Jakarta Dr Patten was advised that most of the laboratory staff would be on leave for 4 days from March 26th. An extended days work was therefore organised for Saturday 24th March from 7am-4pm. Dr Patten left Denpasar on the morning of Monday March 26th to travel to Yogyakarta en route to Jakarta. Dr Patten then returned to the laboratory on April 21st until April 25th to complete the visit.

Equipment supplied:

A Titertek MKII plate reader, Amstrad computer, Epson printer and Voltage stabiliser were delivered to the laboratory from BATAN by Drh Alit. The equipment had been assembled prior to the arrival of Dr Patten.
All equipment was tested and was functioning properly.
Two 50-200ul pipettes had been supplied to Denpasar, one of these was exchanged for the duplicate from Maros.
A Handiwasher was set up without vacuum for dispensing wash solution into the plates.
No systems disk were supplied with the computer although the operating system was loaded onto the Hard disk.
Panacea was not available on the hard disk and the program whilst being used at the laboratory was on a hard disk on a separate computer. Instructions were given for copying the program onto the new unit. The program was installed by Dr Patten on the hard disk on April 23rd.
An ELISA was set up using pre-coated plates and reagents supplied by ACIAR PN83B2 Balitvet. A staff member from the Bacteriology laboratory Denpasar had recently returned from 1 week bench training at Balitvet Bogor.

A short discussion-seminar was held with the staff together with some food technology students visiting the laboratory on the fundamentals of the ELISA test.

The ELISA test performed on the day functioned properly although the substrate reaction using 100ul ABTS substrate without stopping agent was a little slow and developed only after 30-40 minutes rather than the 15-20 minutes expected.

Again as in the other laboratories the conjugate and substrate reacted well in a separate test tube on the bench. All solutions were checked and had been formulated correctly and were at the correct pH. The water being used for diluents was double glass distilled and normal glass distilled water was used in the wash steps.

A quantity of LPS Brucella antigen and Nunc microtitre plates were provided by Dr Patten on his return in April. The antigen and a conjugate previously supplied were titrated by the staff at the laboratory. The antigen was working effectively at a dilution of 1:150 of a 1:100 dilution of a 10mg/ml concentrate solution of antigen. The conjugate was working effectively at a dilution of 1:3000 using ABTS substrate.

Discussions were held with the staff concerning the use of dilution factors and the means of calculating different concentrations and dilutions. It was apparent that this point was causing some problems with the technical staff. Difficulties were also experienced in getting the technical staff to consult the technique sheets supplied for the ELISA. The sheets had been translated into B.Indonesia however the staff were still reluctant to consult the sheets before or during the test.

A total of 99 serum samples collected on the island of Lombok were tested by ELISA and RBPT for Brucella antibodies. Two samples were positive by RBPT and all were negative by ELISA.
An attempt was made to enter the data into a Panacea database however the program was "sticking" on a step in the Replay Procedure set up for use for recording Brucella results. The program disks were copied to be checked on another unit to ensure that they had not been corrupted.

Activities in the Brucella area:

Bali itself is free of bovine brucellosis. Serum samples are received on a semi-regular basis from the B grade laboratories on Lombok and Timor islands. The B grade laboratories test samples by RBPT and submit any positive samples to Denpasar for checking and confirmation. Samples are also received from active service work undertaken by the DIC staff.

It is intended to sample animals on Lombok and Sumbawa islands in the coming year. An Australian aid project has recently commenced in the Nusa Tenggara Timur and Nusa Tenggara Barat islands. This project will aim to establish some monitoring herds which will be sampled on a 3-4 monthly basis. The samples will be available for testing for Brucellosis.

A letter from the Director Keswan was given to Dr Adat concerning the testing of all submitted bovine serum samples for Brucellosis. Dr Adat and Drh Alit did not consider that this would cause any problems in the laboratory.

Follow-up to visit to DIC Denpasar:

(i). Samples of PBS-Tween diluent used at the laboratory were taken to Balitvet and compared in the standard ELISA against the diluents used at Balitvet. The result of the test indicated that the absorbance of the positive and negative control samples were generally not significantly effected when diluted with the Denpasar diluent and that the absorbencies were not significantly different using conjugate diluted with the Denpasar diluent.
Serum Diluent  Bacto  Bacto  Viro  Viro  Bvet
Conjugate Dilt  Bacto  Bvet  Viro  Bvet  Bvet
Pos/Neg ratio  6.9  8.8  13.7  8.7  8.3

Bacto- diluent from BPPH Bacteriologi lab, double glass distilled water
Viro- diluent from BPPH Virologi laboratory, sterile PBS
Balitvet - diluent formulated from glass distilled water

(iii). A manual for the Titertek MKII model supplied to the laboratory was photocopied and sent to Denpasar.
Visit to BPPH Wil III Bandar Lampung South Sumatera

Date of visit:

May 14th Travel from Bogor to Halim airport, transfer to Cengkarang airport for travel (16:30 hrs) to Bandar Lampung-Tanjungkarang airport (17:30 hrs).

May 17th Return travel was by Garuda-Merpati airlines from Tanjungkarang airport (15:55 hrs) to Halim airport (17:00 hrs) and then travel to Bogor (18:00 hrs) by motor-vehicle.

Contact People:

Drh Susilo Drh Tantri Drh Mardiatmi Drh Sayidi Drh Sri Marfialiningsih
Kepala Balai Bacteriology Bacteriology Epidemiology Epidemiology

ELISA:

A 2 hour discussion-seminar was held with the laboratory staff concerning the principles of the test and the protocol for the performance of the Brucella ELISA.

An ELISA test was performed using reagents supplied by Balitvet. Reagents were formulated using water from a metal still as no glass distilled water was available. The test did not perform as expected with little colour reaction for controls or test serums. A glass still apparatus was located in the laboratory store room and was set-up on a gas stove to produce some glass distilled water. A subsequent test conducted on Saturday May 19th performed better however the reaction time was still slow.

The laboratory had only fixed volume pipettes of 25, 50, 75, 100 and 200 ul which caused some problems in obtaining simple dilutions for serum and conjugate. A variable volume pipette of 5 ul to 200 ul and 200 ul to 1000 ul would be of use in the laboratory.

The Dynatech MR300 single channel Plate reader at the laboratory was faulty and would not read/recognise 5-6 wells on the plate. The machine would read all other wells and produce a print-out through the parallel port connection to a Brother printer.

A Dynatech platewasher was set-up and was functioning properly at the laboratory.
A database for recording the Brucella results was loaded onto the XT clone computer at the laboratory. An Autoexec.bat file was created and a hard-disk file menu program was loaded onto the computer. Panacea program files, previously provided by Keswan Jakarta, were also loaded into the computer although the program did not have the correct printer drive program for the Itoh 1500 printer connected to the computer. The epidemiology staff had very limited experience with Panacea and it is anticipated that problems will occur in recording the Brucella data until the staff receive further training in the use of Panacea.

Activities in the Brucella area:

The laboratory tests approximately 3000 serums/year for Brucellosis. The samples are obtained from field submissions (300), quarantine (1000) and active service work (1700). The samples are tested initially by RBPT by which ~30% are positive, positive samples are tested by SAT and macro CFT. The laboratory staff are not familiar with the use of the micro CFT and it was suggested that a scientist could travel to Balitvet to learn the microtitre CFT.

The use of abattoir collections was discussed with the staff, problems of identification of animals and the source of the animals were discussed. Dr Patten suggested that such collections could provide baseline sampling to assist the laboratory in determining the likely prevalence of different diseases or conditions. Whilst it may not be possible to confirm the exact source location of the animals the general locality could be determined. In discussion it was also determined that the Karantina were testing samples from imported cattle whereas it would normally be expected that this should be performed by staff from the DIC laboratory.

Follow-up to visit to BPPH Bandar Lampung

(i). A letter was forwarded to Dynatech laboratories concerning the fault in the MR300 Platerader. Dynatech replied through their Hongkong office giving the address of a local distributor who could service the unit. Dynatech suggested that the reflective sensors for the well positions on the plate that could not be read may be faulty and require servicing-replacement.

(ii). Suspect positive serums from Bandar Lampung were provided to Dr Patten to test at Balitvet. All of the samples were negative in the RBPT, micro CFT and ELISA when tested at Balitvet. It was recommended that the laboratory at Bandar Lampung retest the samples.

(iii). A sample of the metal distilled water produced at Bandar Lampung (B/L) was tested in the ELISA at Balitvet. The
Water was used to formulate serum and conjugate diluent and compared with serum and conjugate diluent used at Balitvet (glass distilled water). The results indicated that the water from B/L was inhibitory to the binding of serum to the antigen as the positive/negative ratio was reduced when the B/L water was used as serum diluent but not when used as conjugate diluent.

<table>
<thead>
<tr>
<th>Diluent</th>
<th>Serum B/L</th>
<th>B/L</th>
<th>Conjugate B/L</th>
<th>B/L</th>
<th>B/Vet</th>
<th>B/L</th>
<th>B/Vet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance 415nm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>1.625</td>
<td>1.552</td>
<td>1.976</td>
<td>1.864</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>0.216</td>
<td>0.215</td>
<td>0.141</td>
<td>0.174</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Pos/Neg ratio</td>
<td>7.5</td>
<td>7.2</td>
<td>14.0</td>
<td>10.7</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Positive serum = 1:2000 dilution of Br15/II 1000iu/ml
Negative serum = 1:200 dilution Bvet neg serum batch 41089
B/L = water from Bandar Lampung used as diluent, B/Vet = Balitvet glass distilled water used as diluent
Visit to BPPH Wil II Bukittinggi West Sumatera

Date of visit:
May 27th Travel from Bogor to Cengkarang airport for travel by Garuda airways (15:00 hrs) to Padang (17:00 hrs) and then by taxi to Bukittinggi (19:30 hrs).

May 30th Travel courtesy of a laboratory vehicle to Padang for travel by Garuda airways (13:00 hrs) to Medan, North Sumatra (14:00 hrs).

Contact People
Dhr Mulyawan Kepala Balai
Dhr Effendi Epidemiology
Dhr Ambar Bacteriology-Serology

ELISA:
An ELISA test was set up and performed by Dr Patten on the first day using reagents supplied from Balitvet. Two plates were tested using water from Balitvet and water from the glass still at BPPH. Both tests worked satisfactorily. A portable ELISA reader on loan from ODA Balitvet Project was used to read the plates as the Behring ELISA reader from BPPH had been sent away for repairs.

A 2 hour discussion/seminar was held by Dr Patten on the basics of the ELISA and the general principles of the test. Discussions were held concerning the use of glass distilled water and the proper cleaning of glassware. It was recommended that all glassware was rinsed in distilled water prior to drying, presently the glassware was only rinsed in tap water and had a white mineral film on the inner surface.

On day 2 the technical staff were supervised in performing an ELISA using some samples from cattle imported from Nusa Tenggara Timur (NTT). The technicians were competent in performing the test.

The laboratory had sufficient equipment to perform the assay. A reader which was previously giving problems was being repaired, once returned then the laboratory would be fully capable of performing the assay.

Dr Patten discussed with Drh Ambar the principles behind titrating antigen and conjugate and some stock antigen was left for Drh Ambar to use.
Activities in the Brucella area:

A database being distributed by Keswan for recording of Brucella results was installed and discussed with staff. The database was similar to that in use at the laboratory although all results were being stored in one dataset rather than having a separate dataset for each agenda (submission) number. Extra fields for SAT results were added to the dataset and the report section was altered to include the SAT results.

A number of serums from animals imported from NTT were tested by ELISA. The animals from a shipment of ~1100, had reportedly been tested negative however 34 were tested +ve by RBPT and 30 were classed as +ve by BPPH on the basis of SAT/CFT titre. 32 of the samples were tested by ELISA and 27 were classed as positive. One sample was from an RBPT -ve animal and the other 4 negative were from animals classed as dubious by BPPH. Several samples were CFT -ve SAT +ve however the ELISA reactions were strongly positive.

A number of hours were spent in discussing with the Kepala Balai and staff the interpretation of serological results and their significance. The culture of samples was discussed however it was determined that the laboratory lacked a CO<sub>2</sub> incubator and did not have CO<sub>2</sub> gas or gas jars for the culture for Brucella.

The laboratory was receiving from 3000 to 6000 serum samples per year for Brucella testing depending on the active service program. The plan for 1990 was to test imported Bali cattle in Riau and Jambi provinces. The testing of a significant percentage of animals was discussed as was the need to obtain reliable information on the distribution of cattle in each Kabupaten. Dr Mulyawan indicated all cattle/buffalo samples would be tested for Brucellosis. Dr Patten also discussed with the staff the use of abattoir survey to perform a cost efficient survey. Problems concerning the availability of staff at the abattoirs and lack of information concerning the source of the cattle was discussed.

Dr Mulyawan raised questions concerning porcine brucellosis which he regarded as a problem in the laboratories area. Dr Patten discussed the diagnosis and control of porcine brucellosis suggesting that the RBPT be used as a herd test and that testing individual animals was unreliable. It was suggested that Dr Mulyawan advise Keswan of the concern about porcine brucellosis as little work was presently being performed on the disease in Indonesia.
Visit to BPPH Wil I Medan Sumatera Utara

Date of visit:

May 30th: Travel by Garuda airlines from Padang West Sumatera (13:00 hrs) to Medan (14:00 hrs).

June 02: Travel from Medan (16:00 hrs) by Garuda airlines to Jakarta (18:00 hrs) and then to Bogor by vehicle (19:30 hrs).

Contact People:

- Dr Made Gunawan: Kepala Balai
- Drh Wati: Bacteriology-serology
- Drh Herlin: Virology-serology
- Drh Meti: Epidemiology

ELISA:

A 2 hour discussion/seminar was held by Dr Patten for the staff at the laboratory as a refresher of the ELISA technique. A test was set up on Thursday May 31st using reagents supplied from Balitvet. A number of suspect positive sera were used in the test. A comparison was made of 2 tests using buffers supplied by Balitvet and buffers formulated using water from a glass still unit at Medan. The two tests worked satisfactorily except that a positive serum that was titrated produced higher than expected readings. The fault was traced to a faulty 2-20 ul Eppendorf pipette that was delivering approx x3 - x4 the selected volume. The pipette could not be corrected and was removed from service. A faulty 10-100 ul Eppendorf pipette was adjusted by Dr Patten and returned to service.

The Dynatech Platereader at the laboratory appeared to function properly although it would not "talk" to an IBM XT clone computer when connected via the serial port. It was not possible to determine if the fault was in the computer, cable or Platereader. The reader was fitted with an internal thermal printer allowing the manual processing of results.

A second test was performed on Saturday June 2nd using a pipette that had been tested for accuracy. Washing steps using a hand held bottle or a Dynatech manual Plate washer were used. One plate performed as expected but the other plate produced low readings. It was suggested that the staff retest the same sera using both washing measures to determine if the error was due to the operator or because of the platewasher or its contents.
Activities in the Brucella areas:

A discussion session was held on Friday June 1st concerning the testing of samples by the laboratory for Br. abortus. Other areas of discussion included problems found in performing the ELISA test, the titration of antigen and conjugate, interpretation of results, testing of samples for Br. suis and the set-up and use of the Brucella database.

The discussions indicated that the laboratory tested between 3000-6000 serums per year depending on the number of imported cattle and the level of active service work. In the proceeding 12 months approx 6000 samples were tested with only 30-70 being positive. The samples were tested by RBPT and SAT. The CFT was not used as the laboratory had no complement. It was suggested that the laboratory attempt to produce some complement using Guinea pigs fed on an ascorbic acid supplemented feed containing a high proportion of green feed.

It was determined that 3-4 active service visits per month were likely in this financial year. The visits would be in the area of Riau province where it was considered Brucellosis may be a problem, Dr Patten suggested that as the number of Brucella positive were small then more baseline data may be obtained by collecting some samples from the main abattoirs in the provinces. Discussions indicated that some problems may occur with identifying the source area of each animal and in having samples collected. Dr Patten suggested that collection could start in association with Dinas Peternakan at the abattoir in Medan and that the problems could be identified from that collection.

Dr Patten also suggested that as the number of RBPT positive samples were small then it would be advised to run an ELISA test at least once every 2 weeks, testing whatever serums were available. Such a program would ensure that the staff became familiar with the test protocol.

Questions were asked about the testing and diagnosis of B. suis infections. Dr Patten indicated that the serological tests were suitable for herd testing only and were unreliable for individual pigs. Once a herd was positive then it was difficult to eradicate the infection and that all animals should be sold for slaughter only. Any negative farms selling breeding animals should be tested on a regular basis to ensure that they remain free of infection. The RBPT using Br. abortus antigen was a test that appeared to be as good as any for screening herds for B. suis and that if positive, the only way to verify the diagnosis was by culture, which if done should be done carefully.

The Brucella database was set up on the Epidemiology computer. A hard disk menu program was also installed. The computer-printer required servicing as the printer would not print-out any material from the computer. The Panacea program was not used extensively in the laboratory as the staff were still waiting for
the proposed training visit by a person from Keswan Jakarta or for the proposed National training scheme for Panacea. The printer, an Itoh 1500 was similar to that at Bandar Lampung and so it is to be expected that problems may occur in the use of the unit with Panacea due to the lack of a correct printer driver program.

Until staff become familiar with the use of the Panacea program it is expected that problems will occur in recording the details of tests in the Brucella database.
Visit to B.P.P.H Wil VI Banjar Baru

Date of Visit:

June 12  Travel to Jakarta from Bogor by motor vehicle, travel from Jakarta (13:30 hrs) to Banjar Masin (16:30 hrs) by Garuda/Merpati airlines MZ532

June 14  Travel from Banjar Masin (15:55 hrs) to Jakarta (17:35 hrs) by Garuda/Merpati airlines MZ533

June 15 Visit Directorate General Peternakan (Keswan) and Project Office ADB-Kalimantan II project. Return to Bogor by motor vehicle.

Contact People:

Dr Kuat Ketaran, Kepala Balai
Drh Astuti, serology laboratory
Drh Achmed, parasitology laboratory
Dian, technician bacteriology/serology laboratory
Selasea, technician serology laboratory

ELISA:

A short discussion was held with the Kepala Balai concerning the purpose of the visit. A 1 hour discussion/seminar was held with the serology staff to outline the protocol of the ELISA. A test was set up at the laboratory using some reagents supplied by Balitvet. The distilled water supply was checked (glass still using a stainless steel heating element) and diluents using distilled water from both Banjar Baru and Balitvet were compared in an ELISA.

The laboratory had available a Wellcome semi-automatic microtitre plate washer which functioned satisfactorily. A Dynatech MR300 plate reader was available but had not been previously used. The machine was checked and was found to be faulty as it would not recognise the well being read. The machine was packed up and transported by hand to Jakarta (June 14th) for checking and repair. Dr Patten handed the unit to the ADB-Kalimantan II project office in Jakarta (June 15th) who had originally purchased the unit.

As the plate reader was faulty it was not possible to read the test properly or to process data from the test. The interpretation and processing of data using the inbuilt applications functions in the plate reader were discussed with and demonstrated to Dr Astutui.

The ELISA test appeared to work satisfactorily although a little slower than anticipated. The laboratory staff performed the test under supervision on June 14th and the test also appeared to work
properly.

It was recommended that the laboratory run the ELISA at least every 2 weeks regardless of the number of serums available for testing.

**Activities in the Brucella area:**

The laboratory received approximately 3500 serums per annum, although a further 4000-5000 samples were tested at Quarantine centers away from the laboratory by either laboratory staff or staff from the B grade laboratories. Any positive samples from this testing were then returned to the A grade laboratory for further confirmation.

Samples for Brucellosis were tested by RBPT and the result confirmed by CFT. A total of 55 suspect or confirmed positive samples were recorded for 1989-1990.

It is expected that the number of active service visits for 1990-91 will continue at the same level as 1989-90 resulting in about 3000 samples. The number of Quarantine samples may be higher for 1990-91 (approx 15,000 samples). It was determined that all slaughtered cattle were imported from South Sulewesi and so abattoir sampling would provide no useful local information.

The interpretation of the RBPT, CFT and ELISA results were discussed with the laboratory staff. It was evident that there was confusion in the interpretation of the results. Dr Patten indicated that he would regard any RBPT positive sample as suspect positive only and that a confirmatory test such as the CFT and ELISA should be performed on all RBPT positive samples. Dr Patten also indicated that the ELISA could be used as a screening\confirmatory test depending on the number of samples received. It was also evident that confusion was present in the field (Dinas Peternakan) concerning the diagnosis of Brucellosis. Cases were mentioned where because an animal had not aborted then it was classed as Brucella negative even though the animal was sero-positive. Dr Patten explained the situation concerning abortion, latent carriers and the use of serology for the diagnosis of Brucellosis.

The use of the Brucella database was discussed with the Kepala Balai. The epidemiology staff member was presently in Yogyakarta receiving training in Panacea. The database diskette was returned to Jakarta as the local computer was an IBM using 750K diskettes and it was not possible to copy the files from the 340K diskette to the smaller format diskettes. The files on the computer were checked and the autoexec.bat file corrected, a hard-disk access fault was corrected and directories were made for several new programs. Panacea was also loaded onto the hard-disk.
Follow-up to visit:

(i). The faulty plate reader was delivered to Jakarta for servicing and repair on June 15th. The return of the unit was left in the care of the ADB-Kalimantan II project office.

(ii). The Brucella database files were copied onto 750K diskettes and returned to the laboratory for use to record Brucella results.
Conclusions

The long time interval between the training course and the distribution of equipment is unfortunate as it has decreased the impact of the 1989 training course on those involved.

Following the visit to the laboratories it would appear that all are sufficiently equipped to perform the ELISA, although faulty readers at several laboratories still require repairs. Faults in 2 of the 4 Plate Readers distributed to BPPH Wates and to PusVetma caused an understandable degree of frustration both with the staff at the laboratories and with the consultant. It is hoped that repairs can be made quickly to the units to return them into service.

The Plate readers at BPPH Bandar Lampung and at BPPH Banjar Baru are also in need of servicing and repairs.

Further ancillary equipment may be of use for some of the Laboratories:

- PusVetma is without a Plate washer. A 200 ul - 1000 ul variable volume pipette would also be of value in the laboratory performing the ELISA.

- The English language systems disks for the Amstrad computers should be provided to the laboratories concerned (Denpasar, Surabaya, Maros).

- Variable volume pipettes are required at Bandar Lampung, and the Platereader at that laboratory requires servicing and repair.

It would appear that the ELISA test would become functional in the laboratories at BPPH Wates, BPPH Maros and BPPH Bukittinggi (once the reader is returned).

BPPH Wates has had a high input from experts since the time of the training course in 1989 and the staff appear to understand the technique and its application.

Dr Isep Sulaiman at BPPH Maros also appears to have an understanding of the test and its application.

The staff at BPPH Bukittinggi have had a number of previous visits from experts and the staff seem to be very familiar with the test.

The staff in the virologi section at BPPH Medan also appear to be familiar with the test procedure however staff in the bacteriologi area of the laboratory and staff at the other laboratories have had limited experience with the use of the ELISA and would benefit from further regular supervision and input from experts familiar with the test.

The continued supplied of reagents as well as the supply of microtitre plates is of concern to a number of the Indonesian
counterparts. Discussions initially held with Keswan and BATAN and subsequently with PusVetma indicated that Pus Vetma could produce an antigen for use in the DGLS laboratories. Such action would help ensure the sustainability of the tests within Indonesia. Support would be needed for the supply of conjugates and substrate and for microtitre trays which are expensive in Indonesia (~US$1.50 - Rp25000/plate).

PusVetma would benefit from further input to assist with the methodology and quality control procedures required in the production of the Brucella ELISA antigen and control positive and negative serum.

Discussions with Keswan resulted in the implementation of a testing scheme of all cattle/buffalo serum received by the regional Laboratories for antibodies to Brucellosis and the use of a standardised reporting system. The establishment of the database should assist in obtaining a clearer indication of the prevalence of Brucellosis in Indonesia. The use of the database is dependant on the laboratories having a working knowledge of the database program "Panacea".

Following the visit it is apparent that 3 to 4 laboratories, namely Wates, Maros, Bukittinggi and possibly Denpasar have this knowledge. The laboratories at Medan, Bandar Lampung and Banjar Baru require training in the use of the program. Further contact with the laboratories during the first year of testing of the samples would help to correct any problems that may develop with the testing and reporting program.

Discussions with staff at a number of the laboratories indicated that animals vaccinated in the field for Brucellosis were not being properly identified. This situation will interfere with the proper interpretation of the test results in the future.

Difficulties in interpretation of results for Brucellosis both in the laboratory and in the field is a cause of continuing problems.

A number of questions indicating a level of concern were raised concerning Porcine Brucellosis, particularly in the Bukittinggi and Medan areas. It would appear that this is a disease that is of concern to these laboratories and as such should receive some consideration by Keswan Jakarta.
Recommendations

Recommendations to counterpart department/Institution.

i) That funds be sought and/or allocated for the laboratories to purchase sufficient microtitre plates to adequately perform the ELISA test.

ii) Steps be taken by DGLS to ensure animals subject to Brucella vaccination are marked in such a way that they are easily identified as vaccinated animals.

iii) A review/workshop be held in 12 months time by Keswan to access the use of the ELISA in the laboratories and to compare the results for Brucella testing using the present methods and the ELISA.

iv) Extension be undertaken to advise all relevant BPPH staff and relevant Dinas Peternakan veterinarians of the fundamentals of Brucellosis including the diagnosis of the disease and the interpretation of serology/laboratory results as well as the Governments current control regulations concerning the disease.

v) Relevant laboratories receive training in the use of the database program "Panacea" such that all laboratories are able to process data from Brucella testing in a standard manner.