ANNEX 11

Progress Report on the Extension Period of the Japanese Technical Cooperation for the ASEAN Poultry Disease Research and Training Centre

PROGRESS REPORT ON THE EXTENSION PERIOD OF THE JAPANESE TECHNICAL COOPERATION FOR THE ASEAN POULTRY DISEASE RESEACH AND TRAINING CENTRE

1. INTRODUCTION

The first phase of the ASEAN Poultry Disease Research and Training Centre project which started on April 17, 1986 ended on April 16, 1991. Following the joint evaluation on the Centre in 1990, the project was extended for another two years i.e. from April 17, 1991 to April 16, 1993. This report summarises the achievements accomplished for the extended period.

2. RESEARCH ACTIVITIES

Following the report of the evaluation team in 1990 a list of projects were proposed to be carried during the extension period. The proposals were supposed to have been discussed between the Japanese government (JICA) and Malaysians. However, no discussions were held and the proposals were annexed into the Record of Discussions on the Extension Period of Japanese Technical Cooperation for the APDRTC , 1991.

The research projects carried out in 1991 and 1992 are as summarised in Table 1 below. Some may be continuation of projects from the first phase. (Titles of projects are as in Appendix 1.) Not all proposed projects were carried out but a number of studies not suggested earlier were conducted as they were considered to be appropriate.

Table 1: Status of projects

PROJECT	PATHOLOGY	BACTERIOLOGY	VIROLOGY	PARASITOLOGY
PROPOSED	4	8	10	6
Completed	4	4	6	4
In progress	0	2	1	0
Not started	0	2	3	2
ADDITIONAL	11	7	5	3
Completed	11	5	4	3
In progress	0	2	1	0
Total completed	15	9	10	7

3. TRAINING

3.1 Malaysians trained in Japan

Training of Malaysian officers and technicians in Japan continued and the breakdown of the figures are as in the Table 2. Further details are shown in Appendix 2.

Table 2: Personnel trained in Japan

DISCIPLINE	# TRAINED
Management	3
Parasitology	1
Pathology	1
Bacteriology & serology	3
Virology	3
TOTAL	11

3.2 Training of Malaysians by Japanese experts at the APDRTC

Dispatch of experts to the Centre has enabled the Malaysian counterparts to be trained in specific techniques. A total of seven experts have been dispatched and another expert in pathology is expected to arrive on February 15, 1993.

The experts that have been dispatched in the years were:

Table 3: Japanese experts dispatched to the APDRTC

DISCIPLINE	EXPERTS	DURATION OF STAY
Bacteriology	Dr. Masashi Eguchi Dr. Masato Kishima	3 months 11 weeks
Virology	Dr. Hiroshi Hihara	3 months
Parasitology	Dr. Tamio Inamoto Dr. Naotoshi Tsuji	3 months 11 weeks
Pathology	Dr. Toshiaki Taniguchi Dr. Minoru Narita	3 months 2 months
Equipment	Mr. Kaoru Sukegawa	12 days

The Malaysian counterparts were satisfied with the experts as the technologies transferred were relevant to the needs of the Centre.

3.3 Training activities for ASEAN scientists

These are activities of the Third Country Training Programme. For the 1991 fiscal year, 1 seminar was held whilst the course had to be cancelled because of poor response from the ASEAN countries. A specialized diagnostic course and a seminar were held the following year (Appendix 3).

4. SCIENTIFIC PUBLICATIONS AND PRESENTATIONS

4.1 Publications

Seven scientific articles were produced in 1991 and another two in 1992 (Appendix 4a).

Table 4: Scientific publications 1991 - 1992

DISCIPLINE	1991	1992*	TOTAL
Pathology	2	-	1
Virology	-	-	0
Bacteriology	3	2	5
Parasitology	3	-	3
TOTAL	8	2	10

* 16 articles were submitted for publication, however, they are still being reviewed. (Appendix 4b).

In addition to the scientific papers, 10 technical manuals written by the Japanese experts and Malaysians were also published. (Appendix 5)

4.2 Presentations (Oral)

1992 saw the active participation of the APDRTC officers in seminars and conferences with a total of eight presentations. (Appendix 6)

Table 5: Oral presentations, 1991 - 1992

DISCIPLINE	1991	1992	TOTAL
Pathology	-	3	3

Virology	-	3	3
Parasitology	3	1	4
Bacteriology	1	3	4
Isolators (SPF)	-	1	1
TOTAL	4	11	15

An annual report covering 1986 - 1991 is being prepared and is expected to be ready before April, 1993.

5. ADMINISTRATION

5.1 Staffing

The Centre has a staff strength of 28 comprising of all categories.

Table 6: APDRTC Staff

POSITION	ADMIN	BACT	VIRO	PARA	PATH	EPID	SPF	TOTAL
Director	1	-	-	-	-	-	-	1
Vet. Off.	-	1	1	1*	1	1+	-	5
Res. Off.	-	1*	1	-	-	-	-	2
Asst.Vet.Off.	-	-	-	-	-	1	-	1
Expt. Off.	-	-	-	-	1	-	-	1
Lab. Asst.	-	1	3	1	2	1	-	8
Vet. Asst.	-	-	-	-	-	1	2	3
Typist	1	-	-	-	-	-	-	1
Junior clerk	1	-	-	-	-	-	-	1
Labourer	1	1	1	1	1	-	2	7
TOTAL	4	4	6	3	5	4	4	30

* Officers are currently pursuing master degrees in the U.K.
\+ Officer undergoing Ph.D. programme in the U.S.

5.2 Running costs

Expenditures incurred upon the Malaysian government for 1991

and 1992 were RM 915,683.38 (wages not included).

Table 7: Expenditure for the APDRTC (1991 & 1992)

OBJECT	1991	1992
Travel and transport	11,974.64	12,000.00
Transport of articles	4,913.63	541.06
Communication & utilities	300,817.60	332,106.24
Rental	0.0	186.00
Materials, maintenance & repairs	58,539.94	55,866.21
Supplies & other materials	102,900.03	80,369.25
Specialised services & hospitality	7,381.80	1,476.30
Capital equipment	1,381.63	0.0
TOTAL	487,909.27	482,545.06

5.3 SPF

The 5th and 6th flocks were managed during the extension period and the eggs were supplied by the Nippon Institute of Biological Sciences. The eggs for the seventh flock are expected to arrive on March 16, 1993.

6. TECHNICAL EXCHANGE TRIP

A trip to the ASEAN countries was made in August 1992 in an effort to plan for the future activities of the Centre. Discussions were held with the relevant officers in the countries. The vast contrasts in the laboratory facilities within the countries and between countries were significant. Requests forwarded by the countries were also different.

Some of the suggestions made were:

* The training courses need to be tailored in a manner that would be practical for the participants to adapt when returning to their countries because of the lack of facilities in their countries.

* Courses on Basic Diagnostic Techniques are no longer required since they have their own in-house training. The duration for the courses on Specialized Diagnostic

Techniques should be lengthened.

* An urgent need to hold courses for the production of antigens and antiserums.

* Dissemination of information on research findings or other technical matters should be done on a regular basis so that advancements or achievements in poultry disease research in the region is made known to all member countries. The APDRTC should act as a coordinator for such activities.

PROPOSED RESEARCH TOPICS

TITLE	STATUS
I ETIOLOGICAL AND PATHOLOGICAL RESEARCH ON POULTRY DISEASES	
1. Immunological response of chickens against infectious bronchitis virus.	
i) Analysis of antibody response using HI and ELISA techniques	Completed
ii) Analysis on correlation between serological titres and protection rates	Not started
2. Antigenic characterization of IB virus	
i) Purification of virus antigens	Completed
ii) Characterization of virus antigens	Completed
3. Pathogenicity and drug sensitivity of *Mycoplasma gallisepticum* isolated in Malaysia in 1991-1992	
i) Isolation of *M. gallisepticum* and drug sensitivity	Completed
ii) Pathogenicity of *M. gallisepticum*	Completed
4. Characterization of *Haemophilus* sp. isolate from chickens in Malaysia	
i) Isolation and identification of *Haemophilus* sp.	In progress
i) Drug sensitivity of *Hpg* isolated in Malaysia	In progress
5. Epidemiological investigation of leucocytozoonosis in Malaysia	
i) Characterization of insect vectors	Not started
ii) Occurrence of leucocytozoonosis and characterization of protozoa	Not started
6. Immune response against *L. caulleryi* in chickens	

i) Humoral immune response	Completed
ii) Cellular immune response	Not started
7. Immunological studies on coccidiosis	
i) Host immune response to coccidia	Completed
ii) Determination of immunological activities on antigens	Not started
8. Pathology of *S. weltevreden* infection in poultry	
i) Localization of *S. weltevreden* in chickens	Completed
ii) Detection of Salmonella antigens in infected chickens	Completed
9. Duck virus hepatitis in ducklings and chickens	
i) Pathogenicity of duck virus hepatitis in chicks and ducklings	Completed
II DEVELOPMENT AND APPLICATION OF DIAGNOSTIC METHODS FOR POULTRY DISEASES	
1. Development of HI test for infectious bronchitis	
i) Establishment of HI test method	In progress
ii) Comparative studies between HI and ELISA reactions	Not started
2. Diagnosis of Marek's disease with neutralization test	
i) Establishment of cell culture technique	Completed
ii) Farm surveys	Not started
3. Preparation of antibodies in rabbits	
i) IB, Reo and ILT viruses	Completed
4. Studies on base line values of ELISA S/P	Completed

ratios for IB, ILT and Reo virus infections	
5. Development of diagnostic methods for infectious coryza and mycoplasmal infection	
i) Detection of specific antibodies	Completed
ii) Detection of *Haemophilus* antigen	Completed
6. Development of immunopathological methods for diagnosis of poultry diseases	
i) Establishment of immunoperoxidase method on paraffin section	Completed
III DEVELOPMENT OF POULTRY VACCINE	
1. Development of vaccine for infectious coryza	In progress
i) Purification of effective antigen	Not started
ii) Immune response of chickens inoculated with purified antigen	Not started

ADDITIONAL PROJECTS

PATHOLOGY

1.	Effect of acidified drinking water on *S. weltevreden* infection in chicks	Completed
2.	Pathogenicity of *S. hadar* isolated from ducks in day-old SPF chicks and ducklings	Completed
3.	Pathogenicity of *S. weltevreden* and *S. hadar* isolated from ducklings and chicks	Completed
4.	Pathogenicity of duck hepatitis virus in 2-week-old ducklings	Completed
5.	Infectious bursal disease	
i)	Pathogenicity of Lukert and J1 strains of IBDV in 2-day-old and 6-week-old SPF chicks	Completed
ii)	Pathogenicity of Lukert and J1 strains of IBDV in 3-week-old SPF chicks	Completed
iii)	Immunosuppressive effects of Lukert and J1 strains of IBDV in 2-day-old SPF chicks	Completed
iv)	Experimental infection of IBDV (isolate 6511) in 2-week-old duclikngs and 3-week old broiler chicks	Completed
v)	Pathological comparison of bursal lesions in conventional broiler chicks inoculated with different strains of IBDV.	Completed
6.	Duck virus enteritis	
i)	Pathology of duck and ducklings infected with duck virus enteritis	Completed
ii)	Immune response of local ducks to an attenuated strain of DVE	Completed

VIROLOGY

1.	Detection of antibodies against reticulo-endotheliosis virus by ELISA	Completed
2.	Detection of antibodies against Marek's disease by immunofluorescence	Completed
3.	Studies on serological tests for DVE	In progress

4. Studies on Newcastle disease maternal antibodies	Completed
5. Improved procedures for IBD virology and diagnostic work	Completed

PARASITOLOGY

1. Survey of coccidiosis in quails	Completed
2. Freeze preservation of *Leucocytozoon caulleryi*	Completed
3. Experimental infection of *L. caulleryi* in indigenous chickens	Completed

BACTERIOLOGY

1. Isolation of plasmids from drug resistant *M. gallisepticum*	Completed
2. Studies on growth rate of *H. paragallinarum*	Completed
3. Studies on the protein profile of local isolates of *H. paragallinarum*	Completed
4. Isolation of plasmids from local isolates of *H. paragalliraum*	Completed
5. Detection of *M. gallisepticum* by PCR	Completed
6. Analysis of duck immunoglobulins	In progress
7. Two dimension electrophoresis of *Salmonella enteritidis*	In progress

APPENDIX 2

MALAYSIAN PERSONNEL TRAINED IN JAPAN

DISCIPLINE	NAME	DURATION
Management	Dato' Dr. Kardin Shukor	12 days (5-17 Nov'91)
	Dato' Dr. Anwar Hassan	13 days (2-15 Feb'92)
	Dr. Yusoff Mohd. Noor	11 days (14-25 Dec'92)
Virology	Mdm. Ong Geok Huai	3 mths (6 Jan-29 Mar'92)
	Mr. Lim Kean Teik	3 mths (30 Mar-2 Jul'92)
	Mdm. Lim Siew Sam	2 mths (27 Sep-27 Nov'92)
Bacteriology & Serology	Mdm. Siti Hajar Ibrahim	2 mths (7 Oct-8 Dec'92)
	Buthtaman Yusoff	2 mths (7 Oct-8 Dec'92)
	A. Karuppaiya	2 mths (7 Oct-8 Dec'92)
Pathology	Mdm. Zuraidah Ahmad	3 mths (6 Jan-29 Mar'92)
Parasitology	Dr. Chandrawathani	2 mths (7 Oct-8 Dec'92)

THIRD COUNTRY TRAINING PROGRAMME

1. ASEAN Seminar on Poultry Diseases and their Control

NO.	DATE	PARTICIPANTS						
		B	I	T	P	S	M	Total
5+	4 - 13 Mar'92	0	2	1	0	0	6	9
6*	17 - 21 Jan'93	0	4	2	3	1	7	17

B:Brunei, I:Indonesia, T:Thailand, P:Philippines, S:Singapore, M:Malaysia

+Theme: Bacterial Respiratory Diseases
*Theme: Infectious Bursal Disease

2. ASEAN Course on Specialized Diagnostic Techniques

NO.	DATE	PARTICIPANTS						
		B	I	T	P	S	M	Total
4+	28 Dec'92 - 22 Jan'93	0	2	1	1	0	2	6

+Diagnostic techniques for IBD

APPENDIX 4a

SCIENTIFIC PUBLICATIONS

1991

1. Mahani, A.H., Yap, H.C. & Shoya, S. (1991): Cryptosporidia in the bursa of fabricius in chickens. *J. Vet. Malaysia.* 3, 7-12

2. Taniguchi, T., Mahani, A.H. & Zaini, M.Z. (1991): Pathological changes in chicks infected with *Salmmonella weltevreden* isolated in Malaysia. *Proc. Jap. Vet. Ass. Cong.* (Gifu)

3. Rahmat, S.M.S., Chikatsune, M., & Parameswaran, S. (1991): A comparative study on the preservation of *L. caulleryi* sporozoites. *Proc. 3rd. Vet. Ass. Malaysia Cong.*

4. Rahmat, S.M.S. & Parameswaran, S. (1991): Maintenance of a colony of *Culicoides arakawae* in the laboratory. *J. Tropic. Biomed.* 8, 201-204

5. Rahmat, S.M.S. & Parameswaran, S. (1991): Isolation and maintenance of *L. caulleryi* - VRI strain. *J. Vet. Malaysia.* 3(2), 65-69

6. Zaini, M.Z., Siti Zaleha, A.T, & Tan, L.J. (1991): Characterization of *Haemophilus paragallinarum* isolated in Malaysia. *J. Vet. Malaysia.* 3(1), 25-30

7. Zaini, M.Z. & Tan, L. J. (1991): The effect of incubation and storage periods on the haemagglutinating activity of *Haemophilus paragallinarum. Proc. 3rd Vet. Ass. Malaysia Cong.* 77-79

8. Zaini, M.Z. & Kanameda, M. (1991): Susceptibility of the indigenous domestic fowl (*Gallus gallus domesticus*) to experimental infection with *Haemophilus paragallinarum. J. Vet. Malaysia.* 3(1), 21-24

1992

1. Zaini, M.Z. & Iritani, Y. (1992): Serotyping of *Haemophilus paragallinarum* isolated in Malaysia. *J. Vet. Med. Soc.* 54, 363-365

2. Yamamoto, K., Kuniyasu, C., Zaini, M.Z. & Tan, L. J. (1992): Bacteriological and serological survey of avian mycoplasmosis in Peninsular Malaysia. *Jap. Agric. Res. Quart.* 25, 278-282

SCIENTIFIC ARTICLES UNDER REVIEW

1. Mahani, A.H., Yap, H.C., Taniguchi, T. & Rahmat, S.M.S.: Bursal cryptosporidiosis in a duck.

2. Mahani, A.H. & Zuraidah, A.: Experimental infection of layers and pullets with *Salmonella weltevreden*

3. Mahani, A.H., Taniguchi, T. & Zaini, M.Z.: A natural outbreak of *Salmonella weltevreden* and the patogenicity of an isolant in chicks

4. Mahani, A.H. & Zuraidah, A.: Experimental infection of layers and pullets with *Salmonella weltevreden*

5. Sharifah, S.H., Mahani, A.H. & Taniguchi, T: Outbreaks of duck virus enteritis (duck plaque) in Malaysia

6. Loganathan, P., Sharifah, S.H., Arunasalam, V. & Mahani, A.H.: Outbreak of virulent IBD in broilers in Malaysia.

7. Rahmat, S.M.S., Chikatsune, M., & Parameswaran: Resistance of chickens to reinfection with *Leucocytozoon caulleryi*

8. Rahmat, S.M.S. & Parameswaran, P.: Pathogenicity of *Eimeria tenella* isolated from a broiler farm in Johore, Malaysia

9. Rahmat, S.M.S., Chikatsune, M. & Parameswaran, S.: Pathogenicity of *Leucocytozoon caulleryi* - VRI.

10. Rahmat, S.M.S. & Parameswaran, S.: Experimental *L. caulleryi* infection in Malaysian indigenous chickens (*Gallus gallus domesticus*).

11. Zaini, M.Z. & Tan, L.J.: Pathogenicity of *Mycoplasma gallopavonis* isolated from chicken.

12. Zaini, M.Z., Eguchi, M. & Tan, L.J.: Detection of *Mycoplasma gallisepticum* in tissue cultures by polymerase chain reaction.

13. Zaini, M.Z., Eguchi, M., Kuniyasu, C. & Tan, L.J.: Determination of antibiotic resistance of *Mycoplasma gallisepticum* by plasmid isolation.

14. Zaini, M.Z.: Current status of infectious coryza in Peninsular Malaysia

15. Zaini, M.Z., Eguchi, M. & Tan, L.J.: Enzyme linked immunosorbent assay (ELISA) for the detection of *Haemophilus paragallinarum*

16. Lim, K.T. et al: Studies on Newcastle disease maternal antibodies in 3-day old chicks

17. Lim, K.T.: Report on fowlpox in Malaysia

TECHNICAL MANUALS

1. Technical Manual for Electron Microscopy
   Masaru Kobayashi (August 1991)

2. Technical Manual for Electrophoresis and Western Blotting
   Chai Kim Kheong, Cheah Ngan Yok, Hiraoki Ohta (August 1991)

3. Technical Manual for Avian Virology
   Masaya Sakata (August 1991)

4. An Outline of ASEAN Poultry Disease Research & Training Centre
   Gan Chee Hiong (September 1991)

5. Supplement to Technical Manual for Electron Microscopy
   Masaru Kobayashi (September 1992)

6. Proceedings of the Fourth ASEAN Seminar on Poultry Diseases and their control: Vaccines and Vaccination
   Gan Chee Hiong (1992)

7. Bibliography of Veterinary Parasitology Publications
   S. Parameswaran & Rahmat S.M. Sheriff (November 1992)

8. Basic Avian Immunology
   C. Kuniyasu (November 1992)

9. Diagnosis of Avian Diseases
   C. Kuniyasu (December 1992)

10. Virology Manual for Infectious Bursal Disease
    Cheah Ngan Yok, et al (December 1992)

APPENDIX 6

PRESENTATIONS (ORAL)

1991

1. **Rahmat, S.M.S.** & Parameswaran, S.: Isolation and pathogenicity study of *Eimeria tenella* in Malaysia. Ann. Sc. Semin. Malaysian Soc. Parasit. Tropic. Med. 9-10, March, K.L.

2. **Rahmat, S.M.S.**, Chikatsune, M. & Parameswaran, S.: A comparative study on the preservation of *L. caulleryi* sporozoites. 3rd Sci. Cong. Vet. Ass. Malaysia, 4-6 Oct, K.L.

3. **Rahmat, S.M.S.**: Studies on leucocytozoonosis: The use of gel diffusion and simple electrophoretic techniques for diagnosis. VRI Seminar, Oct. 15

4. **Zaini, M.Z.**: Poultry mycoplasma and *E. coli* situation in Malaysia. Sem. on Poultry Respiratory Disease and its Control. Sept, 24. K.L.

1992

1. Hihara, H., **Wan, M.K.**, Lim,K.T. Cheah, N.Y. & Lim, S.S.: Antigenic relatedness of Malaysian isolate (352a) and Japanese (J1) strain of infectious bursal disease virus. 4th Vet. Ass. Malaysia Cong. 2-4 Oct., K.L.

2. **Lim, K.T.**, Lim, S.S., Cheah, N. Y., Zabidah, A. & Wan, M.K.: Studies on Newcastle disease maternal antibodies in 2-day old chicks. 4th Vet. Ass. Malaysia Cong. 2-4 Oct., K.L.

3. **Mahani, A. H.** & Sharifah, S. H.: An outbreak of duck viral enteritis in Malaysia. VRI Seminar, April 29.

4. **Mahani, A.H.**, Sharifah, S.H. & Taniguchi, T.: First outbreak of duck viral enteritis in Malaysia. ASEAN Seminar on Poultry Diseases and their Control. 8-13 March, Ipoh

5. Sharifah, S. H., **Mahani, A. H.** & Taniguchi, T.: Outbreaks of duck virus enteritis in Malaysia. 4th Vet. Ass. Malaysia Cong. 2-4 Oct., K.L.

6. **Wan, M. K.**: The use of isolators in chicken experiments at VRI. 4th Vet. Ass. Malaysia Cong. 2-4 Oct.,K.L.

7. **Rahmat, S.M.S.**, Chikatsune, M. & Parameswaran, S.: Pathogenesis of a Malaysian strain of *L. caulleryi*. 4th Vet. Ass. Malaysia Cong. 2-4 Oct., K.L.

8. **Zaini, M.Z.**: Country report on poultry respiratory diseases. ASEAN Seminar on Poultry Diseases and their control, 8-13 March, Ipoh

9. **Zaini, M.Z.** & Yamamoto, K.: Studies on various assays for the detection of antibodies. ASEAN Seminar on Poultry Diseases and their Control. March 8-13, Ipoh

10. **Zaini, M.Z.**: Current status of infectious coryza in Peninsular Malaysia. 4th Vet. Ass. Malaysia Cong. 2-4 Oct., K.L.

11. **Lim,K.T.**: Virological diagnosis of infectious bursal disease. ASEAN Seminar on Poultry Diseases and their Control. 18-20 Jan.,'93, Ipoh.

Note: Names in bold presented the papers.

ANNEX 12

Achievement Status of Research Activities

Achievement Status of Research Activities According to TIP

Subject	Section	Status of Achievement
(1) Etiological and Pathological Research on Poultry Diseases		
1. Immunological response of chickens against Infectious Bronchitis (IB) virus	Virology	
(i) Analysis of antibody response using the HI and ELISA techniques		B
(ii) Analysis on the correlation between serological titers and protection rates		C
2. Antigenic characterization of IB virus	Virology	
(i) Purification of virus antigens		A
(ii) Characterization of virus antigens		A
3. Pathogenicity and drug sensitivity of Mycoplasma gallisepticum isolated in Malaysia in 1991-1992	Bacteriology	
(i) Isolation of M. gallisepticum and drug sensitivity		A
(ii) Pathogenicity of M. gallisepticum		A
4. Characterization of Haemophilus sp. isolated from chickens in Malaysia	Bacteriology	
(i) Isolation and identification of Haemophilus sp.		B
(ii) Drug sensitivity of H. pg. isolated in Malaysia		B
5. Epidemiological investigation of Leucocytozoonosis in Malaysia	Parasitology	
(i) Characterization of insect vectors		C
(ii) Occurrence of Leucocytozoonosis and characterization of protozoa		C
6. Immune response against L. caulleryi in chickens	Parasitology	
(i) Humoral immune response		A
(ii) Cellular immune response		C

Subject	Section	Status of Achievement
7. Immunological studies on coccidiosis	Parasitology	
(i) Host immune response to coccidia		A
(ii) Determination of immunological activities of antigens		C
8. Pathology of Salmonella weltevreden infection in poultry	Pathology	
(i) Localization of S. weltevreden in chickens		A
(ii) Detection of Salmonella antigens in infected chickens		A
9. Duck virus hepatitis in ducklings and chickens	Pathology	
(i) Pathogenicity of Duck hepatitis virus in chicks and ducklings		A
(2) Development and Application of Diagnostic Methods for Poultry Diseases		
1. Developement of the HI test for Infectious Bronchitis (IB)	Virology	
(i) Establishment of HI test method		B
(ii) Comparative studies between HI and ELISA reactions		C
2. Diagnosis of Marek's disease with Neutralization test	Virology	
(i) Establishment of cell culture technique		A
(ii) Farm surveys		C
3. Preparation of antibodies in rabbits	Virology	
(i) IB, Reo and ILT virus		A
4. Studies on base line value on ELISA S/P ratios for IB, ILT and Reo virus infections	Virology	A
5. Development of diagnostic methods for Infectious Coryza and Mycoplasmal infection	Bacteriology	
(i) Dectection of specific anti-bodies		A
(ii) Detection of Haemophilus antigen		A
6. Development of immunopathological methods for diagnosis of poultry diseases	Pathology	

Subject	Section	Status of Avhieve-ment
(i) Establishment of immunoperoxidase method on paraffin section		A
(3) Development of Poultry Vaccine		
1. Development of vaccine for Infectious Coryza	Bacteriology	
(i) Purification of effective antigen		B
(ii) Immune response of chicken inoculated with purified antigen		C
(4) Investigation and Reference Activities		
1. Revised publication of APDRTC Guide book		A
2. Publication of APDRTC Newletter		C
3. Publication of Research Report		A
4. Publication of Poultry Disease Diagnostic Manual for ASEAN Countries		A
(5) Establishment of SPF Chicken Unit		
1. Monitoring of SPF chickens		
(i) Serological monitoring of SPF chicken flock Nos. 4 and 5		A
(ii) Serological monitoring of SPF chicken flock Nos. 5 and 6		B
(6) Other Activities		
1. Preservation of avian pathogens		A
2. Monitoring of feed and water supply for SPF and ECH		A
3. Interaction with Private Sector on Disease Research		B

A : Achieved/Completed

B : In progress

C : Not started

ANNEX 13

Research Activities Progress Report (1991 - 1992)

(1) Virology
(2) Bacteriology
(3) Parasitology
(4) Pathology
(5) SPF Chicken Unit
(6) Experimental Chicken House

Preparation of Purified IB antigen

IB virus (infected allantoic fluid) is clarified by centrifugation at 5000 rpm for 30 minutes. The supernatant is centrifuged at 80,000 rpm for 90 minutes. The sediment is diluted with 2 ml of PBS and layered over a discontinuous sucrose gradient of 50% and 20% and centrifuged at 80,000 g for 2 hours. Virus band over the 50% sucrose is collected and pelleted by ultracentrifugation.

The sediment is suspended in PBS to 1/200 of the original volume of virus and sonicated. The antigen is solubilised by reacting with an equal volume of 2% n-octyl-beta-D-glucopyranoside detergen at room temperature for 1 hour. The resultant antigen preparation is analysed by Western blotting. Results showed 3 bands around 30, 48 and 84 kilodaltons which are comparable to those published data on purified IB antigens.

Detection of antibodies against Infectious bronchitis, Infectious Laryngotracheitis and Fowlpox by ELISA

(i) Infectious bronchitis (IB)

Purified IB antigen was prepared from IBV-M41 strain and coated onto the flat-bottom wells of polycarbonate EIA plates at 0.5 ug per well. The ELISA negative value was determined as the average sample positive (SP) + 3 standard deviations (SD), based on 90 serum samples obtained from SPF chickens of ages 2 weeks and 8 weeks old. This negative value was determined as 0.07 or 0.1 (corrected to 1 decimal place).

Experimental infection of 20 SPF chicks of ages 2 weeks and 7 weeks old with IB virus at $10^3 EID_{50}$ / chick via the intranasal route revealed on ELISA reactor rate of $\geq$ 20% being indicative of a flock infection. Non specific reactions were detected in 2 out of 92 sera (2.2%) SPF sera tested.

Quantitation of IB-ELISA antibodies was carried out by titration of 160 IB immune sera obtained from SPF chickens exposed to IB virus via the intranasal route. Each serum sample was titrated from 1/125 to 1/128,000. The positive-negative (P/N) values for each serum dilution were calculated and plotted against the logarithm of their corresponding dilutions. A series of parallel graphs were obtained for the various serum samples titrated by ELISA. The logarithm of the ELISA titre (ET) was extrapolated for each graph and plotted against the corresponding P/N value of the serum sample at 1/500 dilution. A linear relationship was established between log (ET) and PN 1/500. In other words, the P/N values were proportional to log ET.

Studies to correlate the ET values to vaccination responses and resistance to intra-tracheal challenge with virulent IB viruses will be carried out in March 1993.

(ii) Infectious Laryngotracheitis (ILT)

ILT antigen was prepared from chick embryo fibroblast (CEF) monolayers previously infected with ILT-NS175 virus. The ILT antigen was extracted by treatment with 2% n-octyl-beta-D-glucopyranoside detergent and coated onto the wells of flat-bottom EIA plates. The ELISA negative value was determined as the average SP value + 3 SD, based on the analysis of 180 SPF chicken sera. The value obtained was 0.15.

Experimental infection of 20 SPF chicks with ILT virus at 100 EID_{50} / chick showed an ELISA reactor rate of $\geq$ 25% being indicative of a flock infection. Non specific reactor rate of 4.3% (4 out of 92 sera tested) was detected in SPF sera.

(iii) Reticuloendotheliosis (RE)

RE antigen was prepared from RE virus infected fluid and concentrated by salt precipitation. ELISA was carried out on 90 SPF chicken sera obtained from chicks in the ages of 2 weeks and 8 weeks old. The absorbance values were converted to sample positive (SP) values. The average SP value was 0.039. The ELISA SP cut-off value was 0.039 + 3 std. deviations and this value was = 0.2.

Preparation of IB, REO and ILT antisera in rabbits

IB, REO and ILT antigens were prepared from infected allantoic fluids, embryo extract and cell culture - detergent extracts respectively. They were then further purified and emulsified with Freund's complete adjuvant and inoculated in small doses at multiple sites on the back of rabbits of age 3 months old via the subcutaneous route. Repeat inoculations were carried out over a period of 6 to 10 weeks. The rabbits were monitored for antibodies from the 3rd to the 12th week post inoculation. Those showing AGID antibodies were bled to death.

Both ILT and IB antisera obtained were specific and were concentrated by salt precipitation. The IgG obtained were retested and found to be specific. Some non-specific reactions were detected in the REO antiserum preparation. The antiserum will absorbed by dialysis against normal embryonic extracts and chicken serum, centrifuged and retested for specificity.

Marek's Disease Antibody Detection by Immunofluorescence

Secondary CEF cultures in growth medium were seeded at 50ul (1.5×10^4 cells) onto 7mm. diameter wells of teflon coated glass slides in petri dishes and incubated at 37°C in 5% CO_2 for 24 hours. The medium was then removed and the monolayers were infected with 500 pfu MD virus in 50 ul growth medium and incubated at 37°C in 5% CO_2 for 24 to 48 hours. When CPE was apparent, the medium was discarded into disinfectant and the infected monolayers were washed 3 times in PBS, air dried, fixed in cold acetone for 10 minutes and air dried again. The slides were then stored at - 25°C for short duration until they were used for FA staining.

For the FA indirect staining, the following procedures were used:-

(i)	Positive serum	:	1/40 dilution
(ii)	Negative serum	:	1/20 dilution
(iii)	Test serum	:	1/40 dilution
(iv)	Control	:	PBS is used instead of serum

The infected CEF in the wells were reacted for 30 min at 37°C with the sera and PBS controls at 50 ul/well. The wells were washed 3 times with PBS, air dried and reacted with 50 ul of anti-chicken FITC conjugate (4 units) at 37°C for 30 min. The wells were washed 3 times in PBS and examined under fluorescence microscope for cell-associated immunofluorescence. Results of the indirect FA test for the detection of antibodies are shown below:-

MD antiserum	:	FA positive
Negative serum	:	FA negative
PBS control	:	FA negative

test sera from SPF chickens	:	FA negative
test sera from 3 weeks old unvaccinated quails	:	FA negative
test sera from MD vaccinated chickens	:	FA positive
test sera from MD vaccinated quails	:	FA positive

The indirect FA test was found to be sensitive and specific. Antibodies could be detected as early as 2 weeks post vaccination in chickens and in quails.

Cross FA tests between MD JM antigen (serotype 1) and HVT antigen (serotype 3) were also carried out and the results are shown below:-

Homologous and Heterologous FA titres

Antigen	Antiserum Titres		Contorls		
	JM	HVT	1/40 positive serum	1/20 negative serum	PBS
JM	640	80	+	-	-
HVT	320	1280	+	-	-

\+ means FA positive

\- means FA negative

With respect to JM serotype 1 antiserum, the r1 value

is = 320/640 = 0.5 and the r_2 value with respect to the

HVT serotype 3 antiserum is = 80/1280 = 0.0625

Composite relationship = 100 / 0.5 x 0.0625 = 17.6 which is indicative of very different subtype. The respective D values are 2.83 and 0.35 thus indicating that serotype 1 dominates over serotype 3.

We conclude that with the use of this indirect FA test for the detection of antibodies, serotype 1 and serotype 3 antibodies are better detected using serotype 1 and 3 antigens respectively.

Monitoring of the Specific Pathogen Free (SPF) Poultry Flock

The SPF poultry flock was monitored every 3 months and each monitoring covered approximately 50% of the flock. In the years 1991 and 1992, the 4th, 5th and 6th SPF flocks were monitored for infections according to the schedules listed below:-

SPF poultry flock	Date of monitoring	Approximate age of flock (in weeks)	No. of sera monitored		
			Male	Female	Total
4th	7.1.91	16	35	47	82
4th	7.4.91	29	10	34	44
4th	10.7.91	42	10	34	44
4th	12.11.91	60			23
5th	10.10.91	13			71
5th	20.1.92	27	8	34	42
5th	27.4.92	38			44
5th	28.7.92	53	10	35	45
5th	20.10.92	65	8	30	38
6th	22.9.92	14	35	45	80

The infections monitored and the tests carried out are shown in the table below:-

Infections	Tests Uses
IB, IBD, AE, ILT CELO REO, RE MD Fowlpox,Avian influenza	Agar gel immunodiffusion (AGID) enzyme linked immunosorbent assay (ELISA), serum neutralisation and challenge tests
ND, EDS	Haemagglutination inhibition (HI) test
Avian leukosis	ELISA and non-producer test
MG, MS, pullorum	Rapid plate agglutination test
IC	HI test
Blood protozoa	blood smear examination.
Helminths and coccidia	faecal sample examination
Leucocytozoon	AGID test

Results showed that the SPF flocks were free from all the infections tested.

Other Studies and Tests Carried Out

(i) Studies on serological tests for duck virus enteritis DVE)

An indirect immunofluorescence (IMF) test using FITC conjugated anti-duck IgG was studied for the detection of DVE antibodies in vaccinated ducklings, vaccinated ducklings that survived challenge test and in unvaccinated ducklings that died following challenge with virulent DVE virus.

Results showed that serum samples diluted 1/160 gave good clear-cut IMF readings between negative sera (from unvaccinated ducklings that died from challenge) and positive sera (from vaccinated survivors following challenge).

However, the IMF test gave poor immunofluorescence with sera obtained at 1 week post vaccination, although the ducklings at this time were fully protected against challenge with virulent DVE virus. The probability of IgM incompatibility with anti-duck IgG was questionable. In view of this, the SN test based on residual infectivity after neutralisation was carried out to detect early antibodies following primary vaccination. SN antibodies were detected at 1 week post vaccination by this test at serum dilution of 1/40. Further work is in progress for the determination of the SN Index.

(ii) Studies on Newcastle Disease (ND) maternal antibodies (MAb)

Studies on ND MAb were carried out in 520 chicks of age 3 days old with varying levels of MAb. The chicks were investigated for their responses to live vaccination, responses to challenge with virulent ND virus and their rate of antibody decline in relation to their individual initial MAb levels at 3 days of age. The studies showed that MAb levels of $\geq$ 16 interfered with live vaccination at 3 days of age. In such chicks with MAb of 16, 32 - 64 and $\geq$ 128, satisfactory vaccination may be carried out at 5, 7 - 10 and 12 days of age respectively when their initial MAb levels have declined to $\leq$ 8. Revaccination at 10 to 14 days later is recommended. In terms of protection against challenge, MAb levels of $\leq$ 8, 16-64 and $\geq$ 128 gave no

protection (100% mortalities), partial protection and total protection respectively in 3-days old chicks. Vaccination of young chicks in the face of MAb at $\geq$ 16 stimulated very low levels of immunity as evident from high mortalities following challenge tests. In these birds, revaccination at 1 to 2 weeks later is recommended. Emergency vaccination in the face of an outbreak in inadequately immune flocks is often a compromised procedure carried out mainly to reduce mortalities and losses.

(iii) Improved procedures for IBD virology and diagnostic work

The LSCC-BK3 suspension cell culture was introduced to this institute by Dr. Hiroshi Hihara, Japanese expert attached to the Virology Section for use as an improved method for isolation and detection of IBD virus.

Through the use of these cells, isolation and identification of IBD viruses from field specimens could be completed in 4 days. From March to July 1992, a total of 94 suspected IBD cases were referred to the APDRTC Virology Section for virus isolation. A total of 94 IBD viruses were isolated from these clinical referred cases.

Two-way cross neutralisation studies were carried out between IBDV-J1 strain and one of the local IBDV isolates. Results are shown in the table below.

IBDV antigen	IBD antiserum	
	J1	local strain
J1	SNT = 102,400	20,000
Local strain	SNT = 51200	100,000

With respect to J1 antiserum, $r_1 = \frac{51200}{102400} = 0.5$

With respect to local antiserum, $r_2 = \frac{20,000}{100,000} = 0.2$

Although the J1 antiserum could neutralise the local IBDV better than the local antiserum could neutralise J1 strain, the implication is that the neutralising capacity of J1 antiserum against the local IBDV isolate is only half its homologous capacity. If IBD-J1 vaccines (if availabe) are used in the farm, the effects of interference by maternal antibodies and mishandling of vaccines would become more apparent.

PROGRAMME IMPLEMENTED IN BACTERIOLOGY SECTION (from 1991 - 1992)

A. Etiological and Pathological Research on Poultry Disease

(1) Pathologicity and drug sensitivity of *Mycoplasma gallisepticum* isolated in Malaysia from 1991 - 1992.

(a) Isolation of *M. gallisepticum* from pipped eggs

In an attempt to collect local *M. gallisepticum* isolates to study their pathogenicity and their sensitivity pattern, 152 pipped eggs were collected in 1991 while another 871 pipped eggs were collected in 1992 from hatcheries in Perak. There was no *M. gallisepticum* isolated from all the eggs although the broth used for culture was proven to support. This project could not be carried out due to the unavailability of local *M. gallisepticum* isolate, and therefore, the pathogenicity and the sensitivity of local *M. gallisepticum* isolates could not be determined.

(b) Pathogenicity and drug sensitivity of *M. gallisepticum* isolated earlier than 1990.

Although the pathogenicity and drug sensitivity pattern of new *M. gallisepticum* isolates could not be determined, two *M. gallisepticum* isolates collected in 1979 and 1990 were investigated. Results showed that both the strains did not cause infection in six-day-old SPF chicks, and no death to 10-day-old embryonated eggs. The MIC of these *M. gallisepticum* isolates were determined and compared to a reference strain, S6. A summary of the MIC is tabulated:

Drugs/Antibiotics	MIC (ug/ml)		
	255/79	2320/90	S6
Tylosin	<0.1	3.13	<0.1
Ampicillin	>100	>100	>100
Neomycin	3.13	3.13	6.25
Cosumix plus	>100	>100	>100
Tiamulin	<0.1	<0.1	<0.1
Chlorotetracycline	0.78	25	6.25
Sulfamethoxine	>100	>100	>100
Spiramycin	0.78	25	6.25
Novobiocin	1.56	25	6.25
Lincomycin	0.78	25	3.13

(c) Isolation of plasmids from drug resistant *M. gallisepticum*

M. gallisepticum resistant to tylosin and spiramycin, a courtesy from Dr. C. Kuniyasu was examined for presence of plasmids. Two techniques for isolation of plasmids were carried out and their presence was examined by agarose gel electrophoresis. Plasmids were not detected from these strains which suggest that resistance to antibiotics is not attributed to plasmids.

(2) Characterization of *Haemophilus sp.* isolated from chickens in Malaysia

(a) Pathogenicity and drug sensitivity of local isolates of *H. paragallinarum*

During the two year-period (1991-1992), six *H. paragallinarum* were isolated and identified, while twelve *H. paragallinarum* isolates were collected from 1987 - 1990. All the isolates were serotyped as type A and their pathogenicity as well as drug sensitivity patterns were determined.

The MLD_{50} of *H. paragallinarum* isolates J75, J94 and P1-6 was determined in 10-day-old embryonated eggs. The MLD_{50} was found to be 1010.6 per ml.

H. paragallinarum isolated in Malaysia were tested for drug sensitivity, and compared to that of the reference strain, 221. A summary of the MIC is tabulated:-

Drug	MIC (ug/ml)						
	221	1009	15-2	J94	P1-6	AB20	J75
Tylosin	<0.1	50	3.13	3.13	50	50	25
Anxin	0.78	<0.1	0.2	0.2	0.2	<0.1	<0.1
Neomyson	1.56	0.39	0.39	12.5	0.78	6.25	0.2
Cosumix plus	1.56	0.39	1.56	0.39	0.78	6.25	<0.1
Tiamulin	25	12.5	6.25	1.26	25	100	25
CTC	0.2	3.13	0.2	0.39	0.78	0.78	0.78
Spiramycin	0.39	>100	100	25	>100	>100	>100
Novobiocin	0.2	3.13	<0.1	<0.1	1.56	0.78	0.78
Lincomycin	12.5	12.5	6.25	6.25	12.5	12.5	25

b) Studies on the growth rate of *H. paragallinarum*

Three local *H. paragallinarum*strains J75, J94 and P1-6 and a reference strains, 221 were investigated, for their log phase. All the *H. paragallinarum* isolates showed a log phase within 24 hours except for strain J75 which have a longer log phase of 72 hours.

(c) Studies on the protein profile of local isolates of *H. paragallinarum*

The soluble whole-cell protein of six isolates of *H. paragallinarum* were examined using standardized SDS-PAGE. The patterns were reproducible and all the isolates were similar to that of the reference strain, 221.

(d) Isolation of plasmids from local isolates of *H. paragallinarum*

The molecular characteristic of *H. paragallinarum* isolated in Malaysia was also examined. Nine local isolates and two reference *H. paragallinarum* strains were checked for the presence of plasmids. Results showed that all the local isolates had a common plasmid of molecular mass of 4.8 Md. Among them, three of the isolates had additional three plasmids with molecular masses of 1.8, 1.5 and 1.3 Md while one isolate had an extra plasmid of molecular mass 4.0 Md.

B. Development and Application of Diagnostic Methods for Poultry Diseases

Development of diagnostic methods for Infectious coryza and Mycoplasmal infection

(1) Detection of specific antibodies

(a) *H. paragallinarum* ELISA

The unit had achieved in detecting specific antibodies to *H. paragallinarum* infection through the development of an ELISA which proved to be not only specific but also, very sensitive to *H. paragallinarum* type A. Further studies on the development of ELISA to detect *H. paragallinarum* type C, will be looked at.

(b) Mg and Ms ELISA

ELISA for detection of antibodies against *M. gallisepticum* and *M. synoviae* were earlier developed and improved. However, when compared with and ELISA commercial kit, the kit was found to be superior than our ELISA for *M. gallisepticum* in terms of specificity

and sensitivity. However, comparison of a commercial kit of ELISA against *M. synoviae* was not carried out.

(c) Detection of antibodies by CIEP

The ddetection of specific antibodies to mycoplasmal and haemophilus infection by counter-immunoelectrophoresis (CIE) using tris-HCl buffer and acetate buffer was not successful. Some modification to the preparation of the antigens also did not give any fruitful results.

(d) Dot-on-slide to detect antibodies to *H. gallisepticum*

A similar test to ELISA was developed to detect antibody against *H. paragallinarum* infection using *H. paragallinarum* cultures added to 1% agarose and dotted onto a glass slide. The slide was dried for half a day and blocked with 3% chicken serum albumin in Tris buffer saline pH 7.5. A standard procedure of ELISA was carried out using HRP as substrate. The dot with positive control serum was observed as purple dot under a dissecting microscope while a negative did not. However, the concentration of the antigen was not satisfactory enough to give clear results. The same test should be repeated using higher concentration of antigen.

(2) Detection of Haemophilus and Mycoplasma antigen

(a) Detection of *M. gallisepticum* by FAT

The diagnosis of *M. gallisepticum* by flourescent antibody technique (FAT) is now made possible by the preparation of FITC conjugated to antibody against *M. gallisepticum*

(b) Detection of *M. gallisepticum* by epi-immunoflourescent

Epi-emmunoflourescent using FITC for *Mycoplasma sp.* was performed. This technique was found to be more rapid than the standard method of FAT. However, further development should be carried out to determine their specificity.

(c) Detection of *H. paragallinarum* by FAT

FAT was also carried out to detect antigen of *H. paragallinarum* and the results was found to be quite satisfactory.

d) Detection of *M. gallisepticum* by PCR

A PCR kit developed by a pharmaceutical company in Japan was evaluated by using cultures avian mycoplasmas. This kit was developed to detect mycoplasma contamination in tissues cultures. Results showed that the kit could only detect seven out of nine species of 8 *Mycoplasma sp.* and one *Acholeplasma sp.*, *M. gallinaceum* and *M. gallopavonis* could not be detected. Nevertheless, *M. synoviae* which did not grow on the K-agar, could be detected by the PCR. All the tissue culture samples did not show contamination of *Mycoplasma sp.* The results were confirmed by gel electrophoresis.

(e) Development of DNA probes

For the development of a more specific technique to detect *H. paragallinarum* and *M. gallisepticum* antigen, possibly to make DNA probes for the bacteria, recently, the unit has ventured into the isolation of *H. paragallinarum* and *M. gallisepticum* and to cut DNA by the restriction enzymes and study their nucleic acid patterns.

C. <u>Development of Poultry Vaccine</u>

Efficacy studies on activated infectious coryza vaccines.

A group of SPF chickens was given infectious coryza vaccines containing inactivated cells of *H. paragallinarum* adsorbed onto an aluminium hydroxide gel, while another group of chickens was given vaccine containing cell inactivated by dextran-sulfate. The chickens were challenged with *H. paragallinarum* strain 221 and signs of coryza were observed daily. At day 7 and 14 post challenge, the chickens were killed and their sinuses were swabbed for bacteria isolation. The rate of protection of the vaccines was compared to a cavvines prepared by a Japanese company.

Although all the vaccines gave good protection rate to the chickens, the chickens except those that were given vaccine with dextran sulfate did not develop antibody titres, after vaccination as well as after challenge. However, the chickens vaccinated with *H. paragallinarum* in dextran sulfate develop low antibody titre, measured by HI test, one week after vaccination which diminished after the second week.

Since the experiment did not exhibit production of antibody, it has to be carried out again.

Development of vaccine for infectious coryza could not be carried out yet because the proteins responsible for immunogenicity has not been determined. Whenever SDS-PAGE was carried out on *M. gallisepticum* and *H. paragallinarum*, the Western blotting did not show satisfactory results.

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RESEARCH ACTIVITIES - PAST, PRESENT AND FUTURE

INTRODUCTION

Since the inception of APDRTC, two parasitic diseases namely, coccidiosis and leucocytozoonosis have been the main foci for research as these were deemed to be important.

Coccidiosis constitutes the most economically important parasitic disease of poultry. It has a worldwide distribution and it invariably occurs wherever chickens are raised. In most countries, coccidiosis ranks next in importance to Newcastle disease. Worldwide, it was estimated that as much as US $ 60 - 120 million of annual losses incurred (Long, 1982). In Malaysia, the annual losses amounted to an estimate of US $60,000 arising from about 1% flock mortality.

Leucocytozoonosis is the next important parasitic disease. It has a wide geographical distribution particularly in Asian region. It causes economic losses by retardation of growth, mortalities and reduction or loss of egg production in adult birds.

COCCIDIOSIS

A. SURVEY

At the beginning of our work in coccidiosis studies, we conducted a survey on incidences and prevalences of the disease for the collection of base-line data. It was a two-year project comprising two parts, namely survey on poultry cases submitted to VRI for disease investigation and survey of farms. Briefly, results showed that about 35% of poultry cases submitted were positive to coccidiosis and of these the most common species were *Eimeria tenella* and *E. acervulina* followed by *E. necatrix*, *E. brunetti* and *E. maxima*. This identification was based on morphological characteristics of oocysts and parasitised areas of gut. Results of survey of farms indicated about 87.5 to 100% of poultry farms in Perak were positive, based on faecal floatation for the examination of oocysts. Oocysts were first detected in broiler and layer flocks at 15 and 21 days old, respectively. In the majority of farms, anticoccidial drugs were used. However, high counts persisted in some farms. Thus efficacy of some the anticoccidials used was doubtful and drug resistance was suspected.

B. PURIFICATION/ISOLATION OF *EIMERIA* SPECIES

From most of the faecal samples collected from various farms during the survey, *Eimeria maxima* was not infrequently detected and the densities (oocyst count) of the eimeria appeared unaltered even after instances of anticoccidial drug administration. *E. maxima* was therefore, suspected to be most resistant to anticoccidial drugs. Therefore, an attempt to isolate and purify *Eimeria* species - an integral step in coccidia studies - was primarily targeted at *E. maxima*. Single oocyst multiplication technique as described by Tsunoda, was employed. The technique is based on the depositing of an oocyst on a small slice of agar - usually 2.5% (Noble) - and inoculating in one susceptible chick which is then kept in one small cage in a coccidia-free room, thereupon. With this technique, other species of coccidia namely, *E. praecox*, *E. tenella* and *E. necatrix* were subsequently procured. The identification of the species was based on the pre-patent period, sporulation time, oocyst morphology, clinical signs and pathological changes in inoculated chickens.

C. ANTICOCCIDIAL DRUG RESISTANCE STUDY

E. maxima was used in an anticoccidial drug resistance study against nine types of anticoccidials. Several one-week-old chicks were inoculated with 100,000 sporulated oocysts of *E. maxima* while on medication with the nine drugs. Oocyst production from medicated groups were enumerated, and in relation to that from inoculated unmedicated group, resistance was determined. Of the nine drugs used, three were seen to be ineffective in eliminating *E. maxima* coccidiosis. This ineffectiveness was considered due to resistance of the coccidia. as evidenced by results obtained. This experimental verification of anticoccidial drug resistance exhibited by *E. maxima* therefore, corroborated our suspicion at farm level.

The owner of the farm from which the resistant *E. maxima* strain was detected, was informed of the situation and advised appropriately.

D. PATHOGENICITY STUDY

Using a strain of *E. tenella* isolated from a broiler farm, we conducted a pathogenicity trial by inoculating graded doses of sporulated oocysts into 30 12-day-old SPF chicks. Clinical coccidiosis was first evidenced on day 5. Sixty percent mortality occurred in a group of chickens inoculated with the highest dose of 200,000 sporulated oocysts. Feed consumption of all infected group reduced markedly during the experimental period. Water intake of all groups however, was varied. Relative bodyweight gains of all groups were proportional to doses of inoculum.

E. ESTABLISHMENT OF ELISA FOR SEROLOGICAL DIAGNOSIS OF COCCIDIA

Recently, ELISA was developed and widely used for antibody detection in poultry coccidiosis as well as other parasitic diseases. The assay is sensitive and rapid and is therefore useful for large-scale screening of antibodies to coccidia. Determination of antibodies level will indicate disease status as the level of antibodies, according to Onaga et al, 1985, is related to severity of infection.

In view of the usefulness of ELISA, and with the help of JICA short-term expert, Dr. Tamio Inamoto, we started a study to establish the assay in the laboratory. Since the project was just started, it is still in the stage of development.

F. QUAIL COCCIDIOSIS SURVEY

As a result of a directive mooted in one of the JPPJ meetings of the DVS, as regards the coccidiosis situation in quails in Malaysia, a study was conducted to survey the prevalence of the disease in three states on Peninsular Malaysia, namely Penang, Perak and Johore between late 1991 and early 1992, concurrent with the JICA short-term expert stint.*

Briefly, a total of 66 faecal samples were collected from 12 farms for 128% sucrose floatation of coccidia oocysts, and oocyst count and measurement. Two intestinal samples were also obtained and examined for coccidia and gross lesions. Results showed that 40 samples (60.1%) from 10 farms (83%) were positive for coccidia. Oocyst counts on positive samples were varied with the farms in Johore having the highest range of oocyst per gram faeces of 3,000 to 6,000 and 168,000. Measurement of oocysts, observation of parasitised zones of intestines and gross lesions revealed the prevalence of two species of quail coccidia namely *Eimeria uzura* and *E. tsunodai*. The former was observed to have a range of length of 16.3 - 26.3 um and width of 15.2 - 17.1 and average dimension of 20.9 x 16.7 um while the latter with 17.5 - 23.2 um length range, 13.3 - 17.0 um width range and 19.2 x 15.0 average dimension. None of the farms surveyed used anticoccidial drugs. Therefore, for effective control of the disease, drugs should be administered.

LEUCOCYTOZOONOSIS

A. VECTOR CULTIVATION

In order to initiate extensive studies on leucocytozoonosis, the colonisation of the vector, *Culicoides arakawae*, known as biting midges, is essential. Local wild midges were initially caught, using light traps, and reared in the laboratory. However, not until the introduction

* The results of this study have been presented by the Director of VRI in one of the later JPPJ meetings.

of midges from an established colony of a Japanese strain in March 1990, was success in vector rearing ever achieved fully.

Methods for laboratory colonisation of the midges were adapted from Morii's with some modifications. A paper on the colonisation procedures has been published in the journal of Tropical Biomedicine (8:pp 210-204).

B. ISOLATION AND MAINTENANCE OF *LEUCOCYTOZOON CAULLERYI*

The establishment of the vector colony has corollarily paved the way for studies on leucocytozoonosis. However, for investigations into chicken leucocytozoonosis, an isolate of the causative agent, *Leucocytozoon caulleryi*, is needed. Thus, after the initial exposure of several SPF chicks to the environment, *Leucocytozoon caulleryi*, was isolated for the first time in the country. A full account of the isolation and the subsequent maintenance procedures was published in the Jurnal Veterinar Malaysia, 3(2): 65-69.

C. PATHOGENICITY STUDY OF *LEUCOCYTOZOON CAULLERYI*

Following the isolation of a local strain of *Leucocytozoon caulleryi*, a study was conducted to experimentally determine its pathogenicity. Two types of chickens, i.e. SPF layer breeders and commercial broilers were used. The chickens were inoculated with varying doses of 2×10^1, 2×10^2 and 2×10^3 of fresh *Leucocytozoon caulleryi* sporozoites. Results showed that all infected groups of chickens had 100% infection. The parasite had further proved to be pathogenic to SPF by causing mortality rates of 20, 40 and 100% commensurating the doses of inoculum. In the broilers, no mortalities occurred.

D. FREEZE PRESERVATION OF *LEUCOCYTOZOON CAULLERYI*

With the establishment of maintenance procedures for the first local strain via chicken-vector passages, it would then be possible to isolate other strains in the future. However, it would be difficult to maintain simultaneously many strains of *Leucocytozoon caulleryi* long term. This was because such maintenance entailed infinite labour, time and much costs. Therefore, another study was conducted to establish the preservation of the parasite. The study not only involved preservation procedures, but also compared the usefulness of various media and storage temperatures. Results showed that the best medium was one which incorporated 85% EMEM, 10% chicken serum and 5% glycerol. With this medium, infectivity of the parasite in chickens was 100% after storage in either -196^0C or -80^0C.

E. SEROLOGICAL AND IMMUNOLOGICAL STUDIES ON LEUCOCYTOZOONOSIS

For the serological diagnosis of *Leucocytozoon caulleryi*, AGPT, established by Morii (1972), is routinely used. Yet another test - counterimmunoelectrophoresis (CIEP), established by Fujisaki *et al* (1980), which proves to be more rapid, has been used in our laboratory with modifications. The modifications involved the use of pre-cooled buffers and ice during electrophoresis and the use of large plates that can house large numbers of samples with which have rendered CIEP more suitable for large-scale serological survey of chicken leucocytozoonosis.

Both AGPT and CIEP can be used for semi-quantitative assay of antigens and antibodies. Based on two-fold titration of antiserum, a study was conducted to examine the effects of primary infection with *Leucocytozoon caulleryi* in SPF chickens and their resistance to reinfection. Varying doses of *Leucocytozoon caulleryi* sporozoites were inoculated into several adult SPF chickens. This primary infection produced 75 - 100% infection rate and a maximum of 25% mortality rate. Subsequently, surviving chickens, having antibody titres up to 32 on AGPT, were reinoculated. However, irrespective of antibody titres, all chickens (that were reinoculated) showed 100% resistance. It was therefore, concluded that humoral immunity was not related to resistance of chickens to leucocytozoonosis.

F. EXPERIMENTAL INFECTION OF *LEUCOCYTOZOON CAULLERYI* IN INDIGENOUS CHICKENS

Because the disease, leucocytozoonosis, usually is prevalent among commercial breed of chickens, a study on the experimental infection of indigenous chickens will elucidate the susceptibility of the chickens and hence the pathogenicity of *Leucocytozoon caulleryi*. Forty three-weekold indigenous chickens were inoculated with graded doses of *Leucocytozoon caulleryi* sporozoites. Results showed that infection rates ranged from 60 - 100% while no clinical signs were observed and no mortalities occurred. Parasitemia was observed from days 16 to 24 after inoculation and serum-soluble antigen and their antibodies were detected between days 14 to 16 and day 18 onwards, respectively. Bodyweight gains of three groups of chickens most heavily infected were significantly different compared to uninoculated control.

FUTURE PERSPECTIVE

It has been well documented that infection by *Eimeria* species induces significant humoral and cell-mediated immune responses. However, the actual mechanism of protective immunity is not clearly understood. There are a number of important questions yet remain to be answered, such as, which antigens or epitopes are immunodorminant, and which elicit protective responses; which stages of the parasite present these antigens and to what extent are they common; and do cellular- and antibody-mediated immune responses involve the same antigens ?

Each stage of the parasitic life-cycle is said to be a source of antigenic material and can therefore be the target of host immune response. However, of all the stages, the sporozoites are the main target against which the host protective form of immune response is most directed, as recent studies have shown. Despite this fact, it is still generally accepted that the asexual stages are the ones that could produce the strongest stimulus for development of immunity. I strongly believe so. A lot of studies have been made on sporozoites than on other stages merely because they are easily accessible.

One of the asexual stages is the first generation merozoites. I am particularly interested in this. A lot of information is available on merozoites of the second and third generation but none on the first. From the information gathered, of particular interests are that about 50% of the immunodorminant sporozoite proteins are present on the second or third generation merozoites and that there are no major antigenic differences observed between the two.

The first generation merozoites being closer to the sporozoite in the life-cycle may harbour more immunodorminant sporozoite proteins on their surfaces and hence may be capable of invoking greater immune response compared to the second and third generations. Furthermore, if the immune response can be manipulated, then it is more beneficially and practically directed against the first generation as it is the stage before the onset of clinical signs and mortalities. If the manipulation can surmount the parasite it (the parasite) will be rendered incapable of proceeding to second stage - more particularly referring to *Eimeria tenella* and *E. necatrix* - thus eluding overt adverse host reaction. In other words, the host will be protected from suffering severe clinical signs and death.

I wish to conduct an in-depth study to expound the antigenic nature of the stage and to possibly attempt to utilise the mechanics of its destruction to potentiate protective immunity against the parasite as whole.

In short, I wish to delve into the immunology of chicken coccidiosis for my postgraduate studies. This may include not only the above design but also other possible areas or openings which will very much be dependent on the approval of my would-be supervisor. The extent of on-going research and the capabilities of the laboratory of the place which I will go to, will at large determine the direction and the degree of the work that I will be approved of, doing. Hopefully, at the end of the day, I will be able to learn a lot more about coccidia and on the related research methodologies, so that I will be able to do better research in the future: probably formulation of vaccine.

Future

PROGRESS REPORT OF PATHOLOGY AND ELECTRON MICROSCOPY UNIT APDRTC (1991 and 1992)

PATHOLOGY UNIT APDRTC

1. Pathology of Salmonella infection in Poultry

a) Experimental infection of *S. weltevreden* in chicks and localization of salmonella antigens in tissues

Subcutaneous and oral inoculation of *S. weltevreden* into 3-day-old chicks caused 100 and 33% mortality respectively. Only 20% of birds showed weakness and moribund before died. No mortality or clinical signs observed in naso-ocularly inoculated chicks. Gross and microscopical lesions were seen especially in the visceral organs of the subcutaneously inoculated birds. Salmonella antigens were demonstrated in the necrotic foci of the liver, lung, lamina propria and lumen of cecum and rectum by immunoperoxidase staining.

b) Experimental infection of layers and pullets with *Salmonella weltevreden* and survival of antigens in chickens.

Intramuscular inoculation acaused 100, 30 and 0 percent mortality in 70, 30 and 13 week-old layers and pullets. All the *S. weltevreden* isolation from ovaries and oviduct were from the dead layers. Intramuscular inoculated survivors harbor *S. weltevreden* in the visceral organs and necrotic inoculation sites until 21 dpi. In orally infected groups no mortality or gross lesions were observed. *S. weltevreden* was isolated from the visceral organs until 14 days of infection only.

c) Effect of acidified drinking water on *S. weltevreden* infection in chicks

No adverse effect were observed when day-old chicks were given acidic (pH 3 and 4.5) or alkaline (pH 8.0) drinking water for a period of 20 days. About 98% of chicks inoculated with 0.1 ml of 10^4 CFU/1 ml *S. weltevreden* and supplied with pH 3 drinking water survived until 21 dpi. Only 20 and 10% survival were observed in other groups supplied with pH 4.5 and 6.6 (tap water) water respectively. Mortality was 100% in flock which inoculated orally with 0.1 ml of 10^8 CFU/1 ml inoculum and given pH 3, 4.0 and 6.6 drinking water.

d) Pathogenicity of 2 serotypes of salmonella recently isolated from ducklings and chicks

No clinical signs or mortality was reported from a flock of SPF and conventional broiler chicks aged 2 days, 1 and 2 weeks old inoculated with *S. weltevreden* (isolate from chicks) or *S. hadar* (isolate from ducklings). Only one bird inoculated with 10^8 organisms/ml in the *S. weltevreden* group showed severe gross and microscopical changes. No significant gross changes were seen in birds of all groups inoculated with *S. hadar*. Histologically mild catarrhl inflammation was observed in birds inoculated with 10^8 and 10^5 organisms/ml of both isolates.

At 7 dpi *S. weltevreden* and *S. hadar* were isolated from the vicera and intestine. However at 14 dpi isolations were made from the cecum and rectum for *S. weltevreden* only and from lungs and lower intestine for *S. hadar*.

By immunoperoxidase staining salmonella antigens were detected more heavily in the lower part than in the upper part of the intestine. Electromicroscopical finding showed gram negative bacterial in the cytoplasm of some cells in the lamina propria of the cecum.

e) Pathogenicity of *S. hadar* isolated from ducks in 1-day old SPF chicks and ducklings

Oral inoculation did not cause mortality both in 1 day old SPF chicks and ducklings. However 10% mortality was observed in naso-ocularly inoculated chicks. Heavy mortality of 53% was seen in ducklings inoculated intramuscularly. Peritonitis, liver necrosis, intestinal haemorrhages and congestion of lungs were seen in dead chicks and ducklings. Microscopical findings were necrosis of the liver, spleen and kidney. Sacrified chicks and ducklings necropsied at 7 and 14 dpi revealed a few foci of necrosis in the liver *S. hadar* was isolated from the vicera, cecum and vectum of all inoculated chicks and ducklings until 14 dpi. Isolation of salmonella from cloacal swabs of chicks and ducklings were positives until 14 dpi.

2. Pathogenicity of Duck Hepatitis virus in 2-week-old ducklings

a. Intramuscular inoculation of DHV into one-week old Khaki Campbell ducklings failed to induce clinical signs, gross and histological lesions.

b. Pathogenicity of Duck Hepatitis virus in 2-week-old ducklings

The mortality in 2-week-old duckling inoculated orally with liver homogenate containing DHV is 46%. At 2 days post infection (dpi) 4 ducklings were found dead while one was sick. The sick duckling had extended neck, greenish watery faeces and died later. Another duck died at 3 dpi. At post mortem all livers were enlarged, yellowish and very friable. Few minute pin point haemorrhages were seen on the surface of the liver. Spleen was swollen and mottled. The kidney and small intestines showed severe congestion.

Histologically necrosis of hepatic cells and varying degrees of inflammatory response and haemorrhages were seen on the liver of the ducklings. The lesion on the kidney, spleen and small intestine was severe congestion. DHV is still pathogenic in ducklings after forth passages in chicken embryos.

3. Infectious Bursa Disease - IBD

a. Pathogenicity of Lukert and JI strains of IBDV in 2 days and 6 weeks old SPF chicks.
(A collaborative project with the Virology Sections (APDRTC and VRI)

No significant gross changes were observed in the BF, spleen and cecal tonsils of birds infected orally with IBDV at 2 days or 6 weeks old sacrified on 3 and 8 dpi. Histologically mild lymphocytes depletion was seen in 15% of total BF of 6 weeks old birds. In 2 days old chicks JI strain caused higher incidence of lymphocytes depletion at 3 dpi only. The BF, spleen and cecal tonsils were normal at 8 dpi.

b. Pathogenicity of Lukert and J1 strains of IBDV in 3 weeks old SPF chicks
(a collaborative project as above)

No significant gross changes were observed in the BF, spleen and cecal tonsils of birds infected orally with IBDV at 3 weeks old. About 5% and 10% of the BF showed mild lymphocytes depletion in the Lukert and JI inoculated chicks chicks respectively. No histological changes present in the spleen and cecal tonsils of the birds sacrified at 3, 8 and 14 dpi.

c. Immunosuppressive effect of Lukert and JI strains of IBDV in 2 days old SPF chicks

Young chicks age 2 days old were inoculated with either Lukert of JI strains of IBDV. Vaccination with NDV; F strain was done at 5 days old and challenged with NDV `velogenic' strain 14 days later. Result showed that mortality in the group inoculated with Lukert and JI strain of IBDV was 25 and 37% respectively. The birds died within 3 to 4 days post challenge with the velogenic NDV. The gross and histological lesions observed in dead birds were typical of NDV infection.

d. Experimental infection of IBDV (isolate 6511) in 2 weeks old ducklings and 3 weeks old broiler chicks

Oral inoculation of BF homogenate into 2 weeks old ducklings failed to induce clinical signs, significant gross or histological changes in BF, spleen and cecal tonsils of those ducklings at 2, 4, 6,8 and 14 dpi.

No mortality was observed in a group of 3 weeks old commercial broiler chicks inoculated orally with BF homogenate. Edema, enlargement and haemorrhages of BF were seen from 2 to 5 dpi. At 8 and 10 dpi all the BF examined were atrophied and about half the size of the normal BF. Histologically mild necrosis of a few follicles was evident at 1 dpi. By 5 dpi all follicles were involved with total disappearance of lymphocytes, purulent material accumalation in the medulla, cellular debris in the cortex and edema with inflammatory reactions in the interfollicular areas. At 8 dpi all the dead cells had been cleared from the follicles leaving reticular cells proliferation, macrophages and few lymphocytes in the atrophied follicles. At 10 dapi the interfollicular areas were thickened, lymphocytes repopulation resulting in various sizes of follicles, involution of epithelium and presence of small cysts occured in the BF.

e. Pathological comparison of bursal lesions in conventional broiler chicks inoculated with different strains of IBDV

Field strain (isolate 3529) caused more severe bursal damage in 27 days than in 5 days old chicks. Field strain infection was characterised by acute necrosis of follicles with severe edema and inflammatory reactions in the lamina propria of the BF. Chicks inoculated with the standard strain only showed lymphocytes depletion followed by regeneration of follicular lymphocytes later.

IBD antigens were more readily detected by direct FAT in the BF of chicks inoculated with field strain inoculated at 27-day-old and examined at 3 and 8 dpi than those in 5-day-old chicks.

4. Duck Virus Enteritis (Duck Plague) - DVE

a. Pathology of duck and ducklings infected with duck virus enteritis (DVE) or duck plague virus (First reported case in Malaysia)

Few sick adult ducks from a farm which had flock mortality of 70% were sent to the Pathology Unit for disease investigation. Gross and histopathological lesions were seen in the visceral organs particularly the lymphoid system.

Oral and intramuscular inoculations of the liver homogenates from the above ducks into the young ducklings caused 89 and 95% mortality respectively. The gross and microscopical findings in ducks and ducklings were similar. The liver revealed whitish necrotic foci with or without petechial haemorrhages. Spleen was dark while the thymus and BF were atrophied fresh haemorrhages were seen on the mucosa of the esophagus, proventriculus, small and large intestines especially on rectum and cloaca.

Microscopically intranuclear inclusion bodies were observed in the epithelial cells of digestive and respiratory tracts, reticular cells of lymphoid organs, hepatocytes and enothelial cells of the blood vessels. The liver which were examined electron microscopically showed numerous virus particles in the nuclei and cytoplasm of the liver cells and reticulo-endothelial cells. The presence of naked virus particles and mature virions in the cytoplasm were characteristics of viruses belonging to herpes group.

b. Immune response of local ducks to an attenated strain of Duck Enteritis Virus

Results showed that one time vaccination in ducks aged 1 or 4 weeks only gives about 50% to 62.5% protection against DVE challenge virus of $10^{5.3}$ DLD_{50}/0.1 ml, respectively. The challenge virus was inoculated 2 weeks after vaccination. Higher rate of protection of 75% to 78% were observed in ducks that received 2 vaccinations, 3 weeks from the first vaccination. Mortality was observed within 5 days post challenge with virulent challenge virus in all groups. At autopsy typical lesions of DVE infection were seen in all dead ducks.

Tests to determine the level of neutralizing antibodies in post vaccinated and post challenged serum samples are in progress.

II. Immunoperoxidase technique (IP)

This technique was first introduced by Dr. M. Narita and Dr. H. Haritani from NIAH, Japan. Dr. H. Haritani also attempted to develope IP technique for duck virus hepatitis during his stay as short - term expert in 1987. Most of the IP demonstrations were using antisera (prepared in rabbit) and paraffin sections brought from Japan.

This unit also attempted to prepare its own antisera (rabbit) against DVH, ND and IBD but the IP results were poor. Commercially available antisera such as against *S. weltevreden* has been successfully used in this laboratory. This IP technique was applied in the studies of localization of *S. weltevreden* and IBD antigens in paraffin embedded tissues. This unit had conducted training on IP method for technicians from the regional laboratories.

Although this IP technique is sensitive and specific, but it is time consuming. Availability of antisera, costly reagents and long tissue processing time are limitations for this method. The use of IP on frozen sections should be pursued in the future if the antisera and reagents problems could be overcome.

Another technique which is more frequently used in this laboratory now is the direct flourescent - antibody technique (FAT) on frozen sections. The technique is simple, sensitive, specific and rapid. So far the staff of the pathology unit had undergone training for the preparation of FITC conjugated antisera and FAT. At the moment FAT is used in the studies of IBDV and NDV infections. Preparation of FITC conjugated antisera against ND, IB and IBD are in progress.

The application of indirect-FAT for several avian diseases on frozen sections is still under trial. The advantage of this method is the use of commercially available FITC conjugated IgG while the antisera only needed to be purified before using.

This unit had conducted training on cryostat sectionings and FAT for the technicians from our regional laboratories, ASEAN participants and MTCP participants from other countries.

We hope to use the direct and indirect - FAT for diagnosis of viral diseases in chickens as well as in ducks.

B. ELECTRON MICROSCOPY UNIT

A total of 305 micrographs were recorded with the scanning electron microscopy (SEM). Most samples were parasites from various species of animals that sent by parasitology section of VRI.

The following parasites were identified :-

Fluke from the eye of a chicken	:	*Philophthalmus galli*
Roundworm from a fish	:	Anisakinae
Roundworm from an elephant	:	*Quiloina rennei*
Parasite from cattle	:	*Stephanofilaria kaeli*
A roundworm	:	*Amplicaecum sp.*
Hookworm from an elephant	:	*Bath mostomum*
Hookworm from a dog	:	*Ancylostoma caninum*

Culicoides perengrinis
Culicoides orientalis
Culicoides shortii
Culicoides guttifer
Culicoides huffe

The SEM had enable the researcher to study the three dimensional structure of bacteria such as *Pasteurella multocida*, *Pasteurella anatipestifer*, *Salmonella weltevreden*, *Haemophilus paragallinarum*, *Haemophilus gallisepticum*, *Mycoplasma gallisepticum*, *E. coli* and *Pseudomonas areginosa*. A collection of micrographs of these bacteria was compiled by this unit for future reference.

A parasite which adhere to the epithelium of the bursa of Fabricius of chicken and duck called *Cryptosporidia* was confirmed by the SEM and TEM. This was a first report of cryptosporidiosis in avian species in this country.

For the transmission electron microscopy (TEM) a total of 198 micrographs were recorded mostly from experimental cases. A chronological studies on the ultrastructure of tissues infected by viruses such as duck hepatitis virus (DVH) infectious bursal diseases (IBDV) and duck enteritis virus (DEV) had been done. Infact the duck virus enteritis disease which was first reported in Malaysia in 1991 was confirmed by using the TEM. The causative agent is a Herpes virus was demonstrated in the nucleus and cytoplasm of liver cells. Other viruses that cause diseases such as IB, ND, ILT, EDS, FP and IBH were also seen for the first time by

our researchers. These viruses and bacteria such as *Salmonella sp.* can be demonstrated in situ in ultrathin sections from tissues of infected birds or infected tissue culture. The viruses are also demonstrated after a process of sedimentation and negative stainings. Contagious ecthyma virus was confirmed using negative staining method from scab samples of goat that naturally infected. Work on tissue samples obtained from cattle and fish suspected of virus infection are in progress.

The EM unit has produced 2 technical manual for electron microscopy and 1 compilation of electromicrographic charts of causative organisms of avian diseases. This unit also participated in exhibition of micrographs organised by the Electron Microscopy Society (Malaysia) in 1991 and 1992. Preliminary and introductory courses on EM are given to VRI, APDRTC staff and to all participants that undergone trainings in pathology.

FUTURE PLAN FOR THE PATHOLOGY UNIT
(INCLUDING ELECTRON MICROSCOPY UNIT)

1. In order to upgrade the research this unit needs to do Collaborative work with other sections particularly virology, parasitology and bacteriology unit. They could help to prepare, titrate and purify antigens for inoculation, assay of the serum and isolation of antigens after inoculations. The dose and type of organisms in the inoculum should be standardised in order to obtain useful data from the experiment.

Advance methods has been used by the virology and bacteriology units to differentiate isolates down to the molecular level. Once the isolate identified their pathogenicity should be studied. Work on identification of DVE virus isolates in this country is in progress. The study will also include the attenuated strain and DVE virus from geese.

2. The immunopathological methods is useful in detection and localization of antigens from tissue in situ. Methods such as immunofluorescent antibody (FA) technique and immunoperoxidase (IP) not only sensitive but also specific. However every methods has its constraint eg lengthy procedure and time consuming, besides costly (IP). Other method available for detection of antigens in unfix tissue is by using probe. These probes can be prepared from non-radioactive material or nucleic acids. Evaluation will be done on the existing IP and FAT against the probe methods in future.

3. Very little work has been done on diseases which only serologically detected in this country. Infection in the field usually masked by other diseases such as IBD, ND, IB, Mycoplasma and bacterial infections. The diseases should be studied are chicken anaemia, inclusion body hepatitis, avian nephritis and reticuloendotheliosis. Experimental infections using agents isolated in other countries is possible with the availability of isolators. The data obtained from the chronological studies of pathological lesions could be shared by the ASEAN countries.

4. Although more emphasis are given to rearing of chicken and ducks but other species of poultry are gaining importance. Turkeys and quails are intensively reared now therefore some of their diseases needs to be identified in future. At least the most common diseases such as haemorrhagic enteritis, mycoplasmosis, blue comb in turkey while ND and coccidiosis in quail under local conditions should be investigated. Knowledge on these diseases are obtained preferably through experimental infection.

5. Electronmicroscopes are excellent tool for research and diagnostic but currently the use of these by our researchers are limited. Firstly very few researchers are trained in using the EM and secondly their knowledge in this field is limited. Therefore difficulties arise when examining ultrathin sections using TEM and identifying virus for the first time. There is nobody to refer to and to comment on the works.

However, in future collaborative work with other institution such as Universiti Pertanian should be encouraged. This not only for the exchange of ideas but also to obtain their expertise in EM on various disease agents. To facilitate the identification of viruses and lesions from the ultrathin sections maybe Japanese experts will accept electronmicrographs from here for description and comment.