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Use of immunoassay technologies for the diagnosis and control of foot-and-mouth disease in Southeast Asia

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FOREWORD

The IAEA and FAO, through the activities of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture and their technical co-operation programmes, support the introduction of nuclear and related techniques to improve animal disease diagnosis and surveillance in developing countries.

At a workshop hosted by the Australian Centre for International Agricultural Research (ACIAR) and the Department of Livestock Development (DLD) of Thailand, in Lampang, Thailand, in September 1993, an analysis of the results of an ACIAR project on foot-and-mouth disease (FMD) as well as national reports from twelve other Asian countries clearly demonstrated that the control and eradication of FMD in Asia is both a national and regional problem (vaccination alone costs in the region US $380 million annually). It was concluded that a co-ordinated regional approach was the only realistic way forward for controlling and eventually eradicating FMD from the region. It was agreed that the OIE would lead this co-ordinated regional programme in close co-operation with FAO, ACIAR, other relevant international organizations and national governments.

Results of the ACIAR Project also clearly demonstrated the immense value of ELISA based systems for the diagnosis and control of FMD within Thailand. The meeting, therefore, recommended that an essential component of a regional strategy was to have, as a minimum, ELISA tests for the detection of FMD virus and for assessing the antibody status of livestock population in each country in the region. In support of this concept, the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture established a co-ordinated research project (CRP) with the primary aim of establishing and documenting appropriate mechanisms for introducing and using ELISA based technologies for FMD diagnosis and surveillance in participating countries.

At all times, activities under this CRP have been co-ordinated with national and regional initiatives to control FMD. Under the general auspices of the OIE, closely allied to FAO and ACIAR national projects, this integrated approach has ensured that the FAO/IAEA CRP had focus and direction towards assisting the regional eradication programmes and therefore could substantially and sustainably assist the overall campaign for FMD control.

At the completion of this Project, the region is left with a national ELISA based diagnostic facility in all participating countries verified through the use of an FAO/IAEA External Quality Assurance exercise. This publication documents how this was achieved, it includes some of the national studies that have been undertaken using this capability and documents the results of the external quality assurance programme. The IAEA officer responsible for this TECDOC was M.H. Jeggo of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture.

EDITORIAL NOTE

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SUMMARY

1. SCIENTIFIC BACKGROUND

Foot-and-mouth disease (FMD) is one of the most important diseases affecting livestock in Asia causing losses directly through reduced production (milk and meat and working time for draft animals) and indirectly through loss of export markets due to the presence of the disease in a country. Although vaccination and animal movement control are central to any FMD control programme, an ability to diagnose the disease and monitor the effectiveness of control measures is crucial. The matter is complicated by the presence of a number of sero-types of the virus that cause FMD and the need to identify these quickly in order to trace the source of the outbreak and select appropriate vaccines for control.

Until recently, the diagnosis and identification of virus types and sub-types and the characterization of the antibody response to infection with FMD involved a number of unreliable, cumbersome and expensive procedures including virus neutralization, complement fixation and gel electrophoresis. A further problem is that all these techniques are difficult to standardize and hence interpretation of results is subjective.

For ten years prior to 1993 much work had been focused on the development of ELISA technology to identify and characterize FMD virus types and the host's immune response to them [1]. The use of this technology within the framework of national and regional FMD control programmes has enormous potential to increase the capability of veterinary services to type FMD viruses and to improve the 'match' between vaccine and field strains. The protocols for doing this and the reagents needed have now been fully standardized by the World Reference Laboratory (WRL) for FMD in UK. The Joint FAO/IAEA Programme on developing a system to transfer this technology to developing countries, worked with the WRL to develop FMD antigen and antibody detection 'kits'. The antigen (or typing) kit was successfully validated in nine Latin American countries through collaboration between FAO/IAEA and the Pan American Foot-and-Mouth Disease Center (PAFMDC) in Brazil under an earlier FAO/IAEA Co-ordinated Research Project [2]. This assay is now being used routinely within the framework of national and regional control and eradication efforts in Latin America. In 1994, the 'antibody kit' was similarly introduced into Latin America for vaccine testing and for examining relationships between vaccine and field strains of FMD [2]. It was foreseen at that time that PAFMDC would eventually produce and distribute the reagents required, whilst the FAO/IAEA Programme and the WRL would focus on providing a quality assurance service.

Parallel to the above, in 1993 the recently completed ACIAR[1]/AAHL[2]/Government of Thailand Project on FMD had also shown the immense value of ELISA-based systems for the diagnosis and control of FMD within Thailand. An ACIAR Workshop in Lampang, Thailand, (September 1993) considered the results of this project and reports from 12 Asian countries on their FMD situation. The meeting strongly concluded that, in the absence of effective animal movement controls in this region, individual national efforts would achieve little and that the only realistic way forward to controlling and eventually eradicating FMD in Southeast Asia was to consider a regionally-based approach (national vaccination programmes alone cost the region US $380 million annually). It was recommended that an essential component for such a regionally based control and eradication programme be for each country in the region to have, as a minimum, an ELISA testing capability for the detection of FMD virus and for assessing the antibody status of their livestock populations.

In response to this, a new FAO/IAEA CRP entitled "Improved diagnosis of foot-and-mouth disease in Southeast Asia using ELISA-based technologies" was established with the objective of bringing together the expertise available in the Joint FAO/IAEA Programme, the WRL and the AAHL to help establish an FMD diagnostic capability within countries of the region and subsequently to use this to develop and monitor control and eradication programmes. It was considered from the outset

[1] Australian Center for International Agricultural Research.
[2] Australian Animal Health Laboratory, Geelong.

that this CRP would function within the framework of the OIE/FAO/ACIAR Control and Eradication Programme for FMD in Southeast Asia.

2. OBJECTIVES OF THE CRP

(1) The primary objective was to strengthen the capability of the national veterinary services in Asia to contribute effectively to FMD control through the introduction and use of ELISA-based systems for FMD diagnosis and monitoring. Emphasis was to be placed on transferring FMD typing capability, and subsequently this would be extended to FMD antibody assays for checking vaccines and for monitoring relationships between strains involved in field outbreaks and used in vaccines.

(2) Within the overall objective, the aim would be to award Research Contracts to individual institutes in the region to use ELISA tests in conjunction with other epidemiological techniques to undertake research studies to determine the prevalence and distribution of FMD in the countries concerned, and hence its economic significance.

(3) Following on from this and based on information obtained from the above activity, to develop nationally improved control and eradication programmes and to monitor the effectiveness of these using ELISA methods, within the framework of the ongoing OIE/FAO/ACIAR regional FMD Programme.

As mentioned above, the control and eradication of FMD in Asia is both a national and regional problem. The approach proposed under this CRP was one that was to address both issues. It involved establishing a regional Network of key national FMD diagnostic laboratories in Southeast Asia, which would be supported by FAO/IAEA, but work in close conjunction with national and regional control and eradication efforts to help ensure the attainment of the overall objective of FMD control and eradication. It was anticipated that this approach would maximize the benefits from resources both nationally and regionally provided from a variety of sources (national Governments, FAO, OIE, ACIAR, EU).

This CRP was not a ‘stand alone’ research effort but a critical and key component of an overall programme of regional disease control. Thus, whilst there were specific research studies to be undertaken, there was also a strong element of capacity building and the subsequent use of this capacity to meet national and regional diagnostic and surveillance needs.

The CRP provided a key element of co-ordination. This applied not only to the research studies being undertaken but also to the use of a single standardized and quality assured assay, to standardized surveillance methodologies, to training and to national and regional control efforts. To assist in ensuring a co-ordination with efforts nationally and supported through other Organizations (OIE, ACIAR, EU, JICA) it was agreed that all Research Co-ordination Meetings (RCMs) held under this programme would be held in conjunction with the OIE FMD Sub-commission meetings held in the region. Throughout the implementation of this CRP (1994–1999) there was always a strong link with the OIE initiative to ensure that the support provided through the CRP remained targeted towards the eradication process. It should be noted that the eradication programme is divided into three Phases. Phase 1 is concerned with planning and capacity building linked to socio-economic studies and enabling research. Phase 2 is the implementation control Phase, and Phase 3 is concerned with eradication and verification. Phase 1 is due to run from 1993 to 1999 and the objectives and activities under this FAO/IAEA CRP in conjunction with this Phase are clearly highly appropriate.

3. PROGRAMME IMPLEMENTATION

3.1. Use of standardized ELISA kits and data analysis programs

An essential component of the CRP was that participating institutes adhere to common test protocols, use fully standardized reagents, conform to a common system of result interpretation and

reporting, and participate in an external quality assurance programme (EQAP). All of the above was provided through collaboration between the FAO/IAEA Programme, AAHL and the WRL. An FAO/IAEA technical contact was awarded each year to the WRL for the preparation of biological reagents for the FMD ELISA kits, test sera and antigens used in the EQAP.

3.2. Co-ordination of technical work and inputs

Technical co-ordination was achieved through a process of defining work plans at the RCMs, through the provision of standardized reagents and protocols and of the EQA reagents by the FAO/IAEA Programme, and through the implementation of uniform data analysis and reporting procedures. Training and equipment inputs were also standardized with the overall aim of providing a uniformity of approach and operation to the technical work undertaken. At all times Research Contract holders co-ordinated their activities with those of national FMD control and eradication activities. Joint annual meetings with the OIE FMD Sub-commission helped assure co-ordination of the regional effort.

3.3. FAO/IAEA Research Co-ordination Meetings

Three Research Co-ordination Meetings were held, in 1995 (Thailand), 1997 (Philippines) and 1999 (Cambodia). In 1996, an FAO/IAEA Regional Training Course on ELISA was held in Viet Nam. This involved all Research Contract holders and the timing coincided with that of the annual OIE FMD Sub-commission meeting. In effect, therefore, each year an annual meeting was held involving all Research Contract holders, national official responsible for FMD control and eradication activities in each country, OIE, FAO, FAO/IAEA and ACIAR officials involved in the regional FMD control and eradication efforts and a range of consultants specialized in specific aspects of the overall regional programme.

During the first RCM detailed work plans for the coming year were prepared taking into account both national and regional priorities in FMD diagnosis and control. Training was also provided on the use of the FAO/IAEA FMD kits. During subsequent meetings, results were presented by each Research Contract holder followed by the preparation of work plans for the coming year. Further training was given on approaches to FMD control and epidemiology, computerized software programs, etc.

The final RCM was held in Phnom Penh, Cambodia, from 22 to 26 February 1999 in conjunction with the fifth meeting of the OIE FMD Sub-commission. At this final RCM, papers were given by the ten contract holders (from Malaysia, Philippines, Thailand, Viet Nam, Laos, Cambodia, Myanmar, Sri Lanka, Bangladesh and Hong Kong China) on the work conducted under their contracts during the past five years. These papers form the basis of this TECDOC. During the meeting these presentations were closely linked with national and technical reports on the OIE eradication programme. The achievements of this CRP were universally acclaimed by the representatives of countries attending this meeting and experts/representatives from various international (FAO, ILRI, APHCA) and donor (ACIAR, JICA, EU) organizations supporting the OIE programme and attending this meeting. A set of conclusions and recommendations emanating from this CRP was prepared and is given below but further specific recommendations relating to individual countries are given in the various papers in this publication.

3.4. Future activities

As part of the ongoing Technical Co-operation Programme, the Agency will host an IAEA/FAO Regional Training Course on FMD diagnosis and surveillance in November 1999 in Thailand and under a national IAEA Technical Co-operation Project for Thailand, support is being provided to the newly established OIE Regional Reference Laboratory for FMD in Pak Chong. A new FAO/IAEA CRP on FMD dealing with developing and validating an assay to separate FMD vaccinated from naturally infected animals will commence in 1999. Five of the national veterinary diagnostic

laboratories supported under the current CRP will be involved in the new CRP (Malaysia, Philippines, Thailand, Myanmar, Hong Kong China) assuring continued support for the FMD control and eradication programme in this region.

4. CONCLUSIONS AND RECOMMENDATIONS

4.1. Overall conclusions and recommendations

- All the national laboratories supported under this CRP are now able to diagnose FMD virus (to the type level), detect antibodies to FMD virus and conduct sero-epidemiological surveys.

- A capacity now exists at the national veterinary laboratory level in all participating countries to assist national and regional FMD control and eradication efforts. To retain this capacity will require further inputs of training and equipment (principally reagents and consumables) from external sources, to a varying degree, in all the countries. However, it is foreseen and accepted by the countries involved, that the provision of technical support, of ELISA reagent supply and the operation of an external quality assurance programme will become functions of the newly established OIE FMD Regional Reference Laboratory in Thailand and that a sustained ability to undertake this will be achieved through cost recovery for services and reagents supplied.

- Two EQAP exercises have confirmed the validity of results so far published and all laboratories are now routinely using quality assurance procedures to some extent. Further training is required in quality assurance principles and in the routine application of internal quality control procedures. All national veterinary laboratories should move towards achieving international laboratory accreditation.

- A number of enabling research studies have been undertaken to assist national FMD control and eradication programmes, e.g. evaluation of the vaccination efforts programme in Philippines; molecular tracing of causative viruses in Thailand. Such targeted studies have been critical to national decision making with regard to FMD control. Such studies should continue, but be on a focused basis and clearly targeted towards support for decision making at the national level. The scant resources available for research must be directed towards this goal, and this will require a strong and continual dialogue between laboratory staff and Government veterinary services officials.

- During Phase 2 of the OIE/FAO FMD Eradication Programme, maintenance of a diagnostic and surveillance capability at the national level utilizing ELISA-based technologies will be critical to the success. There are significant opportunities for technical co-operation programmes to assist in this area and to capitalize on what has already been achieved. Such support will undoubtedly contribute towards the overall aim of improving livestock productivity, itself part of creating food security and poverty alleviation in this region.

4.2. Specific technical conclusions and recommendations

- The provision of standardized ELISA kits from a single source (WRL, UK) was a major component in ensuring a rapid and effective transfer of ELISA-based technologies for FMD diagnosis. This not only ensured that all laboratories could be trained in a uniformed manner, but facilitated a comparison of results from country to country, provided for ease of trouble shooting the assay and greatly assisted the quality assurance programme. Whilst it is accepted that in future kits and reagents will be provided from the new OIE Reference Laboratory in Thailand, in the short term it is hoped that the supply of kits from WRL will continue and that this will require external resources to achieve.

- The delivery of kits through the local UNDP office does not work well in many countries and methods to improve this should be developed.

- Control sera in the assay should be produced within the region and significant amounts should be produced in one exercise to ensure control over time and the long term sustainability of the quality assurance programme.

- Emphasis should be placed on the routine plotting of the internal control data. This should be visually charted in the testing laboratory and supplied to a central source (FAO/IAEA/OIE Central Laboratory, Vienna or OIE Reference Laboratory, Thailand) for continual external review. This should greatly facilitate identifying problems with the assay and individual reagents in a timely manner.

- Although the operation of the EQAP is ideally conducted twice a year this is unrealistic. It is recommended that this is conducted once a year and eventually be based on a cost recovery basis. It is seen as essential for trade related issues.

- Results from an EQA round cannot be reported within one month and the current time frame for this needs some adjustments.

- Some reagents in the current assay kits clearly degenerate over time. Whilst such reagents can be adjusted this does require re-titration of the assay with a subsequent loss of reagents and may well affect analytical sensitivity (although it is unlikely to affect diagnostic sensitivity). It is recommended to look into this and consider ways of reducing this effect (i.e. through the use of stabilizing agents).

- It is critical that assays are set up to detect antibody or antigens (FMD viruses) that occur locally. A continual assessment of virus strains that occur in each country needs to be undertaken through the submission of samples to the WRL or when capable, the OIE Regional Reference Laboratory. In some countries support is needed for the dispatch costs of samples to the WRL.

- It is vital to continue to match viruses isolated from outbreaks to the vaccines being used. This requires submission of viruses from outbreaks to the WRL or OIE Regional Laboratory for sub-typing and matching to currently available vaccines.

- In the longer term, to assist virus isolation and typing it is foreseen that tissue culture facilities will be required in a number of, if not all, national diagnostic laboratories. Consideration should be given for resources to develop this capacity.

- The FAO/IAEA software program EDI (for transfer and management of ELISA generated data) continues to create interfacing problems between the ELISA reader and a computer. It is recommended that alternative approaches be developed to overcome this problem.

- The response of pigs to vaccination is still poorly understood with particular regard to an ELISA monitored response. It is recommended that studies be undertaken to correlate virus neutralization data to that of ELISA and to relate this to protection and vaccination. Whilst it is appreciated that some information does exist in this area, this requires consolidation.

- There is an urgent need to evaluate the duration and impact of maternally derived antibodies on the ELISA response, on vaccination and on protection. It is recommended that studies be undertaken in this area in several countries in this region.

– There is almost no information of the role of the water buffalo in terms of its response to challenge with FMD and on the ability of this species to be a carrier. Given the critical importance of this with regard to long term eradication it is strongly recommended that studies be undertaken as a matter of urgency to better understand the course of an FMD infection in water buffalo.

THE GLOBAL FOOT-AND-MOUTH DISEASE SITUATION DURING 1998 AND ITS RELEVANCE TO CONTROL AND ERADICATION EFFORTS IN SOUTHEAST ASIA

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Abstract

THE GLOBAL FOOT-AND-MOUTH DISEASE SITUATION DURING 1998 AND ITS RELEVANCE TO CONTROL AND ERADICATION EFFORTS IN SOUTHEAST ASIA

This paper reviews the recent successes in the control of FMD in Europe, South America and southern Africa and highlights the lessons to be learnt from those experiences, which could be applied to Southeast Asia to promote the control and eradication of the disease in that region.

1. THE GLOBAL SITUATION OF FOOT-AND-MOUTH DISEASE (FMD) DURING 1998

1.1. Europe

The former USSR republics of Georgia, Armenia and Azerbaijan were the only countries in Europe to report FMD during 1998. The outbreaks in Georgia and Azerbaijan were caused by type O virus, those in Armenia by a type A virus, shown by the All Russian Research Institute for Animal Health, Vladimir, to belong to same genotype as the type A Iran/96 strain, which has been circulating in Iran and Asiatic Turkey.

1.2. South America

The Hemispheric Eradication Programme in South America proceeded successfully during 1998 pushing FMD further northwards on the sub-continent. Chile, Uruguay, Surinam, French Guyana and Guyana retained their status as FMD-free, non-vaccinating countries. Argentina, Paraguay and the States of Santa Catarina and Rio Grange do Sol in the south of Brazil were also free (with vaccination). The veterinary authorities in Argentina intend to cease the annual vaccination of cattle by the end of March 1999. The countries in South America in which FMD was reported in 1998 included Ecuador, Colombia, Venezuela, Brazil and Bolivia.

1.3. Africa

No reports of FMD were received this year from Morocco, Algeria or Tunisia. Outbreaks of type O did, however, occur in Egypt. Further to the south, type O outbreaks took place in Uganda, Tanzania and Malawi. Sero-type A strains received by the WRL from Gambia and Senegal in West Africa, and from Eritrea in East Africa (Table I), were found to be antigenically different from existing vaccine strains. Eritrea and Uganda suffered type SAT 2 outbreaks also.

1.4. West Asia

The A Iran/96 variant of type A virus continued to spread in Iran and Asiatic Turkey. Homologous vaccines have been developed by private laboratories in Europe and Asiatic Turkey and by State laboratories in Iran and Turkey. Type O virus was isolated by the WRL from samples from Asiatic Turkey, Lebanon, Kuwait, Bahrain and Saudi Arabia (Table I) and reported by Qatar, Kazakhstan and Kyrghystan.

1.5. Central and East Asia

Type O virus was identified by the WRL in samples from Taiwan Province of China, Pakistan, Nepal, Bhutan, the Philippines, Hong Kong and Myanmar. Type O continues to cause sporadic outbreaks on Luzon and Leyte islands in the Philippines. Type A virus was found in samples from Thailand and Nepal and Asia I in samples from Pakistan and Myanmar (Table II). The People's Republic of China reported outbreaks in Yunnan Province but samples were not sent to the WRL. Malaysia did not report any outbreaks of type A during the year. No type C viruses were identified in

samples submitted from the region to the WRL. Indonesia, Singapore, Japan and South Korea remained free of the disease.

2. CONTROL AND ERADICATION OF FMD IN EUROPE

This century has seen dramatic improvement in the FMD situation in Europe. For centuries the disease ebbed and flowed according to the rise and fall of the naturally acquired immunity of the livestock populations. Century after century most countries in Europe suffered tens, even hundreds of thousands of outbreaks per year. This century there were particularly serious epidemics in the 1920's and 1930's. After the Second World War the incidence declined but then it surged again in the early 1950's [1].

The first major impact of control measures occurred in the mid-60's when the Netherlands introduced mass annual vaccination of its national cattle herd. France and Germany followed soon afterwards then the majority of other countries. Although methods for the large-scale production vaccines had been developed and used with success earlier [2], they had been administered haphazardly; this was the first time that vaccine was applied in a systematic manner.

TABLE I. OIE/FAO WORLD REFERENCE LABORATORY FOR FMD*
CUMULATIVE REPORT FOR JANUARY TO DECEMBER 1998

	No. of	FMD Virus Sero-type							No virus
Country	Samples	O	A	C	SAT1	SAT2	SAT3	ASIA I	isolated
Bahrain	8	8	–	–	–	–	–	–	–
Bhutan	2	2	–	–	–	–	–	–	–
Burkina Faso	9	–	–	–	–	–	–	–	9
Cambodia	10	10	–	–	–	–	–	–	–
Eritrea	12	–	2	–	–	7	–	–	3
Gambia	52	–	10	–	–	–	–	–	42
Greece	10	–	–	–	–	–	–	–	10
Hong Kong	5	1	–	–	–	–	–	–	4
Iran	28	15	12	–	–	–	–	–	1
Italy	18	–	–	–	–	–	–	–	–
Kuwait	3	2	–	–	–	–	–	–	1
Lebanon	17	14	–	–	–	–	–	–	3
Malawi	2	2	–	–	–	–	–	–	–
Myanmar	2	1	–	–	–	–	–	–	1
Nepal	8	6	–	–	–	–	–	–	2
New Zealand	11	–	–	–	–	–	–	–	11
Pakistan	12	1	–	–	–	–	–	3	8
Philippines	18	16	–	–	–	–	–	–	2
Rwanda	6	3	–	–	–	–	–	–	3
Saudi Arabia	43	12	–	–	–	–	–	–	31
Taiwan (China)	13	2	–	–	–	–	–	–	6
Tanzania	10	9	–	–	–	–	–	–	1
Turkey	44	9	29	–	–	–	–	–	6
Uganda	21	4	–	–	–	2	–	–	15
Yemen	15	12	1	–	–	–	–	–	2
TOTAL	379	129	54	–	–	9	–	3	161

* Institute for Animal Health, Pirbright Laboratory, Woking, Surrey, UK.

TABLE II. OIE/FAO WORLD REFERENCE LABORATORY FOR FMD. REPORT FOR JANUARY 1999

Country	WRL reference	Animal species	Date of Collection	FMD sub-type
Bhutan	BHU 3/98	Bovine	NK	O
	BHU 4/98	Bovine	NK	O
	BHU 5/98	Bovine	NK	O
	BHU 6/98	Bovine	NK	O
Hong Kong	HKN 6/98	Porcine	07.12.98	NVD
	HKN 7/98	Porcine	10.12.98	O
	HKN 8/98	Porcine	10.12.98	O
	HKN 9/98	Porcine	16.12.98	O
	HKN 10/98	Porcine	18.12.98	O
	HKN 11/98	Porcine	18.12.98	O
	HKN 12/98	Porcine	30.12.98	O
	HKN 1/99	Porcine	05.01.99	O
LAO PDR	LOA 1/98	Bovine	00.11.98	O
Mauritania	MAU 1/99	Bovine	19.01.99	NVD
	MAU 2/99	Bovine	19.01.99	NVD
	MAU 3/99	Bovine	19.01.99	NVD
	MAU 4/99	Bovine	19.01.99	NVD
	MAU 5/99	Bovine	19.01.99	NVD
	MAU 6/99	Bovine	19.01.99	NVD
Philippines	PHI 19/98	Porcine	NK	O
	PHI 20/98	Porcine	NK	O
	PHI 21/98	Porcine	NK	O
	PHI 22/98	Porcine	NK	O
	PHI 23/98	Porcine	NK	O
	PHI 24/98	Porcine	NK	NVD
	PHI 25/98	Porcine	NK	O
	PHI 26/98	Porcine	NK	NVD
	PHI 27/98	Porcine	NK	NVD
	PHI 28/98	Porcine	NK	NVD
	PHI 29/98	Porcine	NK	NVD
	PHI 30/98	Porcine	NK	O
	PHI 31/98	Buffalo	NK	NVD
	PHI 32/98	Buffalo	NK	NVD
	PHI 33/98	Porcine	NK	NVD
	PHI 34/98	Porcine	NK	NVD
	PHI 01/99	Porcine	NK	O
Saudi Arabia	SAU 44/98	Ovine	00.12.98	NVD
	SAU 45/98	Ovine	00.12.98	NVD
	SAU 46/98	Ovine	00.12.98	NVD
	SAU 47/98	Ovine	00.12.98	NVD
	SAU 48/98	Ovine	00.12.98	NVD
	SAU 49/98	Ovine	00.12.98	NVD
	SAU 50/98	Ovine	00.12.98	NVD
	SAU 51/98	Ovine	00.12.98	NVD
Tanzania	TAN 1/99	Bovine	13.01.99	SAT2
	TAN 2/99	Bovine	13.01.99	NVD
	TAN 3/99	Bovine	13.01.99	NVD
	TAN 4/99	Bovine	13.01.99	NVD
	TOTAL 48			

NK – not known NPF – 3rd. February 1999.

The success of the policy quickly became apparent as the prevalence of outbreaks began to decline. Within a decade the number of outbreaks in Western Europe fell from more than 20 000 to less than 4000 per year. The decline continued and by the 1980's the number was below 400 most years [3].

Success did not result from vaccination alone, other control measures had been brought into operation. These included booster vaccination around foci; the prevention of the movement of animal and products around infected premises and the heat treatment of waste food to prevent the circulation of virus through the feeding of swill. A range of safeguards were introduced at harbours and airports to reduce the risk of virus entry from other countries in livestock and animal products. As the prevalence of disease was reduced it became economically viable for an increasing number of countries to apply stamping out.

Control policies were not uniform, however, and while the majority of the countries on the continent vaccinated their cattle populations prophylactically and, in the event of an outbreak, applied ring vaccination and total or partial stamping out, in the others routine vaccination was not routinely undertaken and stamping out and movement restricts were the main actions taken against the disease.

The next major change in European policy was instigated in 1986 when the Commission of the European Community (CEC) decided that the methods for controlling FMD would have to be harmonized throughout the Community, well in advance of 1 January 1993, the date set for the commencement of free movement of livestock and animal products across the borders of the Community, and the creation thereby of the Single Market. The CEC, having consulted expert groups who examined over a two year study period the two options of non-vaccination and pan-vaccination, and the risks and cost–benefits for each, decided in 1989 that the preferred option was one of non-vaccination. Consequently, the Member States that had applied routine vaccination were instructed to abandon the policy so that a period of at least 12 months could lapse before the commencement of the Single Market on 1 January 1993. Some countries stopped vaccination during 1990, the remainder had complied by 31 December 1991.

In deciding in favour of the non-vaccination policy the CEC took into account the cost–benefit calculations of an expert group who, on the basis of an 11– year retrospective analysis, proposed three scenarios during the 10 years following the cessation of vaccination (i) the best case of 13 primary outbreaks without secondary outbreaks; (ii) the central case of 13 outbreaks and 273 secondary outbreaks; and (iii) the worst case of 13 primary outbreaks and 1963 secondary outbreaks [4].

Strong points against the continued use of vaccine was the evidence that around 38% of the outbreaks in the Community during 1977–1987 study period were ‘home grown’, i.e. had originated from within the Community. These were attributed either to faulty (incompletely inactivated) vaccine or escapes from vaccine production laboratories [4]. Nucleotide sequence analysis and comparison of vaccine and outbreak strains of virus provided the evidence for those conclusions [5].

During the first five years after the cessation of vaccination the Community suffered two primary outbreaks. The first in Italy in 1993 caused 56 secondary outbreaks and the second in 1994 in Greece resulted in 94 secondary outbreaks. Stamping out and movement control were the main control methods used. The total cost of the two episodes was approximately ECU 10 million [6].

An economic evaluation of the non-vaccination policy concluded that since 1991 it had saved the agricultural industry about ECU 135 million in vaccination costs per annum at 1987 prices and that the total savings to the Community agriculture converted to 1996 prices was around ECU 800 million. The value of the additional exports and freedom of trade in the Single Market was probably many times higher [6].

The Eastern Bloc countries of the former USSR, being anxious to preserve their export trade to Western Europe, decided that they too would have to cease vaccination, which they did around the same time as the Community. In the years immediately after adoption of that policy, the former Eastern Bloc suffered a number of outbreaks. Bulgaria had single outbreaks during 1991 and 1993, which were both controlled by stamping out, movement restrictions and ring vaccination.

The eastward extension of the non-vaccination policy during 1990–1991 ended at the Russian Federation, which held to its policy of strategic vaccination. Under that system vaccine is routinely

applied around the Moscow region and along its borders with the Transcaucasian Republics in the south to Mongolia and China in the east. The Russian Federation had its last outbreak in 1995, on a pig farm near Moscow, which was linked to pig meat imported from China contaminated with a strain of virus closely related to isolates from Hong Kong, considered to represent those circulating in China [7]. Stamping out and ring vaccination were used to eradicate the disease.

The only other outbreak of FMD reported in a European country during 1995 was in European Turkey where an outbreak of type O was confirmed close to the border with Greece and Bulgaria. By nucleotide sequence analysis it was shown that the type O virus was very similar to those isolated previously in Greece in 1994 and in Bulgaria in 1991 and 1993.

During May and June 1996 two more type O outbreaks were reported in European Turkey, one in the south and another in the north of Edirne province. One month later a series of outbreaks began across the border in the neighbouring Greek Prefecture of Evros. The strains of virus were identical and transfer across the border was most probably due to the movement of infected small ruminants. The same strain of type O virus also caused an outbreak in Bulgaria a short distance north of the border with European Turkey, but how it reached this area is not known [7].

Europe was also affected by type A virus during 1996. The first outbreak was confirmed on 24 May in Albania in the District of Korcha in the southeast of the country. Ten villages were affected. The clinically affected animals were immediately destroyed. The other susceptible animals in the infected premises/villages were slaughtered later. The outbreak strain was shown to be very closely related to a strain circulating in Saudi Arabia and India. These results and the finding of buffalo meat on the bone pointed to India as the probable origin of the epidemic [7]. Ring vaccination with A22 vaccine was carried out covering a zone of approximately 50 km radius. On 25 June an outbreak was reported in the Former Yugoslav Republic of Macedonia (FYRM) and shown later to be due to the same strain of virus as that in Albania. An epidemic followed, comprised of seventeen outbreaks in the Skopje District and one in Titov Veles District. The control measures consisted of the stamping out of the cattle in the 18 villages and two rounds of vaccination. The veterinary service of the Federal Republic of Yugoslavia reported FMD in Kosovo, close to the border with the FYRM on 7 July. Later a total of 101 villages were diagnosed to be infected. The WRL was unable to identify or isolate any FMD virus from tissue samples or to demonstrate specific antibody in 131 serum samples submitted for diagnosis. Control consisted of stamping out without vaccination [8].

During 1997–1998 and in 1999 up to the time of writing (February) FMD has been restricted in Europe to the former USSR Republics of Armenia, Georgia and Azerbaijan. Type A virus, closely related to the type A Iran/96 strain circulating in Iran and Asiatic Turkey was isolated in Armenia during 1998. The outbreaks in Georgia and Azerbaijan were type O.

2.1. Lessons learnt from European experiences

The control of FMD in Europe is a success story from which a number of lessons can be learnt. These include the following:

- The greatest risk of spread of FMD is associated with movement of infected animals, next is the movement of infected animal products.
- The factor which exerts the strongest influence on animal trade movement is price. The flow of trade will be towards the region or regions where the price is highest.
- Control will be more difficult for countries which share land borders and so they should co-operate with their neighbours in a regional control programme.
- Conversely, control will be easier for countries which are isolated by geographical barriers, i.e. seaways or high mountains. Such countries can be more independent in their control policy.
- The availability of potent, safe vaccine and a high vaccination coverage are essential if the prevalence of disease is to be reduced.
- Vaccination alone will not be sufficient to achieve a high impact on disease prevalence, it must be supported by zoo-sanitary measures.

– Cost–benefit analyses are valuable in assisting decision-making about the control options to implement.

3. CONTROL AND ERADICATION OF FMD IN SOUTH AMERICA

FMD was transported to the southern part of South America from Europe around 1870, most probably by infected cattle. Within a short period the disease became widely distributed through the beef production areas of Argentina, Uruguay, southern Brazil and Paraguay. The disease reduced livestock productivity and curtailed live animal exports from most of the sub-continent until recent years when some countries, having eradicated the disease, could export live animals and fresh meat. Previously, very few live animals could be exported to FMD free countries, and generally only after extended quarantine and testing. Beef had to be deboned and other products had to be processed in such a way that FMD virus would be inactivated.

Chile was the first country in South America to complete a programme of FMD eradication. It was given its impetus in 1969 when the terms of a loan by the Interamerican Bank of Development were agreed with the Government of Chile so that it could implement a national plan for control and eradication. The objectives of the plan were: to vaccinate 94% of the bovine population older than three months every four months; to implement zoo–sanitary measures and to promote a publicity campaign for the plan beginning in the south of the country and progressing northwards in a gradual manner [9].

The number of recorded outbreaks during the decade before the start of the plan was 7009. It was reduced to 1684 in the next decade and of that total 1061 outbreaks were registered in the first year (1970) of the plan. By 1972 the number of outbreaks had fallen to fewer than 50. Most of the outbreaks from 1972 to 1974, from 1976 to 1977 and in 1978 were considered to have originated from imported animals — smuggled through the Andean mountains from Argentina. Steady progress in control was maintained and in January 1981, Chile declared freedom from FMD.

The total cost of the plan was US $35.71 million. The Chilean Government contributed 56.3% of the cost, the livestock industry 40% and the Interamerican Bank of Development 3.7% as a loan. The estimated gross benefit was US $94.88 million, i.e. a cost–benefit ratio of 1:2.66 and a net benefit of US $59.17 million (Report 1981).

In 1974, Colombia initiated a plan for the regional FMD when a co-operative programme was agreed between the Ministry of Agriculture of Colombia, the Colombian Agricultural and Livestock Institute (ICA) and the Department of Agriculture of the USA (USDA). The Colombian Ministry of Agriculture delegated to ICA the planning and implementation of the actions, which led to the creation of the ICA–USDA Programme.

The activities of the programme were focused in a region in the northern part of Colombia that borders Panama and extends for a short distance along the Caribbean Sea towards the north-east and along the Atlantic Ocean to the south-west. The region was subdivided into areas. During the next two decades the FMD situation was steadily improved so that currently there is a free non-vaccinated area, surrounded by several areas that are disease free with vaccination and then vaccinated, buffer areas. The region is protected from the surrounding endemically infected region by a network of border control posts located on the principal land and river ways. There is also a system of fixed and mobile control posts between the areas inside the protected region. The animals in the different areas carry ear-tags of different colour and the main purpose of the checkpoints is to ensure that livestock move from areas of higher to lower health status only. The personnel at checkpoints also inspect vehicles to make sure that animal products are not being moved illegally.

In the areas where cattle were routinely vaccinated the policy in the early stages of the programme was to apply vaccine three times per year. When the potency of the vaccines improved, the frequency was reduced to twice per year. More recently, with wider use of oil-adjuvant vaccines it has been reduced to just once a year. Before owners could move their cattle they had to obtain a certificate confirming that they had been appropriately vaccinated. These practices are universal throughout South America.

In April 1987, the 5th Inter-American Meeting on Animal Health at the Ministerial Level (RIMSA V) issued Resolution XIII, which entrusted the Panamerican Health Organisation (PAHO)

and the South American Commission for the Control of FMD (COSALFA) with preparing the hemispheric programme for eradicating FMD, including adequate mechanisms for its implementation. This meeting approved the creation of a Hemispheric Committee for the Eradication of FMD consisting of a representative from the Government of each of the following sub-regions: the Southern Cone, the Andean Region, the Amazon, Central America, the Caribbean and North America, also one or more representatives of the producers of each of the above sub-regions.

The main objectives of the Hemispheric Programme for the Eradication of FMD are (i) to eradicate FMD from the American Hemisphere, (ii) to prevent its introduction into free areas and (iii) to settle new livestock areas, especially the Amazonian Sub-region, thereby preventing the introduction of FMD virus and other alien pathogenic agents and at the same time respecting the ecological integrity of those areas. The programme has been very successful and the disease has been pushed progressively northwards. The last confirmed outbreaks were in June 1990 in Uruguay in April 1994 in Argentina, in September 1994 in Paraguay and in December 1993 in the States of Rio Grande do Sul and Santa Catarina in Brazil. Uruguay has been disease free without vaccination since 1996. Argentina plans to stop vaccination by the end of March 1999. As a result of the higher health status of its livestock and animal products, Uruguay has gained access to the lucrative markets of the United States and Japan. Argentina and the other countries of South America should soon be able to follow suit.

3.1. Lessons learnt from South American experiences

In a few parts of South America there is a similarity between the husbandry systems and the epidemiology of FMD and those in Europe. However, for the most part the systems differ and so different control procedures have evolved. Particular lessons, which have been learnt in South America, are:

- The need for control measures to be harmonized and implemented on a multi-national basis and for the countries in a region to co-operate at all levels.
- The need for politicians to be involved in high level review and planning meetings to maintain the support for programmes.
- Livestock producers should be given the opportunity to participate at all levels of review and planning activities as they can make important contributions to the success of programmes.
- It may be beneficial to proceed in a step-wise manner from one area to the next with the progression being from the higher towards the lower health status.
- Regions which have attained a high health status should be protected by installing safeguards to prevent the re-introduction of virus from regions of lower health status.
- It is essential to achieve a good vaccination coverage with potent, safe vaccines containing antigens appropriate to the region where they will be applied.
- Animal movement must be controlled. Colour-coded ear tags can help to identify the origin of animals.
- Publicity campaigns through schools and the media are important to obtain the compliance of farmers with control programmes.
- Specialist advice, training and diagnostic activity should be provided through a network of national laboratories linked to a regional laboratory.
- Regular cost–benefit analyses are valuable to assess the progress of a programme and to persuading sponsors and livestock producers to continue their support.

4. CONTROL OF FMD IN SOUTHERN AFRICA

The history of FMD in southern Africa, its main epidemiological features, its economic impact and the measures used for control has been comprehensively reviewed by G.R. Thomson [10]. He included ten countries in his paper viz. Angola, Botswana, Lesotho, Malawi, Mozambique, Namibia,

South Africa, Swaziland, Zambia and Zimbabwe. With the exception of Lesotho, FMD has been reported in all of the countries since 1931, the year when the disease re-appeared in the region after an absence of several decades for a reason that has not been explained. Between 1971 and 1980 the total number of epidemics was equivalent to those during the previous twenty years. An unusually high incidence in Angola and Mozambique accounted for the major part of the 1971–1980 total, which was probably a consequence of civil unrest and disruption of the infrastructure in both countries.

Since the 1980's the incidence of disease in the region has declined considerably. In Angola and Mozambique this may have been due to the dramatic reduction of the livestock populations as a consequence of military actions in farming and wildlife areas. The more favourable disease situation elsewhere in the region has been attributed by Thomson [10] to improved disease control, in particular since the late 1970's of locally produced vaccine of good quality. Some countries of the region have not had outbreaks for many years. South Africa has not had an outbreak in its domestic livestock since 1983, whilst Botswana's last episode was in 1980.

There are some epidemiological features of FMD that are particular to southern Africa, the uniqueness of the virus types in the region (Southern African Territories SAT 1, 2 and 3) and the central role of the African buffalo in the epidemiology of the disease. Areas within the region which are prone to outbreaks caused by SAT type viruses are associated with higher densities of buffalo whereas outbreaks of type O and A, generally in the north of the region, are usually associated with established trade routes. While nucleotide sequencing has provided evidence linking several outbreaks in cattle with buffalo the technique has also demonstrated that some SAT 2 outbreaks, in Zimbabwe for example, had originated from carrier cattle. Investigations of carrier cattle in Zimbabwe have shown that they can be persistently infected for up to three years [7].

Although the details are not fully understood, it is believed that FMD infection in southern Africa is maintained within buffalo herds and that they are the source from which the virus occasionally spills over into other wildlife species and domestic livestock. Therefore, the major strategy for protecting domestic livestock is to keep buffalo away from areas of livestock production. This is done by the containment of buffalo behind game fences. For some distance beyond the fences cattle are vaccinated to create a buffer zone. The vaccines contain the strains of virus considered to be circulating in the buffalo. These are identified by capturing and probing sampling the buffalo at intervals. Beyond the buffer zone cattle are left unvaccinated and in several countries, e.g. Botswana, Namibia, Swaziland and Zimbabwe, these constitute the FMD free zones from which beef is exported to the European Union. This trade is a valuable source of hard currency for those countries.

4.1. Lessons learnt from southern African experiences

Fencing is an effective method for controlling the movement of wildlife and cattle and preventing the spread of virus from buffalo to domestic livestock. However, fences may severely impair the migration of certain wildlife species. It is important to use vaccines of good quality and they should contain antigens related to the primary threat which in southern Africa are the SAT types circulating in buffalo.

There is a need to monitor the antigenic profiles of the SAT viruses circulating in buffalo populations and it would be beneficial to develop a regional approach to define the extent of intra-typic antigenic variation of the SAT viruses in circulation and to standardize the methods for rapid strain selection for vaccines.

5. RELEVANCE OF THE EXPERIENCES OF OTHER REGIONS TO SOUTHEAST ASIA

Since FMD has been controlled and eradicated from most of Europe and a large part of South America, the focus for control and eradication has shifted to Southeast Asia. The drive for this has come from different quarters: from international organizations and from individual countries which have recognized the need to increase agricultural productivity to meet the demands for more protein to feed the rapidly expanding populations; from certain countries which want to eradicate the disease to increase their hard currency earnings through increased export, in particular of pig meat and pork products to Japan and finally pressure from vaccine producers who face a declining market elsewhere.

Southeast Asia can learn lessons from the experiences gained in the control and eradication of FMD in other regions of the world but lessons can also be learnt from campaigns within Southeast Asia itself, for example from the experiences of Indonesia which mounted a very successful programme during 1974–1981 which lead to the eradication of the disease from Bali and Madura in 1978, and from South Sulawesi and East Java in 1981. The last case of FMD was reported in Kebumen, Central Java in December 1983, while the last vaccination in Java against FMD was at the end of 1985. All of Indonesia was declared free in 1986 [11].

The successful campaigns in Europe, including the former USSR, South America, southern Africa and Indonesia have certain elements in common which should be considered when plans are being formulated to control and eradicate FMD in the Southeast Asian region. These include:

- Each of the countries of the region should formulate a national plan for the control and eradication of FMD which has the legal and financial support of the Government and the appropriate resources at all levels, i.e. personnel and technical support to effectively undertake and sustain the activities of the campaign through to the achievement of its final objectives. Guidelines for formulating national contingency plans for FMD have been provided in a document prepared jointly by the CEC, OIE and the European Commission for the Control of FMD and published by FAO [12].
- The technical requirements of the campaign, i.e. surveillance, diagnosis, implementation of control measures, vaccine availability and delivery systems etc. must be given sufficient resources, if they are to be effective. There should be a central fund which is protected against the possibility of regional economic crises.
- From the earliest possible stage, representatives of the livestock industry in each country should be invited to participate in control campaigns and be involved in decision-making at all levels.
- Countries in the region should benefit from the establishment of regional groups to develop common control strategies, especially those which share land borders with their neighbours.
- The control of the movement of livestock within and between countries will be essential if the areas which have achieved a high health status are to be protected against re-introduction of virus from areas of lower status. This will require a knowledge of livestock trade movements and probably checkpoints and barriers to reinforce the controls. Colour-coded ear-tags have been found to be useful in several parts of the world for identifying the origin of animals and in helping to deter illegal movement.
- Adequate supplies of safe, potent vaccines of appropriate antigenic specificity are essential to reduce the prevalence of disease to levels where it will be economically acceptable to cease vaccination, implement stamping out and move towards the final goal of virus eradication.
- Campaigns should have a publicity group, whose main responsibility is to ensure that farming communities and the livestock industry are aware of the campaign and its potential benefits.
- The progress of a campaign should be evaluated at regular intervals including the production of ‘running’ cost–benefit analyses.

6. REQUIREMENTS FOR CONTROL WHICH ARE UNIQUE TO SOUTHEAST ASIA

The domestic livestock in Southeast Asia have several special features. The domestic pig predominates throughout the region and the water buffalo population is more numerous there than in other parts of the world. Pigs, especially, play an important role in the epidemiology of the disease and so there is a requirement for safe, good quality vaccines in sufficient quantity to protect them. There is a need for the establishment of internationally accepted protocols for testing FMD vaccines for pigs and for an independent body to take responsibility for overseeing the procedures. These shortcomings were clearly illustrated during the 1997 FMD epidemic in Taiwan Province of China and highlighted during the last meeting of the OIE Sub-commission for FMD in Southeast Asia [13]. The issues have been brought to the attention of the OIE Standards Commission and the proposals of that body are awaited.

Infected pigs have been defined as amplifier hosts for FMD virus [14]. In the European context this was with reference to the role of the pig in excreting enormous quantities of airborne FMD virus, which under certain climatic and epidemiological conditions can result in an explosive spread of the disease. While the evidence suggests that airborne spread of FMD is not a common event in Southeast Asia, the pig still fits the definition of an amplifier host in that it is frequently the species that is primarily infected by virus circulating in contaminated waste food, which then leads to the initiation of outbreaks. The adoption of procedures to prevent the spread of FMD virus through waste food will be essential if the virus is to be eradicated from Southeast Asia.

Another feature of the epidemiology of FMD that appears to be currently unique to Southeast Asia is the occurrence of species-adapted strains, in particular strains which are highly adapted to pigs. This has been recognized in Taiwan Province of China, the Philippines and Viet Nam [13,15]. The capability of pig-adapted strains to cause very serious economic impact and the need for an early warning of their presence, therefore, were highlighted at the last meeting of the OIE Sub-commission for Foot-and-mouth Disease in Southeast Asia [13].

The part which the water buffalo plays in the epidemiology of FMD in Southeast Asia has not been fully investigated and is worthy of further attention, especially to know more about the maintenance and persistence of FMD virus in that species and whether there are special requirements for vaccines to be effective.

7. DIAGNOSTIC REQUIREMENTS TO ACCELERATE FMD CONTROL IN SOUTHEAST ASIA

Under the FAO/IAEA sponsored Co-ordinated Research Project entitled “Improved diagnosis and control of FMD in Southeast Asia using ELISA-based technologies” the methods required to detect FMD viral antigen and antibody were successfully introduced into the national FMD laboratories of Southeast Asia. The priority activity for the laboratories of the region should be to use their diagnostic and surveillance capabilities to support national control and eradication schemes. The veterinary authorities should ensure that their field officers make maximum use of laboratory support to investigate all suspected cases of FMD. Unfortunately, at present the number of samples collected is too few in most of the countries in the region for conclusions to be drawn about the true prevalence and incidence of disease and for assessments to be made about the appropriateness of the antigens in vaccines. When outbreaks occur, therefore, judgement of the suitability of vaccine is based on whether vaccination prevents further spread or not. This is a high-risk strategy and one that will need to be changed if control is to be more effective. Planning and accurate costing of resources for campaigns will not be possible until comprehensive and reliable surveillance data are available.

When national laboratories are routinely using their diagnostic and surveillance tests they should consider expanding their capabilities to acquire a tissue culture capability. A few laboratories have already taken this step. A tissue capability enables a laboratory to isolate viruses from field samples, to grow them and send aliquots to the regional laboratory or the WRL for antigenic and genomic analyses. Laboratories with the capability can also confirm ELISA results by using virus neutralization tests. The liquid phase blocking ELISA is highly sensitive and ideal for screening large numbers of serum samples. However, a small number of samples will inevitably give equivocal results and so further testing by virus neutralization, the definitive confirmatory test, is necessary to obtain a final result. Clearly, this requires a tissue culture capability.

The antigenic characterization of field isolates has two functions (i) to confirm the appropriateness of current antigens in vaccines and (ii) to determine if there is a requirement for a new strain to be included in the vaccines. These activities fall within the remit of a regional laboratory. However, that does not preclude the possibility of a national FMD laboratory undertaking those activities should it have the capability. The WRL remains willing to provide additional support if it were required.

Nucleotide sequencing has been shown by the WRL, some national FMD laboratories in Europe and the FMD laboratory in South Africa to be a valuable tool for identifying the origin of outbreaks. The technique is also very useful for many research activities. There would be scope to use the technique in Southeast Asia for molecular epidemiological purposes and possibly for research, for

example to investigate the duration of persistence in the water buffalo. The method requires specialist knowledge, equipment, reagents and access to sequence data banks. It is expensive and so the potential benefits would have to be balanced against the cost. In the author's opinion, Malaysia, the Philippines and Thailand are countries where there could be grounds for using the techniques —primarily for molecular epidemiological investigations of the origin of outbreaks.

Donaldson and Kihm [16] reviewed developments in diagnostic methods and other techniques, which could accelerate the control and eradication of FMD. They pointed to the need for a reliable, practical, rapid and sensitive method to differentiate infected from vaccinated animals. The applications of the test are two-fold. Firstly, when a country or zone has not reported any outbreaks of disease for some months and the veterinary authority is considering the possibility of ceasing vaccination then the test can be used to verify, that virus is no longer circulating. Secondly, when an FMD-free country or zone experiences an outbreak and uses emergency ring vaccination in the face of disease, the test can be employed to test vaccinated animals before they are allowed to leave the vaccination zone to ensure that they are not carrying virus. In the Southeast Asian context the author suggests, that there would be applications for the test in the Philippines, Malaysia and Thailand.

Several different types of test have been developed to differentiate infected from vaccinated animals. Most depend on the fact that cattle which have been infected with FMD virus can be differentiated from those which have been vaccinated on the basis of the detection of antibody to one or more of the non-structural (NS) proteins of the virus. During the period 1994–1997 the CEC sponsored a concerted action programme in which several EU laboratories collaborated to investigate the potential of using assays measuring antibody to the NS proteins of FMD virus to differentiate infected from vaccinated animals. A number of national FMD laboratories worldwide pursued similar objectives during the same period. At a meeting held at the Institute for Animal Science and Health, Lelysad, the Netherlands, on 28 and 29 April 1997, the findings were presented and discussed [17]. The most promising results have been obtained with an indirect ELISA, which uses as antigen the NS polyprotein 3ABC expressed as a fusion protein in *E. coli*. Measuring antibody to 3ABC on a herd basis is useful to detect exposure of vaccinated herds to live virus and herds so identified can then be examined for the presence of virus. However, there are serious limitations to the reliability of the use of antibody to NS proteins for the detection of carrier animals, especially at the individual animal rather than the herd level, and further work in this area is required. This will be among the topics that will be addressed during the next FAO/IAEA Co-ordinated Research Project on FMD.

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ESTABLISHING A FOOT-AND-MOUTH DISEASE LABORATORY NETWORK IN SOUTHEAST ASIA

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Abstract

ESTABLISHING A FOOT-AND-MOUTH DISEASE LABORATORY NETWORK IN SOUTHEAST ASIA

The Joint FAO/IAEA Division has established an effective laboratory network in Southeast Asia to support the diagnostic requirements of the Southeast Asian Foot-and-mouth disease control campaign (SEAFMD). All laboratories have a capability to accurately detect and type foot-and-mouth disease virus antigen in clinical specimens and to conduct the screening test for detection of serum antibodies against the endemic sero-types of the virus.

1. INTRODUCTION

An international workshop on the diagnosis and epidemiology of foot-and-mouth disease (FMD) was conducted in September 1993 at Lampang, Thailand, by the Department of Livestock Development (DLD), Thailand, and the Australian Centre for International Agricultural Research (ACIAR). A series of recommendations were developed by the participants to help guide regional animal health administrations and international agencies with foot-and-mouth disease control. One working group considered the requirements for laboratory diagnosis to support a national and regional FMD control programme. Two of the essential capabilities of a national diagnostic laboratory that were set down were an 'ability to diagnose FMD and sero-type by ELISA techniques' and the 'capacity to detect and quantify antibody to FMD virus for evaluation of vaccine, sero-surveillance and animal import/export testing by ELISA'. As one of the international organizations represented at the workshop, the FAO/IAEA Joint Division agreed to support the development of the national FMD diagnostic laboratories in the Southeast Asian region through a Co-ordinated Research Project (CRP). The FMD ELISA technology CRP has close links with the regional campaign to control FMD (SEAFMD) being co-ordinated by the Office International Des Epizooties (OIE). For example during the course of the FMD ELISA CRP, the contract and agreement holders have participated in three meetings of the OIE Sub-commission for FMD control in Southeast Asia (SEAFMD).

2. OBJECTIVES OF THE FAO/IAEA CO-ORDINATED RESEARCH PROJECT

The CRP had three objectives. The first was to establish the capability and capacity to conduct the FMD antigen detection and sero-typing ELISA in each of the laboratories designated as the national FMD laboratory by the countries in the programme. The second was to assist with the design of a project to apply the technology to a field and to monitor its implementation in each of the participating countries. The third objective was to facilitate analysis and presentation of the findings of the project at various meetings and in this publication. A further objective, which developed during the project, was to introduce the internal and external quality assurance programmes for the serological assays.

3. METHODOLOGY

The development, initiation and implementation of the CRP were as follows.

(1) Identifying the participating countries: Governments in the Region were invited to participate. Since the programme commenced Hong Kong has been handed back to the People's Republic of China.

(2) Identifying the Contract holders: Contract holders were nominated by the collaborating institution/organization in each country.

(3) Identifying the Agreement holders to provide the appropriate mix of technical support for the programme.

(4) Conducting an initial meeting (February 1995) to design the contract projects and identify both the equipment and the training needs.

(5) Supplying the necessary equipment and reagents.

(6) Conducting an ELISA training workshop and introducing the Electronic Data Interface (EDI) program for capturing and analysing ELISA data from both assays (February 1996).

(7) Assisting with the design of field studies linked to an identified national need.

(8) Establishing E-mail communication with most of the contract holders.

(9) Instituting the External Quality Assurance Programme (EQAP) assist with quality control, especially of the serological assays.

(10) Evaluating progress through presentation of data to the OIE Sub-commission (February 1997).

(11) Evaluating final progress reports through presentation of data to the OIE Sub-commission (February 1999).

(12) Supervising the preparation of the final reports for publication in this Technical Document.

4. ACHIEVEMENTS

(1) There is a national laboratory operating and contributing to regional FMD reporting system in each of the seven countries participating at the OIE project.

(2) In Malaysia, the Department of Veterinary Services has been able to use the technology to monitor serological status of animals moving into the country, and to monitor the effectiveness of routine vaccination campaigns.

(3) In the Philippines, there has been a close synergy of the inputs of this CRP and the inputs of the FAO FMD control project.

(4) A network of individuals with skills in interpretation of laboratory results and improved technical understanding of the issues surrounding sero-surveillance has been developed. This network will become a source of regional technical expertise for the future FMD control campaign.

(5) Overall understanding of FMD epidemiology has been enhanced as a result of the laboratory outputs, especially the contribution to routine reporting to the SEAFMD Regional Co-ordination Unit.

(6) The EQAP has been instituted and all the Contract holders have participated. Furthermore, there is a high priority given to continue support for the EQAP, indicating that the principle has been successfully inculcated into the normal laboratory practices of the contract holders.

5. CONSTRAINTS

(1) Delivery of equipment and reagents: At the beginning of the programme some countries experienced difficulties with adequate notification of deliveries of reagents, so that necessary formalities for importation were completed prior to dispatch of reagents. This matter was quickly dealt with and all participants report that a smooth process is now in place.

(2) Installation of equipment: Some of the participants experienced difficulty with the early version of the electronic data interface (EDI) program and the configuration of the cable connecting the ELISA reader to the computer. However, these problems were dealt with and no participants experience difficulties with data transfer and processing.

(3) In general, the equipment provided by the programme has proved to be reliable and remains functional with a minimum of maintenance. One participant who experienced problems with the ELISA reader could not get any service in the region and had to return the equipment to Vienna. The positive side is that a service is available and the equipment could be repaired and returned within a reasonable time.

6. FIELD STUDIES.

Application of the ELISA technology to field investigations has been variable. Certainly all the laboratories have a capability to provide a sero-typing service to enable the veterinary service to report on the FMD status of field outbreaks. This capability in one instance is blocked by lack of support for other laboratory functions.

The laboratory service represents a link in the chain of the veterinary service's capability, but the output from the national laboratory is dependent on the field service being able to undertake adequate disease outbreak investigations. The laboratory capacity is under-utilized where the field service does not have the resources or training to visit field outbreaks to conduct investigations, or where field personnel are not trained to submit proper specimens for diagnosis. There is also the problem that laboratory based personnel do not have the resources at their disposal to participate in investigative field work and so make full use of the potential to provide useful epidemiological information to field services. In Myanmar and Indochina there is a need to substantially increase the resources for animal health services to improve co-ordination between laboratory and field operations and increase the utilization of the capability of both.

7. TRAINING

The training workshops have bridged many of the gaps from the technical standpoint. The programme has provided the participants with some basic information on the fundamentals of epidemiology to assist in the design and implement better quality sero-monitoring projects. ELISA wet workshops have introduced the tests at a technical level. Many participants, however, lack a solid basic education in the science that underlies the technology so that troubleshooting and interpretation of data can present a problem. This deficiency can be overcome by continued utilization of the technology and by checking former participants through further training workshops. Long term availability of the technical backstopping on the Internet will assist (this option is not available to all countries at present) as will continued formal and informal interaction between laboratory personnel.

It is noticeable that one or two of the persons trained in the programme have been able to extend their technical training and understanding beyond the FMD control programme and set up ELISA for other pathogens. Such initiatives represent very successful technology transfer. In most instances, there is reluctance to apply the technology widely, because it may mean lack of control over equipment (examples where other staff experimenting with the technology have caused expensive, but more importantly difficult to repair, damage to ELISA readers). It may also reflect the sheer lack of access to resources to undertake other tests. The great advantage of the FAO/IAEA FMD CRP has been the availability of the well characterized and technically supported ELISA kits from the World Reference Laboratory for FMD at Pirbright, United Kingdom.

The nominated participant from the government service of some countries has not always been the same, or sometimes was not a person involved directly in day-to-day diagnostic laboratory work, so some of the external inputs have missed the desired target. Where the same participant does not attend continuously, the programme looses momentum. It does also to some extent diminish the importance of the programme in the national context, as the personnel issues take precedence over the technology transfer issues. However, this constraint has been more or less overcome and the programme successfully established in all cases.

8. CHALLENGES FOR THE FUTURE

The major challenge for the future will be to ensure that the government services provide enough support to enable continuance of the diagnostic service, that has arisen from the CRP. Funds are required to purchase reagent kits and also to maintain equipment, an area often overlooked. If the laboratories can charge fees for services this would help ensure long-term survival. The SEAFMD Sub-commission will be a venue for the veterinary services to report on the condition of their national diagnostic service in the context of the regional FMD control programme. It is likely that FAO/IAEA will continue to participate in the Sub-commission and monitor these investments.

At the moment, there is no functioning Regional Reference Laboratory (RRL) for FMD. The construction of a bio-containment laboratory at the FMD Centre Pak Chong, Thailand, by DLD is well under way, and it is likely, that this extension of the national laboratory will become the RRL within the next three years. It will be a significant benefit to the regional programme if this laboratory is designated by OIE and becomes a regional centre of excellence for FMD diagnosis and training. Such a RRL could undertake the regional EQAP as well as produce and supply diagnostic reagents for ELISA. There would be great benefit in conducting a regular technical meeting for laboratory personnel supporting the regional control programme separately from the SEAFMD Sub-commission meeting.

The FAO/IAEA Joint Division plans to initiate a further project on FMD diagnostic tests to distinguish vaccinated from infected animals. This technology has great promise to unravel some of the key epidemiological issues with FMD control in the region.

9. RECOMMENDATIONS

(1) That the participants operating in the national FMD laboratories, especially those supporting the OIE SEAFMD campaign are surveyed from time to time to determine any cause that might have resulted in cessation of the ELISA diagnostic programme.

(2) That the FMD Centre in Pak Chong, Thailand, supply reagents for the FMD typing ELISA to other countries in the region.

(3) That the FMD Centre Pak Chong, Thailand, become a regional training centre for the use of ELISA technology in FMD diagnosis.

(4) That the FMD Centre Pak Chong Thailand becomes responsible for the EQAP for the laboratories in the region, especially those participating in the SEAFMD campaign.

(5) That the OIE Sub-commission conducts a regular technical meeting to discuss FMD diagnosis and epidemiology.

(6) That the participants of the CRP prepare a regional IAEA TCP to provide further critical support for national FMD diagnostic programmes, to continue the EQAP and to develop an effective regional supply of diagnostic kits and reagents.

EPIDEMIOLOGICAL CONSIDERATIONS IN THE SURVEILLANCE AND CONTROL OF FMD IN SOUTHEAST ASIA

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Abstract

EPIDEMIOLOGICAL CONSIDERATIONS IN THE SURVEILLANCE AND CONTROL OF FMD IN SOUTHEAST ASIA

If control and ultimately eradication of FMD is going to be achieved in Southeast Asia, regional co-operation between the various countries that share common borders will be required. Control programmes need to be flexible so that they can target the issues that are likely to give the greatest improvements in control with the available resources. In order to design these dynamic control programmes, communications must be strengthened, epidemiologically sound information needs to be routinely collected and analyses must be conducted. Control effectiveness should be monitored so that weaknesses in present programmes can be identified. Short-term and long-term strategies must be developed concurrently so that control programmes can readily proceed from one stage to the next.

1. INTRODUCTION

Traditional foot-and-mouth disease (FMD) control in endemic countries typically involves the implementation of phased programmes incorporating mass vaccination and various movement control measures. The goal is usually to reduce the number of outbreaks to the point where FMD-free zones can be established, maintained and gradually increased in size until the whole country or region is free.

The objective of the mass vaccination programme is to immunize a sufficiently high proportion of the susceptible farm animal population on a regular basis, such that herd immunity effectively protects the entire population. In practice, the aim should be a minimum of 80% vaccination coverage. The vaccine used should be of high quality, contain sufficient antigenic payload of the particular FMD vaccine strain(s) appropriate for the region and be safe (i.e. contain no non-inactivated FMD particles — which effectively means formalin inactivation should no longer be used). Vaccine should only be administered by trained vaccinators and attention should be given to the cold chain. Young animals should be given two vaccinations approximately one month apart, once they reach about 3 months of age (i.e. once maternal immunity has waned). From then on, they should be given a booster dose on a regular basis depending on the duration of immunity. Additional ring vaccination can be applied around the affected zone in the face of an outbreak.

The objectives of movement control measures are to prevent the introduction of infection into free areas, to ensure infection does not spread from outbreak zones, and generally to limit the movements of animals of unknown vaccination or infection status. Permission to move animals may be subject to a vaccination certificate. Quarantine stations may be established at strategic locations to observe and/or vaccinate animals of unknown status.

In addition to these measures, outbreak investigations by trained investigators can help identify weaknesses in vaccination and movement control programmes.

2. CONSTRAINTS IN SOUTHEAST ASIA

There are several constraints in Southeast Asia that are limiting the adoption of control measures against FMD. These include:

– Either insufficient vaccine is produced to meet the demand for mass vaccination campaigns or else the countries cannot afford to purchase the number of doses required.

– Movement control is extremely difficult to implement, as animal trading is viewed as a fundamental component of village-based economies.

– Government veterinary services often do not have sufficient resources to implement the measures.

– Some of the countries in the region have long shared borders with other endemic countries.

– There is generally poor understanding of the disease by farmers and traders.

3. EPIDEMIOLOGICAL APPROACH

Given the above constraints, the only way to make progress is to adopt an epidemiological-based approach that will lead to better targeting of the available resources. The motive for this approach is the premise that knowledge of disease processes can be derived from information (that is extracted from raw data via analysis) when it is coupled with experience. Experience comes from having feedback mechanisms that continually evaluate the effectiveness of existing control programmes. Hence, an epidemiological approach must be an information-based approach.

FMD is recognized as a regional problem and, therefore, will require a co-ordinated response. The establishment of the OIE Regional Co-ordination Unit (RCU) and Reference Laboratory are thus of crucial importance to the Region, as has been the case with the PANAFTOSA centre in Latin America.

Communication between field staff and national state veterinary offices as well as between countries and the Co-ordination Unit will need to be strengthened to facilitate the flow of information. It is stressed, that information flow should be two-way, so that there is both reliable surveillance data coming from the field and at the same time, field staff are kept up-to-date with the national and regional situations. Therefore, investments in improving communication systems are important, including telephone, facsimile and the emerging Internet-based technologies (e.g. E-mail, News groups and Web-based reporting systems).

In order to capture the field and laboratory investigations and allow a broad range of analyses to be carried out, an information system needs to be developed. This should incorporate a geographic information system (GIS) to permit spatial analyses to be conducted. For most of Southeast Asia, the village is the key unit of interest, therefore, data collected should primarily be at a village-level. The type of data collected should include outbreak data (data about actual outbreaks), demographic (information about the animal population in the area), spatial data (geographical co-ordinates) and information on suspected risk factors. A suggested list of data items to consider include:

– Name of village and geographical location (easting & northing or latitude & longitude)
– Dominant production system (e.g. rice crops)
– Number of animals at risk by species
– Number of animals with disease by species
– Time course of the epidemic
– Virus type and strain
– Village vaccination history
– Degree of movement of livestock
– Interaction with neighbouring villages
– When animals last came into the village
– Whether initial disease was in moved or resident animals
– Proximity to markets / transportation routes / neighbouring country border.

The same information system should be used throughout the particular state veterinary service. Wherever possible, data entry should be conducted as close to the ‘front-line’ as possible, whether it is the field or the laboratory. This will give the front-line staff more ‘ownership’ of the system and ensure the data is up-to-date. If computer networks or communications systems permit, the main database should be at a central location, but with remote access by multiple concurrent personnel provided. Such a design will ensure, that there is only one copy of the database.

It is important that negative reports as well as positive results be recorded. Historically, very little statistical ‘control’ information is ever documented (i.e. information about villages in the

outbreak zone that are not affected). This makes the calculation of true incidence rates extremely difficult.

Effort should be made to acquire or build national and regional GIS datasets of features such as villages (names and locations), transport corridors (roads, railways), livestock markets, border crossings etc. In many countries, these types of digital files are already available, perhaps within other Government departments. If not, there are several options for building them, including digitizing of paper maps, equipping field staff with global positioning systems (GPS) or conducting remote sensing (involving the interpretation of satellite imagery).

Assembling these national datasets may seem like a daunting task, but the long-term advantages are well worth it. Similarly, when starting out with a new database system, the rewards in terms of the ability to conduct sophisticated analyses may seem a long way off. However it is important that a start is made. Effort should go into keeping field and laboratory staff fully informed with regular feedback, so that staff enthusiasm does not diminish, especially, when it may appear that unimportant data is requested. Making data recording easy and exciting, such as through the use of well-constructed Web pages may be one solution (see Fig. 1. showing a map-based recording system).

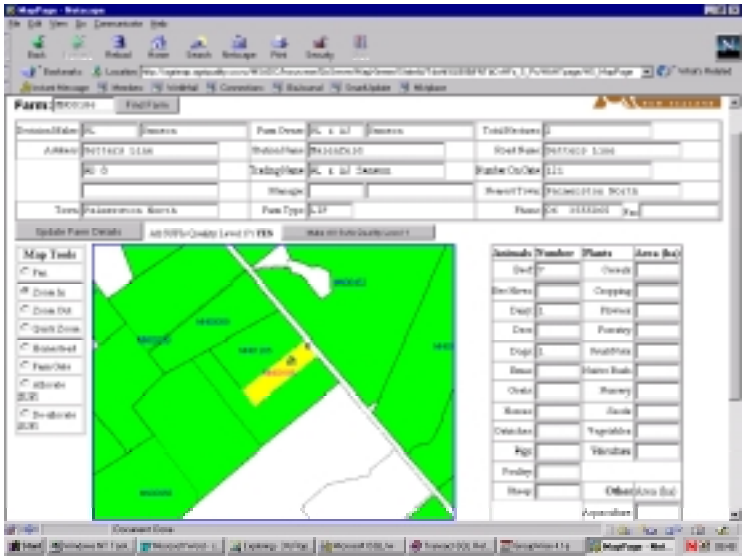

FIG. 1. An example of a map-based Web reporting system, designed for capturing information about farms in New Zealand.

When the data collected through outbreak investigations and control programme implementation is combined with the information held in the national spatial datasets, the investigative power becomes significant. The capabilities include:

– Calculation of true incidence rates or disease prevalence, as denominator data is available.
– The links between disease control activities and FMD incidence can be identified.
– Weaknesses in control programmes become obvious.
– A broad range of potential risk factors can be included in the analyses.
– Local or national economic cost–benefit studies become possible.
– Planning for future control programmes is facilitated.

4. AN EXAMPLE FROM THAILAND

Using data collected by the Department of Livestock Development (DLD), Thailand over a three year period from 1995–1997, Dr. Tippawon Teekayuwat and Dr. Dirk Pfeiffer, Massey University, have developed a prototype GIS to demonstrate the range of analyses that can be conducted when routine data is combined with national spatial datasets. Figures 2–5 illustrate descriptive, analytical and predictive capabilities.

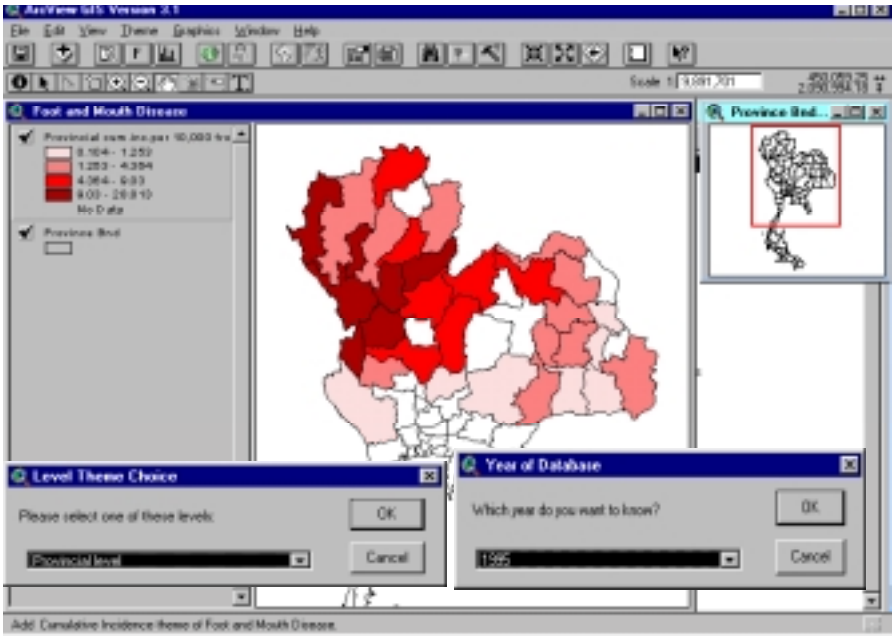

FIG. 2. Screen presenting a choroplethic map of cumulative incidence of FMD at a provincial level for a selected year.

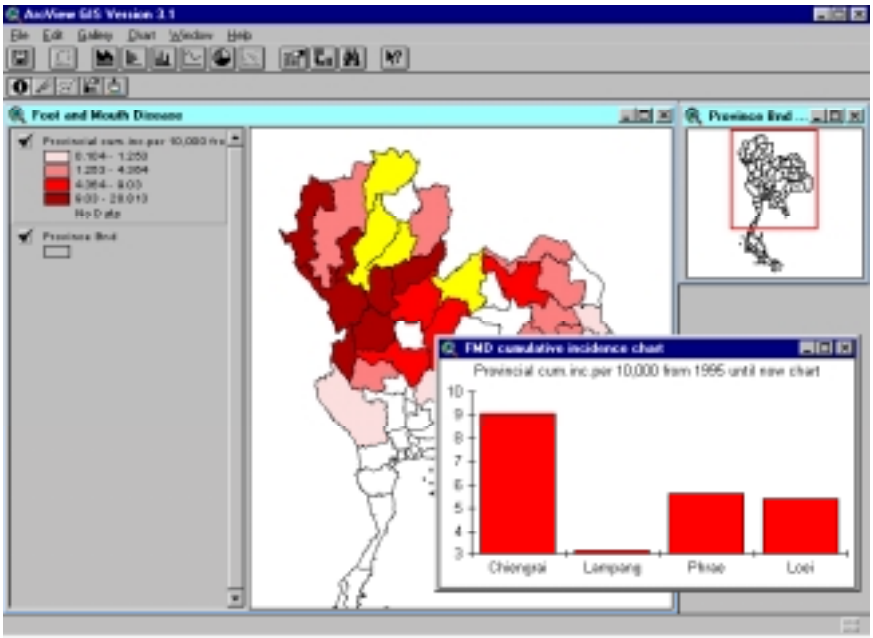

FIG. 3. Screen showing graph of provincial cumulative incidence for selected provinces.

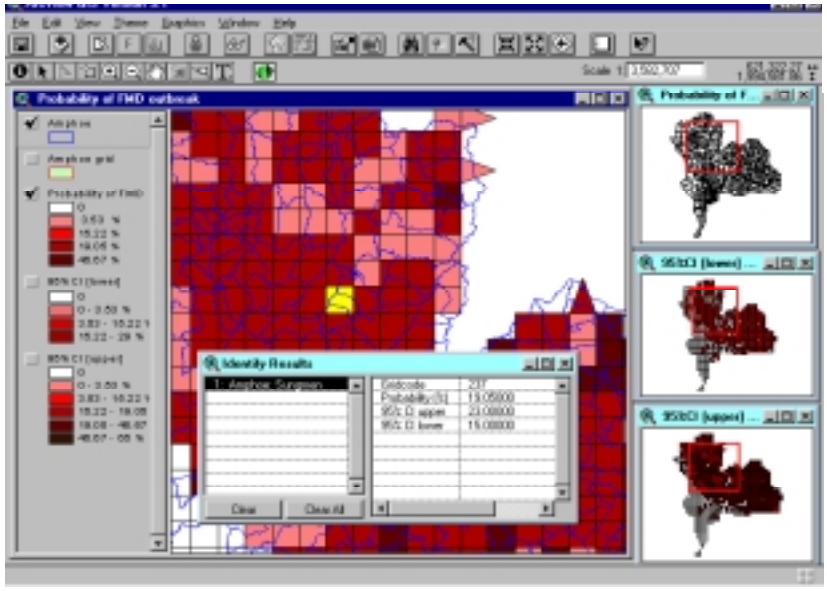

FIG. 4. Grid map of the probability of an FMD outbreak at an Amphoe level, including 95% confidence intervals, based on classification and regression tree (CART) analysis.

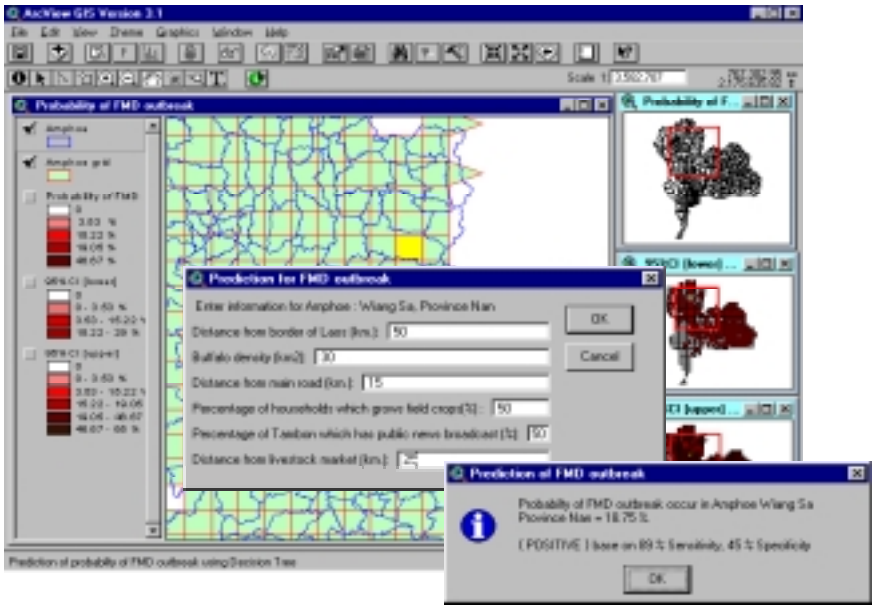

FIG. 5. Screen illustrating predictive capability. The user selects an Amphoe for investigation. The user can then examine and modify the values of the significant independent variables and the system will calculate the probability of an outbreak of FMD in the ensuing year.

5. DISCUSSION

This paper encourages an epidemiological approach to FMD control in Southeast Asia. The components of this include the OIE RCU and Reference Laboratory, improved communication and information systems. This will require some investment in the establishment of databases, communication systems and training in epidemiology for key staff. However, the payback over the medium and longer terms should be significant. The outcomes will be the ability to identify important risk factors for FMD in the various zones of Southeast Asia, the ability to target risk factors that will provide the most improvement in disease situations using existing resources and improved feedback from control activities (including the identification of successes and failures).

In order to capitalize on these benefits, control programmes must be flexible and innovative, so that different risk factors can be targeted at the different stages of the overall FMD control and eradication programme in Southeast Asia. Veterinary services must be creative in their thinking and be prepared to work with other Government Departments and Agencies. For example, some of the national datasets mentioned above, such as a village database, could serve multiple uses. Similarly, FMD control at the village level could be combined with other extension activities, e.g. haemorrhagic septicaemia (HS) vaccination.

Planning and data collection to support future operations should be conducted concurrently with the implementation of short-term control activities. In this way, 'down-time' between the various phases will be minimized, and continual progress will be more achievable. The DLD example shows that meaningful information can be extracted within 2–3 years of data collection.

In conclusion, the experiences from Latin America prove that FMD eradication can be achieved. There, the PANAFTOSA centre provided regional co-ordination and acted as the main data warehouse. In addition, it conducted viral, diagnostic and epidemiological research. Meanwhile, the countries in the region demonstrated strong national commitment to FMD eradication. Other key points are that independent quality control of vaccine efficacy was established, complete and regular vaccination coverage using trained vaccinators was implemented in various zones and movement control measures were consistently applied. As success was achieved, initially in Chile, but more recently in Uruguay, Argentina and southern Brazil, the emphasis has progressively shifted from vaccination to stamping-out. The rewards include an expanding export trade in animal products into markets that were previously closed.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the International Atomic Energy Agency for supporting our participation in the development of FMD control strategies for Southeast Asia. We would also like to thank Dr. Tippawan Teekayuwat and Dr. Dick Pfeiffer, Massey University, and the Thai Department of Livestock Development for allowing us to describe their GIS-based analysis system.

THE EXTERNAL QUALITY ASSURANCE PROGRAMME (EQAP) FOR THE FAO/IAEA ANTIBODY FMD ELISA IN SOUTHEAST ASIA

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Abstract

THE EXTERNAL QUALITY ASSURANCE PROGRAMME (EQAP) FOR THE FAO/IAEA ANTIBODY FMD ELISA IN SOUTHEAST ASIA

The external quality assurance programme (EQAP) consists of three equally important items: the questionnaire, the monitoring of the internal quality control data and the external quality control test panel. The EQAP is conducted twice per year. The first round of the EQAP for the FAO/IAEA liquid phase blocking ELISA being used in Southeast Asia was carried out in 1995–1996. A total of 10 laboratories from Asia participated. The round consisted of three FMD sero-types (O, A and Asia) for both, the antigen and antibody ELISA. No interim report was produced, but results were communicated on an individual basis. The second round was initiated in late 1998. Participating laboratories were the same as in 1996. This time the EQA proficiency exercise consisted only of one sero-type (O_1 Manisa) for the FMD Antibody ELISA. The results of this round are presented in this report. All participants replied to this EQA exercise. Out of ten participants six laboratories returned all EQA components (EQC results, IQC data and questionnaire). Four laboratories did not return the complete set of the EQA panel. Three out of five EQC samples achieved an overall agreement of 100%. Two EQC samples were excluded from the evaluation because a minimum of 80% of agreement between participants was not achieved. In comparison to the reference values for the EQC samples as established by the World Reference Center (WRC), UK, the participating laboratories produced lower PI values for the three positive samples 2, 8 and 5 and higher PI values for the negative samples 10 and 9 resulting in a decreased binding ratio. Nevertheless results show that all laboratories are able to detect a strong positive sample and no equivocally result was obtained for the two negative samples. Weak positive sera close to the cut-off are problematical for a minority of the laboratories, which tested these samples negative. The closer the sample comes to the cut-off the less agreement between laboratories is reached and the number of false negative results is likely to increase. These findings result in reduced assay sensitivity. Nevertheless, most of the laboratories correctly identified all EQC samples indicating that the assay itself works properly and that the majority has a good proficiency for conducting the assay. Most of the IQC data were located within the established upper and lower control limits indicating that the majority of laboratories have a good routine use of the assay and that the ELISA is under control. From the information supplied in the questionnaire it can be concluded, that more attention must be given to quality control/assurance issues (e.g. auto-measuring of IQC data and calibration procedures). Comparing the results from the FMD96a and this round it is concluded that the number of responses and the quality of EQC and IQC results has improved considerably. Detailed information on the results of this EQA round have been published in an interim report entitled: "The external quality assurance programme for use with the FAO/IAEA FMD Antibody ELISA (EQAP/FMD/1998a)".

1. INTRODUCTION

For any testing laboratory it is essential, that assurance can be given that the test results produced are valid and reliable. It is also very important, that results are comparable between different laboratories involved in similar assessments. Many diagnostic tests contain an element of subjectivity in their interpretation of results and this renders both internal and external assurance difficult to operate. One of the distinct advantages of an ELISA-based system is the objectivity of reading the results and the ability to process data using a computer. Thus, it is possible to incorporate a high level of internal quality control for every ELISA test plate used. Indeed, internal quality control is now a routine operation for most laboratories utilizing FAO/IAEA ELISA based testing systems [1].

Equally important is the determination, whether a laboratory is giving the correct interpretation of the results, even if the assay is shown to be functioning correctly. The procedures for establishing the assurance that the test results provided from a laboratory are reliable, form the basis for an external quality assurance programme (EQAP).

In September 1994, an FAO/IAEA consultants meeting was convened with the aim of extending and further improving the EQAP for veterinary laboratories in developing countries utilizing FAO/IAEA ELISA kits. The meeting focused on establishing procedures that would lead to 'recognition' of veterinary laboratories as competent in utilizing FAO/IAEA ELISA kits for specific diseases and tasks. The conclusions and recommendations of this meeting are contained in the report "Establishment of external quality assurance procedures for use with FAO/IAEA ELISA kits" [2].

This improved EQAP for veterinary laboratories is based on i) proof of the presence and use of quality assurance/quality control systems, ii) the continual satisfactory performance of processes and

output and iii) participation in external quality control test rounds. To obtain such proof, the EQAP consists of three critical elements as detailed below.

1.1. Survey questionnaire

A questionnaire-based survey of individual laboratories is utilized to provide a regular system for monitoring the presence and use of the key quality elements. It is a mandatory requirement, that all laboratories participating in the FAO/IAEA EQAP should complete and return such a questionnaire. The information gathered through the questionnaire is updated at least once per year by the officer in charge[1] in the participating laboratory. The satisfactory presence of the relevant key elements is determined by the EQAP Co-ordinator in close collaboration with the appropriate Technical Officer of the Sub-programme in Animal Health and Production of the Joint FAO/IAEA Programme and forms an essential part of the assessment of the participating laboratory.

1.2. Internal quality control (IQC)

It is mandatory that laboratories fulfill the requirements for IQC as specified in the designated standard assay protocol. These include the use of appropriate reference standard control sera, the application of test acceptance criteria, the monitoring of test performance through the use of control charts, and the provision of relevant data for third party assessment. IQC data are used to assess the repeatability and precision of the test conducted in that particular laboratory [3]. These data can be used by the test operator to detect trends and shifts in test performance [4].

1.3. External quality control (EQC)

External quality control involves proficiency testing, i.e. inter-laboratory comparisons between two or more laboratories. For inter-laboratory proficiency testing, each laboratory conducts the designated test method on a defined panel of test samples, the EQC panel. Identical panels of test samples are dispatched to the participating laboratories for concurrent testing. The proficiency testing is conducted twice per year.

In February 1998, a consultants meeting entitled “The FAO/IAEA External Quality Assurance Programme (EQAP) and Movement Towards a Generic Veterinary Diagnostic Testing Laboratory Accreditation Scheme” was convened to consider the design, impact and proposals for future implementation of the current FAO/IAEA EQAP for animal disease diagnosis and make recommendations with regard to its central purposes and future direction. In addition, the Consultants considered the broader question of a generic QA ‘accreditation’ scheme for veterinary diagnostic testing laboratories that could be made available through international, regional, or national organizations as appropriate to the country of interest. This broader discussion was stimulated by the fact, that few developed and no developing countries have nationally organized schemes to measure and recognize the QA systems and technical competence of veterinary diagnostic testing laboratories, but such a scheme is of vital importance to the quality of policy and decisions and actions taken on national animal health issues and the international trade of livestock and livestock commodities. It followed that, in the Sub-programme’s role as a Collaborating Center to the Office International des Epizooties (OIE or World Animal Health Organisation), it would be appropriate to consider the FAO/IAEA EQAP within the broader scope of an international scheme for veterinary diagnostic laboratory accreditation for two reasons 1) to use information learned through the design and implementation of the FAO/IAEA EQAP to assist in the appropriate development of an international scheme and 2) to ensure that the FAO/IAEA EQAP objectives and procedures are in harmony with international QA guidelines as they develop in this area [5].

The objectives of the EQAP effort were and remain to a) develop reference data for the assessment of new FAO/IAEA diagnostic assay performance in the field, b) determine the user’s general QA status and specify assay proficiency, c) enhance the user’s QA awareness and culture, d) provide an organized and transparent mechanism to enhance the national and international credibility of the user’s laboratory. In addition, the data developed through the FAO/IAEA EQAP can be used from a programmatic perspective as baseline data for a) the development of appropriate

[1] The officer responsible for the diagnosis and monitoring of rinderpest in an EQAP participating laboratory.

intervention strategies, b) monitoring project implementation, and c) evaluation of project impact during and after the project's conclusion.

It is recognized that the FAO/IAEA EQAP is programmatic in nature and is designed to assist counterpart laboratories to bridge the gap between what they have now and formal national or international recognition of quality management and technical competence.

The first round of the EQA for the FAO/IAEA liquid phase blocking ELISA was carried out in 1995/1996. A total of ten laboratories from Asia participated. The round consisted of three FMD sero-types (O, A and Asia) for both, the antigen and antibody ELISA. No interim report was produced, but results were communicated on an individual basis.

The second round was initiated in November 1998. Participating laboratories were the same as in 1996. This time the EQC proficiency testing exercise consisted only of one sero-type (O1 Manisa) for the FMD Antibody ELISA. The results of this round are presented in this report. To assure confidentiality participating laboratories were identified with a code number.

2. MATERIALS AND METHODS

Many parties are involved in the different steps of the EQAP and great effort from each participant is needed to assure final success. An overview of the different steps and involvement for the EQAP is shown in Fig. 1.

2.1. Questionnaire

Laboratories, which had already completed the questionnaire during a former EQA round, received a copy of their completed questionnaire and were asked to review and, if applicable, update the information. Laboratories, which had not completed or returned the questionnaire during former EQAP round were sent a new questionnaire and were asked to complete and return it.

The questionnaire consisted of the following 9 categories:

- A : Administrative information
- B : General information on other diagnostic activities performed in the laboratory
- C : Laboratory facilities
- D : Maintenance and calibration of equipment
- E : Handling of test results
- F : Monitoring of IQC data
- G : Laboratory staff
- H : Other quality assurance procedures within the laboratory
- I : Availability, specifications and usage of computers.

2.2. Internal quality control (IQC) data

The IQC data provide valuable information on the test performance in an individual laboratory. The IQC data for the FAO/IAEA antibody FMD ELISA consists of the four replicates of the antigen control (Ca), of the high positive control (C++), of the medium positive control (C+), and of the negative control (C-).

Prior to incorporation into the ELISA, the IQC samples were tested extensively under different circumstances at the Institute for Animal Health (IAH), Pirbright Laboratory, UK, using the same ELISA to establish the upper and lower control limits (UCL and LCL). These control limits are provided with the FACT SHEET of each new ELISA kit.

As part of the EQAP, the participating laboratories received "ELISA DATA SHEET FOR THE IQC DATA OF THE FMDV ANTIBODY SREENING/TITRATION ASSAY" to fill in the IQC data for each sero-type including other relevant information e.g. date and plate number. Additionally an Excel spreadsheet was distributed at the final RCM in Cambodia to be used for storage, analysis and display of IQC charts.

For IQC evaluation, the mean and ± 2 STD (standard deviations) of the 4 values of the 4 wells is taken for the Ca as OD values. For C++, C+ and C- the mean and ± 2 STD of the 4 values of the 4 wells is taken as percentage of inhibition (PI values).

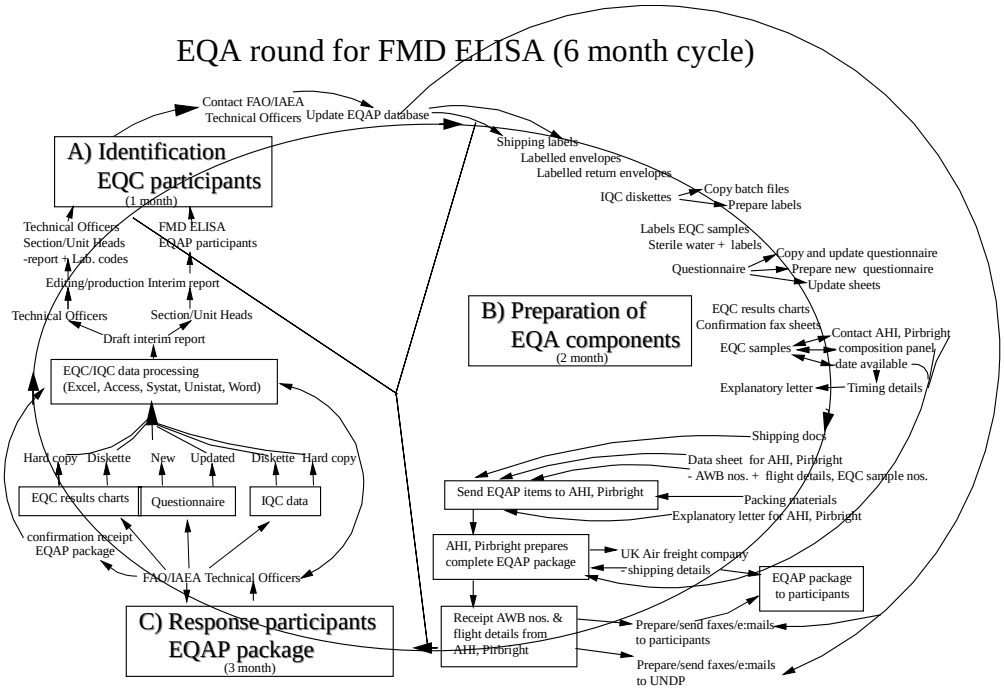

FIG. 1. Overview of the FMD ELISA EQA round

2.3. External quality control test panel

The external quality control (EQC) test panels consisted of five freeze-dried serum samples, three positive samples and two negative samples. The EQC test panels for this round were prepared and dispatched by Dr. Nigel Ferris, IAH. The positive serum samples were derived from experimentally immunized cattle. The test samples, 1.0 ml serum per aliquot, were freeze-dried in one batch at IAH. The samples were tested prior to and after freeze-drying. Each test sample was subsequently labelled with a unique code number. Hence each laboratory received uniquely coded unknown test panels.

The laboratories were requested to reconstitute the EQC samples on the day of testing using attached distilled water and to treat and test these samples in a manner identical to that of their field sera. The laboratories were requested to provide the EQC results in terms of OD and PI values on two separate ELISA data sheets (screening and titration) including IQC data of the ELISA plate and — if possible — to submit a full computer printout of the test plate.

2.4. Distribution

In November 1998, the EQC test panels were dispatched together with the ELISA kits from IAH, principally by international courier to the UNDP offices in the different countries. All participating laboratories and their associated UNDP offices were notified by fax/telex/e-mail of the date of dispatch. The laboratories were urged to collect the EQC test panel from their respective UNDP Offices as soon as possible. Since the sera were freeze-dried, it was not expected that the time or temperature experienced during shipment would add any unwanted variables.

It is assumed that after a maximum of two weeks post-shipment the Technical Officer receives the confirmation fax. After receipt of the confirmation fax the laboratory is given three weeks to produce and return results resulting in a maximum of five weeks from shipment of the EQA panel to the receipt of results.

Results from some laboratories were received very late by the EQA co-ordinator. These laboratories are requested to pay attention that their results are received on time during the next rounds to assure that they will be included in the report. In general, laboratories are requested to adhere more strictly to the deadline for submission of results.

3. RESULTS

All participants replied to this EQA exercise. Out of ten participants six laboratories returned all EQA components (EQC results, IQC data and questionnaire). Four laboratories did not return the complete set of the EQA panel. Three out of five EQC samples achieved an overall agreement of 100%. Two EQC samples were excluded from the evaluation because a minimum of 80% of agreement between participants was not achieved. An overview of the results as received by the EQA co-ordinator is given in Table I.

TABLE I. OVERVIEW OF EQA RESULTS AS SENT BY PARTICIPANTS IN ROUND FMD98a

Lab. Code	EQC	IQC	Questionnaire
1	√	√	√
2	√	√	√
3*	√		√
4**	√	√	√
5	√	√	√
6	√	√	√
7	√	√	√
8	√	√	
9***		√	
10	√		
Total 10	9	8	7

IQC: Internal quality control data
EQC: External quality control data
*did not supply titres
** did only supply titres
*** laboratory reported not having received the panel

3.1. Questionnaire

Nine laboratories (1, 2, 3, 4, 5, 6, 7, 8, and 10) returned the completed and/or updated questionnaire during the two EQA rounds. No questionnaire information was received from laboratory 9. A summary is given below.

3.1.1. Pipettes, tips and ELISA readers

Most laboratories use pipettes from Finnpipette (3), followed by Biohit Proline® (2), Titertek (2), Labosi (3), BDSL (2), Costar (1), Pasteur (1) and Sealpipette (1). Most frequent are tips from Costar (3) followed by Biohit Proline® (2), Gibson (1) and Traceplastics (1). Most of the laboratories use single-channel in the range of 5–50 µl (6), 50–250 µl (7) and 250–1000 µl (4) and multi-channel pipettes in the range of 5–50 µl (8), 50–250 µl (7) and 250–1000 µl (2).

The Immunoskan Plus from BDSL is the most frequently used ELISA reader (7), followed by Dynatech (2), (Multiskan MCC/340 (1), Multiskan Plus Mark II (1) and Pasteur (1).

3.1.2. Power supply/air condition

Six laboratories indicated to have power supply problems. Four laboratories have regular power problems of less than 12 hours and two of irregular duration. Five reported to have power problems on an irregular and one on a weekly basis. Three laboratories have no problems regarding power supply. Four reported problems of no power at all, one reported voltage instability. One laboratory indicated a

'typhoon' as the source of its power problem. No laboratory reported power problems taking longer than 12 hours. Four laboratories use a stabilizer. The majority (7) have access to an emergency power supply and have access to a generator (7). One does not have access to a generator. All have air-condition (9).

3.1.3. Handling of test results and ELISA manuals

With regard to plate reading and calculation of ELISA results the majority use the EDI programme (6), four laboratories indicate to calculate manually raw OD values read with Procomm and two use ELISA software from IAH and Australia.

Three laboratories indicate to use a computer to link test results with other details of source sample using EPI-INFO (1), PANACEA (1) and Excel (1). Four laboratories do not link test results through a computer but manually through a laboratory logbook. Information from laboratory 10 could not be evaluated. The majority of the laboratories (6) monitor their IQC data to determine whether the ELISA plate readings are 'within' limits and can be accepted. Laboratory 7 indicates to monitor its IQC data with a special software programme QCEL. Laboratory 6 has produced its own Excel spreadsheet to monitor IQC data. Laboratory 1 does not monitor IQC data and the information supplied from laboratory 10 could not be evaluated.

All laboratories (9) use the ELISA manual from IAH, Pirbright/FAO/IAEA.

3.1.4. Sample storage

All laboratories (9) store the serum samples at -20°C in most cases using Cryopreservation vials. Additionally, one laboratory uses Nalgene storage system and one laboratory indicates to use another system. Seven laboratories have access to -80°C freezers and two to Liquid Nitrogen facilities. Laboratory 4 indicates that it has access to -70°C and laboratory 5 indicated that it has no access to -80°C but that it has sharing facilities for -20°C. Four laboratories reported to establish a serum bank. Three indicate that they do not establish a serum bank. Information from laboratory 4 could not be evaluated.

3.1.5. Water quality, filters/columns and equipment calibration

All laboratories (9) have access to deionized (5) and/or distilled (4) and/or double distilled (3) water. Seven of them reported to have a maintenance schedule in place. Filters/columns are changed in the following manner: 'according to manufacturer' (2 laboratories), 'once per month' (1 laboratory), one laboratory 'not regularly', one 'according to machine sometimes', one laboratory 'every six month' and one laboratory 'every three years'.

The majority (8) reported that no equipment calibration is carried out. Only one laboratory indicates to calibrate its pipettes and reader.

3.1.6. Laboratory staff training

Laboratory staff has an average of 3.5 years of experience with ELISA (minimum one year to maximum five years). Additional FMD ELISA training was received by seven analysts through participation in FAO/IAEA training courses (4), on the job training given by local experts (3), visiting FAO/IAEA experts (5) and other type of training, e.g. fellowships (1).

3.1.7. Computer/data processing

All laboratories reported that a computer is used for reading of ELISA plates and/or storage of data. Most of the machines are equipped with a 486 processor (6), followed by three Pentium and one 386 processor.

3.1.8. Diagnostic techniques

Besides ELISA the following diagnostic techniques are in place.

For detection of FMDV agent/antigen virus isolation (4), inoculation in suckling mice (2), CFT (1), PCR (1), CSPA (1).

For serological antibody detection to FMDV: VIA (2), NT (2), CFT (1), AGID (2).

The main purpose of testing is for control as part of an eradication programme (8) and for disease diagnosis (8).

3.2. IQC data

A total of eight laboratories (1, 2, 4, 5, 6, 7, 8, 9) submitted IQC results for sero-types A, ASIA, C and O in this round. No IQC data were received from laboratories 3 and 10 (Table II).

From the IQC data analysed it can be concluded that most of the internal controls are within the limits as established at the World Reference Laboratory (WRL), UK, These limits are given in Table III.

TABLE II. IQC DATA RECEIVED PER LABORATORY AND SERO-TYPE

Laboratory code	Sero-type			
	A	Asia	C	O
Lab 1	√	√		√
Lab 2	√	√		√
Lab 3				
Lab 4	√		√	√
Lab 5	√	√	√	√
Lab 6	√	√		√
Lab 7	√	√		√
Lab 8				√
Lab 9	√	√		√
Lab 10				
Total	7	6	2	8

TABLE III. IQC LIMITS

	LCL	UCL
Ca (OD)	0.8	1.9
Ca (PI)	-25	25
C++ (PI)	90	100
C+ (PI)	50	90
C- (PI)	0	49

LCL = lower control limit
UCL =upper control limit

Most of the laboratories supplied IQC data per sero-type written on a uniform datasheet, which was supplied together with the EQA panels. Some laboratories are very efficient in monitoring their IQC data and in applying regular calibration procedures, e.g. laboratory 7 uses QCEL for monitoring ELISA and laboratory 6 has produced an Excel spreadsheet, which is used to produce IQC charts. This spreadsheet was slightly modified (e.g. 2 STD for intra-assay variation instead of 1 STD) and handed out to the participants at the final RCM in Cambodia to be used for further analysis and storage of their individual IQC data. From the IQC data supplied it can be concluded that most of the internal controls are within the limits as established at the WRL. Individual IQC data (per laboratory and sero-type) and basic statistics can be viewed in the interim report entitled “The external quality assurance programme for use with the FAO/IAEA FMD antibody ELISA (EQAP/FMD/1998a)” [6]. The plotted

error bars are indicative of the 'within plate' variation, i.e. the variation between the 4 values of one control sample on one ELISA plate. The variation between the mean values of a control sample on different ELISA plates provides information on the 'between' plate variation. As one aims for a high test precision, both types of variation should be as small as possible and not exceed the UCL and LCL.

3.2.1. Inter-laboratory comparison of IQC results

Figures 2a–d show IQC data per sero-type based on the summary statistics given for each laboratory. The graphs represent the mean value of each control (OD Ca, PI C++, PI C+, PI C-). The bars represent 2 STD. Upper and lower control limits as established by the WRL are shown as a dot line. Results from Figures 2a–d can be summarized in three categories:

I. Mean (dots) and 2STD (bars) within control limits (UCL, LCL).

II. Mean (dots) are within control limits, but 2STD (bars) outside.

III. Mean (dots) and 2STD (bars) outside control limits.

The length of the bar is a measure for the degree of variation.

Table IV resumes the results as shown in Figs. 2a–d giving an evaluation based on the three categories mentioned above.

Results show that Laboratory 1, 2 and 6 fall within category I, showing, that most of the mean and most of the 2 Standard Deviations fall within the established upper and lower control limits. The assay seems to be under control.

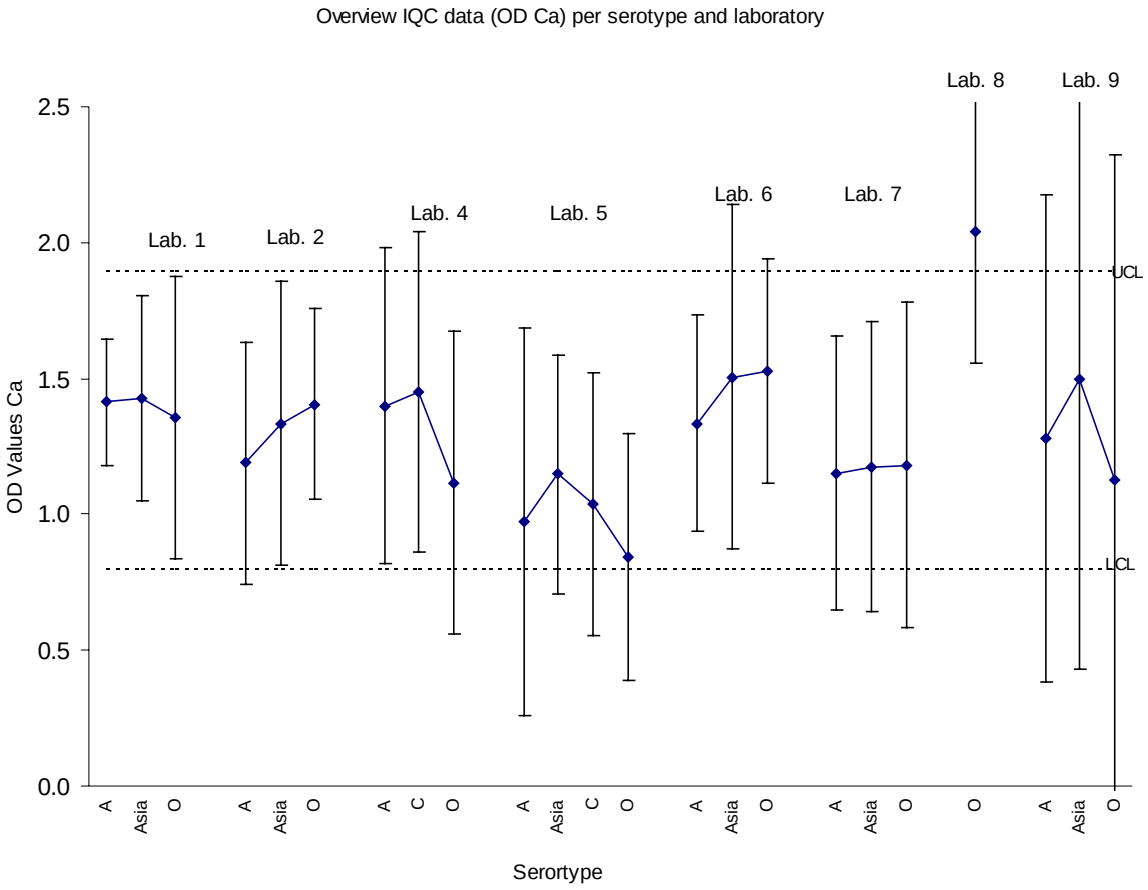

FIG. 2a. Inter-laboratory comparison of OD Ca IQC data

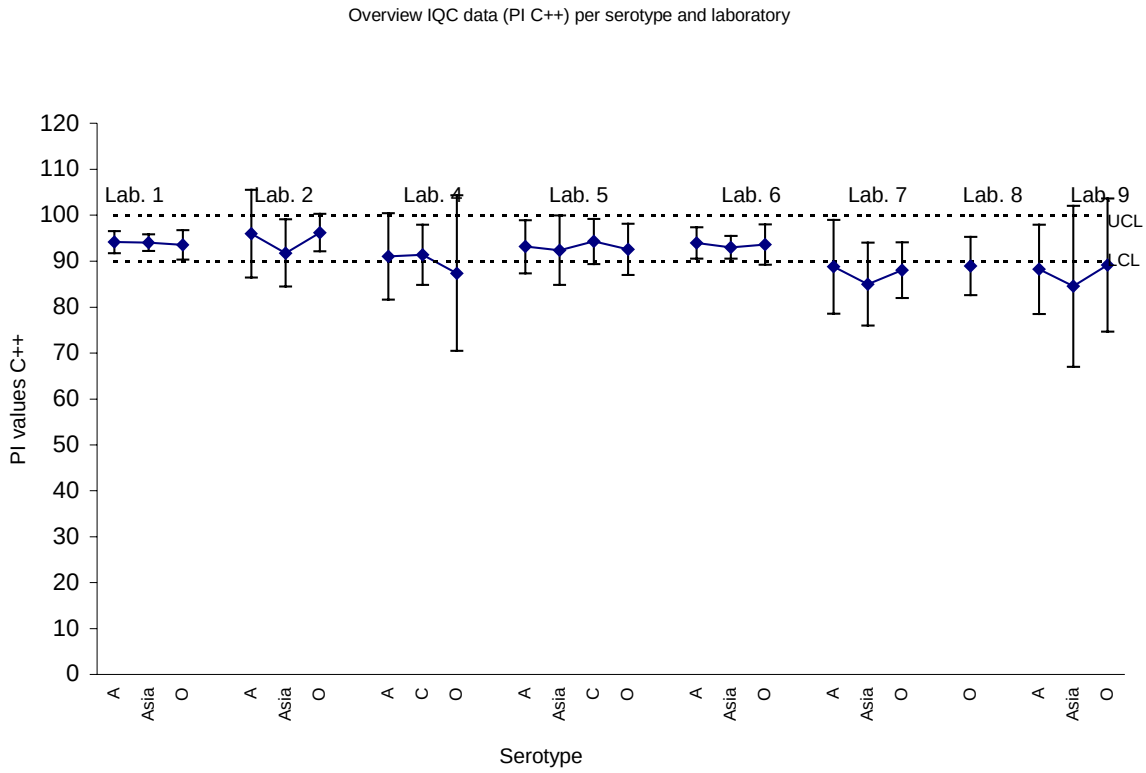

FIG. 2b. Inter-laboratory comparison of PI C++ IQC data

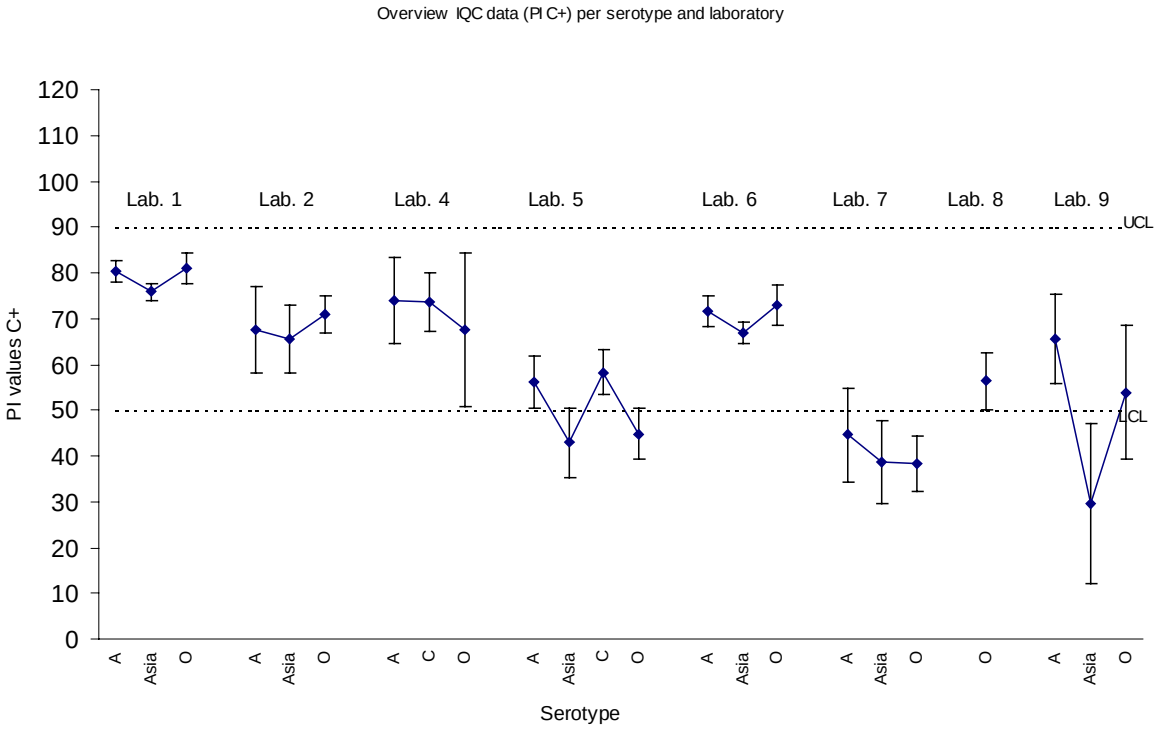

FIG. 2c. Inter-laboratory comparison of PI C+ data

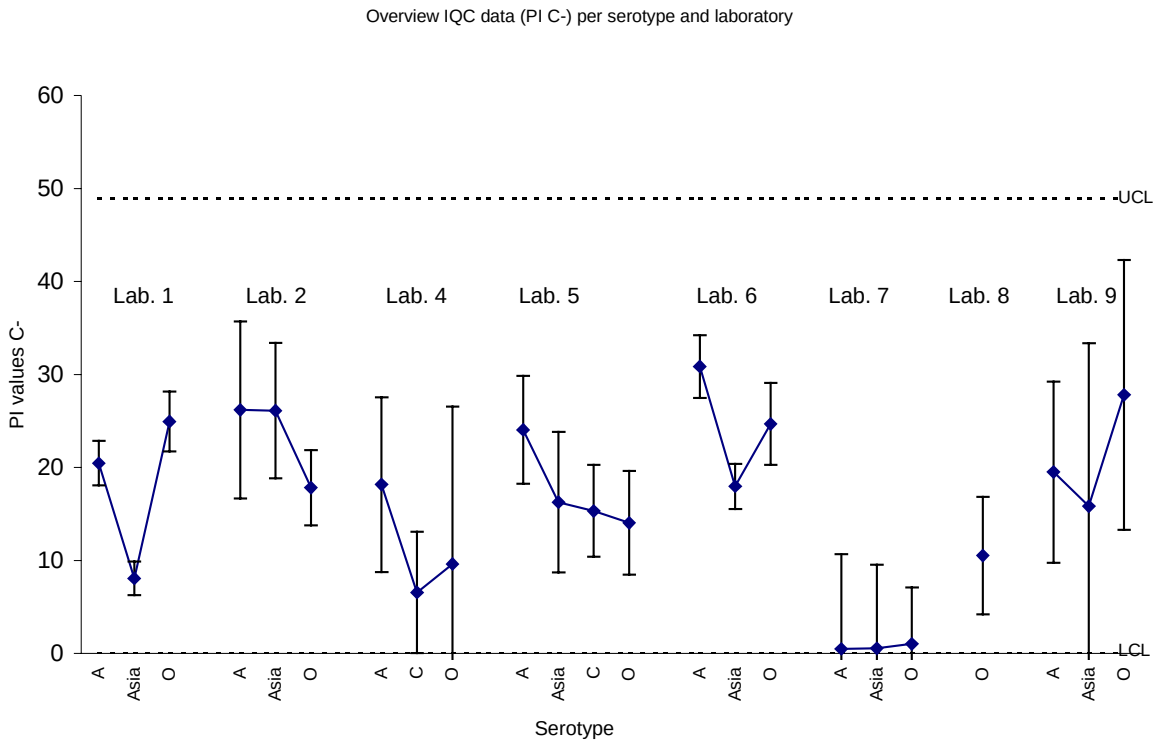

FIG. 2d. Inter-laboratory comparison of PI C- IQC data

TABLE IV. OVERVIEW OF CATEGORY PER SERO-TYPE AND LABORATORY

Sero-type A

	Laboratory code									
	1	2	3	4	5	6	7	8	9	10
Category I	x	x				x				
Category II				x↓	x↓		x↓	x↓↑		
Category III										

Sero-type Asia

	Laboratory code									
	1	2	3	4	5	6	7	8	9	10
Category I	x	x				x↓				
Category II					x↓					
Category III							x↓		x↓	

Cont. Sero-type C

	Laboratory code									
	1	2	3	4	5	6	7	8	9	10
Category I										
Category II				x↓	x↓					
Category III										

Sero-type O

	Laboratory code									
	1	2	3	4	5	6	7	8	9	10
Category I	x	x				x				
Category II				x↓	x↓		x↓	x↑	x↓	
Category III										

↑, ↓= Values tend to be too low, high.

Laboratories 4 and 5 fall within category II, showing that most of the mean values are within limits but that some of the 2 Standard Deviation bars go outside the established limits. There is a clear tendency to produce too low values. The length of the bars indicates that the variation occasionally is too wide. As shown in Fig. 2c, the mean IQC C+ data from laboratory 5 for sero-type Asia and O even fall below the lower control limit. Reduced assay sensitivity for both assays (Asia and O) may result in an increased number of wrong negatives.

Laboratory 7 falls within category III because most of its mean values and 2 Standard Deviation bars are below the lower control limits, e.g. IQC values for all three sero-types for C++ and C+ are below the lower control limit. Reduced assay sensitivity for all three assays (A, Asia and O) may result in an increased number of wrong negatives.

Laboratory 8 has submitted IQC results for sero-type O only. It has produced the highest OD values. Some of the mean and 2 STD values fall above (Ca OD) and others below (PI C++) the control limits. Therefore, it falls within category II and III.

Laboratory 9 falls within category III, because a number of the mean values and many of the 2 STD bars fall outside the lower control values. The length of the bars is also very wide indicating considerable variation.

In general, it is concluded that most of the mean data are within limits. Nevertheless the bars of some laboratories fall outside the lower control limits. The size of variation should be less. This observation confirms the results of the EQC data where in general lower values than the ones established by the WRC were obtained. Applying a 50% cut-off the predictive value, sensitivity and specificity of the assay is acceptable, even if some of the values are close or somewhat below the limits. It is recommended to monitor the IQC data on a routine basis as a tool to observe any drastic shift of results and take corrective steps before the assay goes out of control.

3.3. External quality control test panel

3.3.1. Screening assay

This time the EQC samples consisted of three positive sera and two negative sera of sero-type O1 Manisa as shown in Table V.

TABLE V. EQC SAMPLES

Sample 2	Sample 8	Sample 5	Sample 10	Sample 9
+++	++	+	-	-

Results are based on EQC data supplied from 90% of the laboratories (Laboratories 1, 2, 3, 4, 5, 6, 7, 8 and 10 (Fig. 3). From laboratory 9 no EQC results were received. Per definition an EQC sample is included in the overall evaluation if an agreement of at least 80% is reached between participating laboratories. This was the case for three out of five samples (Table VI). For samples 2, 10 and 9 100% of agreement between laboratories was established when a cut-off of 50% was applied. For sample 8 and 5 only 78% respectively 67% agreement between laboratories was established. Therefore, these two samples were excluded.

In comparison to the reference values for the EQC samples as established by the WRL the participating laboratories produced lower PI values for the three positive samples 2, 8 and 5 and higher PI values for the negative samples 10 and 9 resulting in a decreased binding ratio. Nevertheless, results show that all laboratories are capable to detect a strong positive sample and no equivocally result was obtained for any of the two negative samples. Weak positive sera close to the cut-off are problematical for a minority of the laboratories, which tested these samples negative, e.g. laboratories 5 and 6 (28%) obtained negative results for sample 8 and laboratories 5, 6 and 7 (33%) obtained negative results for sample 5. The closer the sample comes to the cut-off the less agreement between laboratories is reached and more false negative results appear. These findings result in a reduced assay sensitivity compared to the values of the test supplied by the WRL.

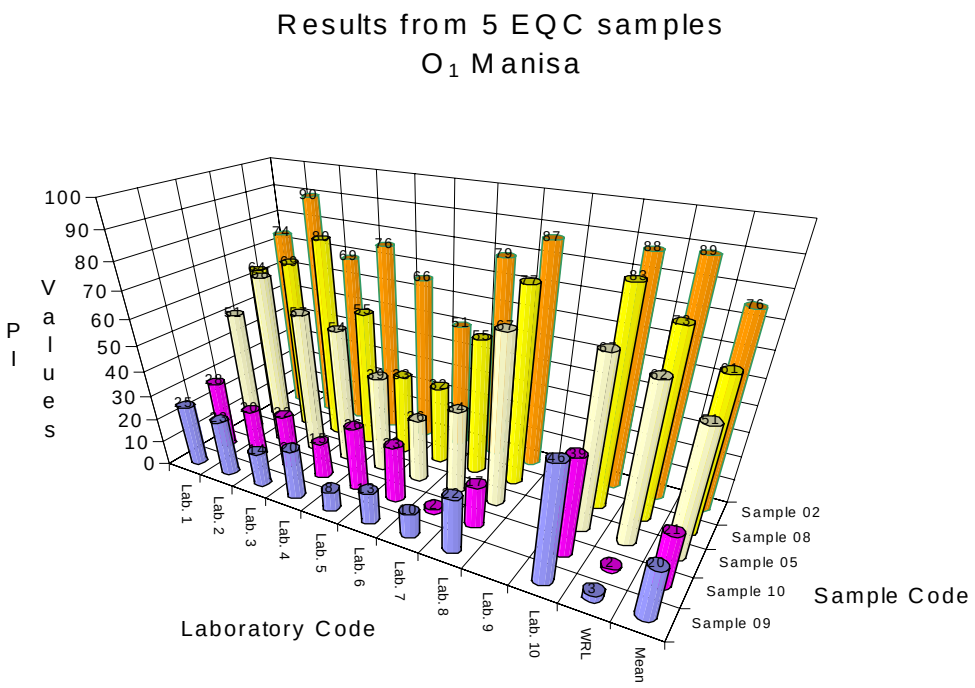

FIG. 3. Results from 5 EQC samples per laboratory

3.3.2. Titration assay

Titres for sero-type O1 Manisa were received from six laboratories (Laboratories 1, 2, 4, 5, 6, and 8) as shown in Table VI. Titres > 1/32 are positive. A good correlation between PI values and titres was observed.

TABLE VI. EQC RESULTS EXPRESSED IN PI VALUES, TITRE AND AS QUALITATIVE DATA (+, -)

	Sample 2			**Sample 8**			**Sample 5**			**Sample 10**			**Sample 9**		
Lab. code	PI value	Titre	++,+,-	PI value	Titre	++,+, -	PI value	Titre	++,+, -	PI value	Titre	++,+, -	PI value	Titre	++,+, -
1	74	1/112	(++)	64	<50	(++)	51	<50	(+)	28	<50	(-)	25	<50	(-)
2	90	1/181	(++)	69	1/90	(++)	68	1/45	(++)	20	< 1/32	(-)	23	< 1/32	(-)
3	69		(++)	80		(++)	57		(+)	22		(-)	14		(-)
4	76	1/90	(++)	55	1/90	(++)	54	1/45	(+)	15	< 1/32	(-)	20	< 1/32	(-)
5	66	>1/32< 1/64	(++)	33	>1/32< 1/64	(-)	39	>1/32 <1/64	(-)	26	<1/16	(-)	8	<1/16	(-)
6	51	1/45	(++)	32	< 1/32	(-)	26	< 1/32	(-)	23	< 1/32	(-)	13	< 1/32	(-)
7	79		(++)	55		(++)	34		(-)	2		(-)	10		(-)
8	87	1/181	(++)	77	1/45-1/90	(++)	67	1/45	(+)	17	<1/32	(-)	22	<1/32	(-)
9*															
10	88		(++)	83		(++)	67		(+)	39		(-)	46		(-)
WRL	89			73			62			1.5			3		
Mean	76			61			51			21			20		

*no EQC data supplied

Basic statistics in Table VII indicate that the mean results as received from nine laboratories for the five EQC samples are still in agreement with the results established by the WRL, even if in general lower values were produced for the positive samples and higher values for the negative samples. The coefficient of variation is higher for the two negative samples as for the positive samples.

TABLE VII. BASIC STATISTICAL DATA ON RESULTS (PI values) OF EQC DATA

	Sample 10	Sample 9	Sample 5	Sample 8	Sample 2
Mean	21.33	20.11	51.44	60.89	75.56
Standard Error	3.37	3.81	5.14	6.31	4.16
Median	22.00	20.00	54.00	64.00	76.00
Mode	#N/A	#N/A	67.00	55.00	#N/A
Standard Deviation	10.10	11.42	15.42	18.94	12.48
Sample Variance	102.00	130.36	237.78	358.86	155.78
Range	37.00	38.00	42.00	51.00	39.00
Minimum	2.00	8.00	26.00	32.00	51.00
Maximum	39.00	46.00	68.00	83.00	90.00
Coef. variation (%)	47.34	56.77	29.97	31.11	16.52
No. of participants	9	9	9	9	9

3.3.3. Youden plots

Figures 4a–c show the EQC results for positive samples 8, 5 and 2 as they are plotted in a simplified Youden diagram. Such a diagram consists of a rectangular plot, on which the individual laboratory's results for two samples are represented by one dot. The X-axis (horizontal component) of each dot is the laboratory's result for sample X, while the Y-axis (vertical component) contains the result for sample Y. The small rectangle inside the Youden diagram represents the mean ± 1 STD range for both samples.

The Youden diagram helps to identify systematic versus random differences between laboratories. Laboratories with systematic error components are either in the upper right hand quadrant (as formed by the line for the means of both samples) or in the lower left-hand quadrant. A laboratory with results positioned in the upper right hand quadrant and outside the +1 STD range could indicate that the laboratory's values for both positive samples are too high, possibly due to an increased level of diagnostic sensitivity of the assay in that laboratory. A laboratory positioned in the lower left quadrant of the diagram and outside -1 STD range could indicate that the laboratory obtained results too low for both positive samples as a result of a decrease in diagnostic sensitivity of the assay. Laboratories reporting results indicating random error are located either in the upper left hand or lower right hand quadrant and outside the ± 1 STD range.

The diagonal distribution of values indicates that there is a positive correlation between both samples. The mean value for all positive EQC samples from all laboratories is considerably lower than the values established by the WRL. The result is a reduced diagnostic sensitivity for some laboratories in comparison to the WRL, e.g. the results for laboratories 5 and 6 fall outside the 1 STD quadrant in the lower left extreme indicating that these laboratories consistently produce low results, thus lowering the diagnostic sensitivity of the assay. Both laboratories identified EQC samples 8 and 5 wrongly as negative. Other laboratories are located within the 1 STD quadrant (Lab. 1 and 4) or close to the upper right 1 STD (Lab. 2, 3, 8, and 10) indicating an elevated diagnostic sensitivity, e.g. laboratory 10 has produced the highest PI values for all samples producing constantly high results. Its result for the negative EQC sample 9 (PI 46) comes close to the cut-off (50%). Thus, it is likely that this laboratory may produce wrong positive results due to its systematically elevated diagnostic sensitivity. Laboratory 7 is located outside the 1 STD only for sample 5 (Figs. 4a and 4c). This laboratory has identified wrongly negative only in sample 5. This may be due to a random error.

Reasons for the loss of positivity in some laboratories have to be discussed, e.g. handling, storage dilution of EQC samples.

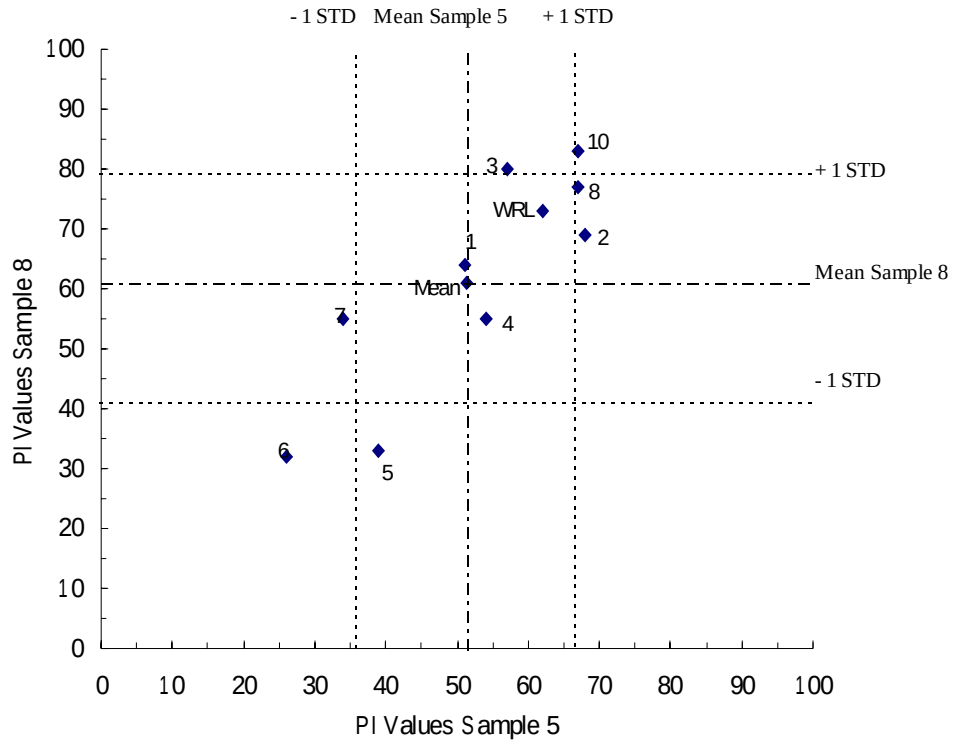

FIG. 4a. Modified Youden plot sample 8 and 5

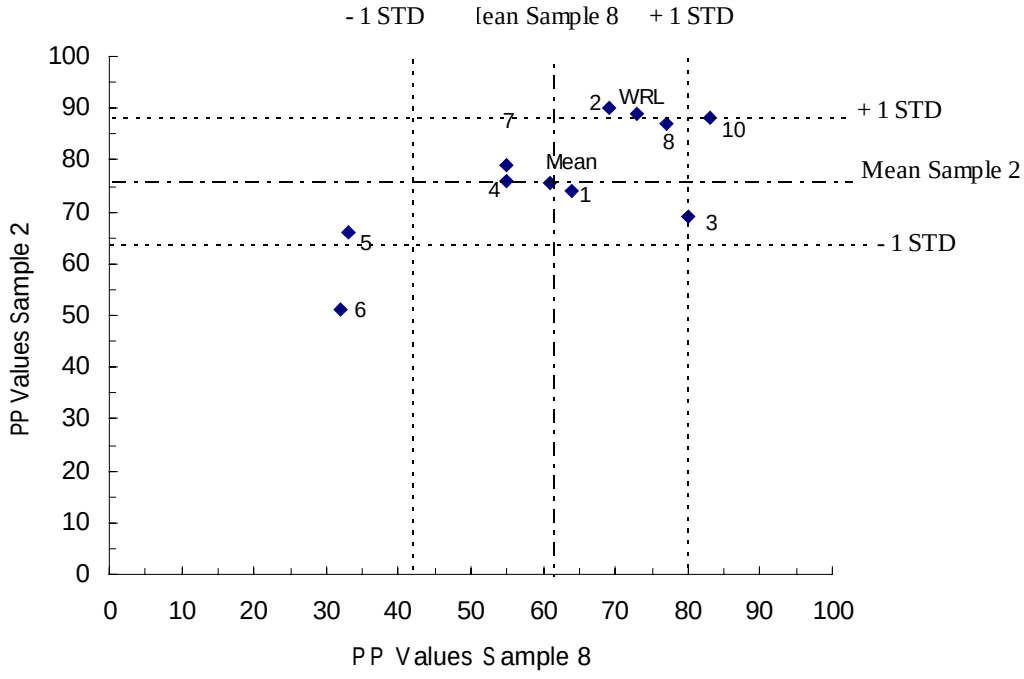

FIG. 4b. Modified Youden plot sample 2 and 8

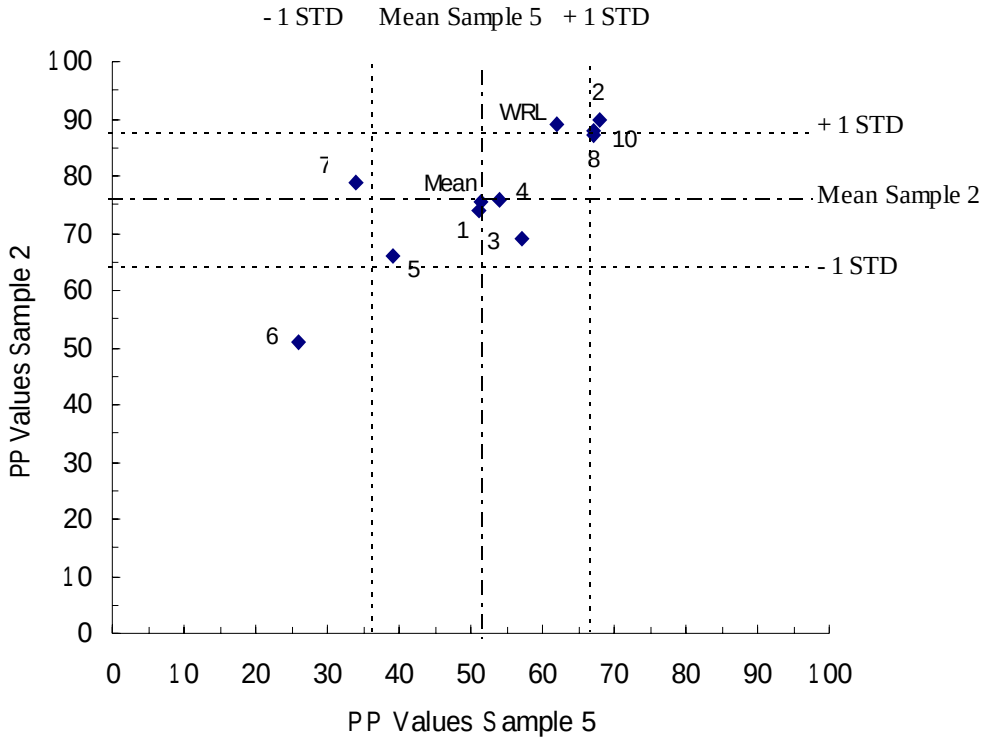

FIG. 4c. Modified Youden plot sample 2 and 5

4. SPECIFIC CONCLUSIONSAND RECOMMENDATIONS

Quality control/quality assurance procedures are essential to testing laboratories as they provide confidence in test results, as well as informing test operators of unacceptable trends in assay performance. The assurance that the test results produced are reliable is not only of importance to the test operator or owner of the animal, but also for all outside interested parties. To achieve this, the Animal Production and Health Sub-programme of the Joint FAO/IAEA Division has initiated an external quality assurance programme.

The return of nine EQC test panel results (90%) was very good, although, the objective is to have 100% returns. The EQC test panel was distributed together with the ELISA kit using a courier service.

4.1. Questionnaire

It can be concluded, that several laboratories still need to improve their IQC practices, i.e. they should pay attention to the monitoring and analyses of the IQC data and should regularly check the calibration of their ELISA equipment. To enhance the monitoring of the internal control data a document entitled “Internal Quality Control (IQC) of Competitive Enzyme Linked Immunosorbent Assay for Measurement of Antibodies against Rinderpest and Peste des Petits Ruminants Viruses using charting methods” is being prepared [4]. Since the principles of analysing and displaying these data as daily or summarized data charts is similar to all type of ELISA (indirect or competitive) this information can be used as a baseline for auto-measuring IQC data. Additionally an Excel spreadsheet for the monitoring of IQC data has been produced and was distributed at the final RCM in Cambodia. Until other software becomes available, the laboratories should use this software for the storage and analysis of their IQC data.

The majority of the laboratories (8) informed that they do not calibrate their equipment. This is a very critical issue, since pipetting errors may be the reason for any kind of variation in an assay. All

ELISA equipment (reader, pipettes etc.) should be checked and, if necessary, calibrated following the procedures as outlined by the producer or the respective protocol. Guidelines have been developed to assist counterparts in checking the calibration of pipettes and ELISA readers and a document entitled "The Laboratory Wizard – A practical loose-leaf edition guide for all who want to share and update ordinary information reported from technical staff of diagnostic laboratories world-wide" assists in a number of good laboratory praxis issues [7].

Different products and versions of ELISA software are in use. The latest version of the FAO/IAEA ELISA software EDI should be installed as soon as possible and older versions of EDI deleted. Different ELISA manuals are in use. It is necessary that only the manual that was received together with the ELISA kit be used.

For the majority of the laboratories power supply, e.g. no power or voltage irregularities, is still a problem. This has direct impact on the laboratories which use air-condition. Temperature fluctuations may be a reason for inconsistency of results and improvement in this area is necessary.

The majority change filters and cartridges**,** but the criteria for the frequency and intervals of change are not clear. Criteria for the change of filter should be more objective (e.g. conductivity control or manufacturer's recommendation). A more uniform and controlled approach towards criteria and frequency in change of filters and cartridges is necessary. The quality of water must be checked on a more consistent basis to eliminate this important issue as a possible reason for assay variation.

Some of the information supplied in the questionnaire was not clear and could not be evaluated. Participants are requested to fill in the questionnaire with more accuracy, since the information supplied is critical for the EQA co-ordinator.

4.2. IQC data

Some laboratories are very efficient in monitoring their IQC data and in applying regular calibration procedures, e.g. laboratory 7 uses QCEL for monitoring ELISA and laboratory 6 has produced an Excel spreadsheet, which is used to produce IQC charts. But for most participants there is an obvious need to improve the routine monitoring of ELISA in most laboratories. These laboratories should concentrate on storing and analysing ELISA results electronically, e.g. using EDI, Excel spreadsheets. An IAEA document entitled "Internal Quality Control (IQC) of Competitive Enzyme Linked Immunosorbent Assay (C-ELISA) for measurement of antibodies against Rinderpest and Peste des Petits Ruminants (PPR) viruses using charting methods" [4] is being prepared to assist analysis of IQC data in the laboratory. Availability of IQC data electronically will ease the work of the EQA co-ordinator considerably.

As part of establishing quality control/quality assurance procedures within a laboratory, the test operators should maintain control charts themselves. For the EQA rounds the laboratories are requested to submit copies written on such control charts or electronically e.g. on diskette or as an E-mail attachment with all relevant information of the last ± 40 plates for external assessment.

As explained in detail in the ELISA manual, the assay data are expressed in OD and PP values for the Ca, and in PP values for the C++, C+, C-. These data are used to determine whether or not the test performed within acceptable limits of variability and therefore, whether or not the test data may be accepted for any given ELISA plate.

Obviously, the test operator should aim to minimize both the 'within plate' and the 'between plates' variation. While it is likely, that the assay will still give a correct positive or negative value to the test sera, if the value of a control falls just outside the upper and lower control limit, the results as such are questionable. The assay must be examined in this situation and the cause for the failure to obtain controls within the limits determined and corrected. It is not acceptable to carry on testing sera with controls consistently falling outside the limits. Something is clearly wrong and it must be investigated and resolved.

The latest EDI version should be installed in the computer as soon as possible and older versions (e.g. EDI) should be deleted. EDI will during installation overwrite any present older EDI version and will also create a new subdirectory ‘eqstat.qc’ for the automatic storage of IQC data. The existing subdirectory ‘Instatqc’ or ‘Eqstat.qc’ and its file(s) will remain unchanged.

In a number of cases, unacceptable low IQC values for the Ca, C++ or C+ were detected. This may result in a reduced test sensitivity. These laboratories also had problems in correctly identifying two of the positive EQC samples. Nevertheless, comparing the results from the EQA exercise in 1996 and this round the laboratories have improved in producing reliable results as an increasing number of the IQC results are within the UCL and LCL. In some cases, there is still considerable variation. These laboratories should take notice of the ‘within plate’ and ‘between plate’ variation in their IQC results and should initiate all necessary measures to reduce that variation. The most likely causes for variation of the IQC data are given below.

4.2.1. Water quality

Data from the questionnaire shows that the majority of the laboratories are using distilled and deionized water. The frequency of cleaning or replacing filters and columns varies from laboratory to laboratory, depending on the type of distiller/deionizer used. The test operator should ensure that the filter/columns are changed as advised in the manufacturer’s documentation. If the test operator still suspects water quality to be a problem, it is suggested that, if available, an alternative water source is utilized for the ELISA and results are then compared.

4.2.2. The test operator

Where more than one operator performs the test, it is almost inevitable that greater variation in results will occur. As long as test operators obtain good test results, there is no problem. However, as part of quality assurance, the laboratory should aim for high repeatability and precision. Therefore, it is suggested that test operators carefully compare their results with respect to IQC data and identify any differences. In this way, possible variations in the technique of performing the ELISA may be highlighted and necessary steps taken to decrease the variation.

4.2.3. Pipetting precision

This is an important factor in variation, particularly where small volumes are being pipetted. Often it is the major cause of the differences in variation observed between test operators.

From the inter-laboratory comparison of IQC data it is concluded that most of the mean data are within limits. Nevertheless, the bars of some laboratories fall outside the lower control limits. Variation should be less. This observation confirms the results of the EQC data where in general lower values than the ones established by the WRL were obtained. Applying a 50% cut-off the predictive value, sensitivity and specificity of the assay is acceptable even if some of the values are close or somewhat below the limits. It is recommended to monitor the IQC data on a routine basis as a tool to observe any drastic shift of IQC data and take corrective steps before the assay goes out of control.

4.3. The EQC test panel

In comparison to the reference values for the EQC samples as established by the WRL the participating laboratories produced lower PI values for the three positive samples 2, 8 and 5 and higher PI values for the negative samples 10 and 9 resulting in a decreased binding ratio. Nevertheless, results show that all laboratories are capable to detect a strong positive sample and no equivocally result was obtained for any of the two negative samples. Weak positive sera close to the cut-off are problematical for a minority of the laboratories, which tested these samples negative, e.g. laboratories 5 and 6 (28%) obtained negative results for sample 8 and laboratories 5, 6 and 7 (33%) obtained negative results for sample 5. The closer the sample comes to the cut-off the less agreement between laboratories is

reached and more false negative results appear. These findings may result in reduced assay sensitivity as compared to the values of the test supplied by WRL. Nevertheless most of the laboratories correctly identified all EQC samples, indicating that the assay itself works properly and that the majority has a good proficiency for conducting the assay.

Titres for sero-type O1 Manisa were received from six laboratories (Laboratories 1, 2, 4, 5, 6, and 8) as shown in Table VI. Titres >1/32 are positive. A good correlation between PI values and titres was observed.

4.4. EQAP/FMD96a and FMD98a

Overall results show that participating laboratories have a good proficiency in ELISA performance. Comparing the results from the FMD96a and this round it is concluded that the number of responds and the quality of EQC and IQC results have improved considerably.

4.5. Recognition

To draw conclusions on the recognition status of the participating laboratories more information is needed. This can be achieved through further EQA rounds.

Recognized laboratories will receive an FAO/IAEA recognition document. OIE and FAO are informed accordingly.

5. GENERAL CONCLUSIONS AND RECOMMENDATIONS FOR THE FAO/IAEA EQA PROGRAMME

(1) Following the conclusions and recommendations of a consultants meeting entitled: “The FAO/IAEA External Quality Assurance Programme (EQAP) and Movement Towards a Generic Veterinary Diagnostic Testing Laboratory Accreditation Scheme” the three pillars of the FAO/IAEA EQA programme remain IQC, EQC and information supplied through a questionnaire, but the focus will be on quality management and documentation of specific laboratory activities through standard operating procedures (SOPs). Participation in the EQAP will assist in creating a quality management working environment, which will assist participants — especially from developing countries, who do not count with a national accreditation body — to bridge the gap between what they have now and formal national or international recognition of quality management and technical competence [5].

(2) Understanding of the principles of assay validation still widely differs. The basis for any EQA participation is a correctly validated assay. The paper from R. Jacobson “Validation of Serological Assays for Diagnosis of Infectious Diseases” is recommended as a guideline to assist the continuing process of assay validation [3].

(3) For the continued success of the programme, it is of vital importance that participating laboratories keep to the time limits set by the EQAP Co-ordinator regarding confirmation of receipt of the EQC test panel and the returning of results. If a laboratory foresees problems in keeping to the time limits, it is the responsibility of the laboratory to contact and inform their FAO/IAEA Technical Officer immediately.

(4) The target of the EQAP is 100% participation of laboratories including the return of questionnaire, IQC, and EQC data. This involves extensive communication between the counterparts and the FAO/IAEA Technical Officer. As the EQAP becomes more of a routine for all involved, it is expected that a higher percentage of returned results can be achieved. To avoid wasting time tracing lost results, EQAP materials, it is recommended that a courier service be used wherever possible.

(5) The questionnaire is considered an essential component of the EQAP. It is urged that the laboratory officers complete the questionnaire as accurately as possible. The information gathered with the questionnaire will require regular updating by the laboratory officer in charge, in close collaboration with the test operator, and should be done at least once a year. In some cases the information provided by the laboratory might need some further clarification. This will be determined by the EQAP Co-ordinator and FAO/IAEA Technical Officers on an individual basis during future EQAP rounds.

(6) The maintenance and calibration of ELISA equipment needs improvement in most laboratories. Relevant documentation has been produced and is being distributed through the EQAP Co-ordinator and FAO/IAEA Technical Officers [7].

(7) When significant hard ware/software changes are made in respect to the computer linked to the ELISA reader, the 'Instatqc' subdirectory and its file(s) should not be deleted but copied to a diskette to save the accumulated IQC data in the '\Instatqc\br.qc' and/or 'Instatqc\bri.qc' files for future reference and retrieval.

(8) To avoid the 'IQC data' of 'experimental' assays becoming part of the valid, routine IQC data and to enable the EQAP Coordinator to differentiate between the possible different types of data submitted on the diskette, it is very important to clearly identify these 'experimental' assays through the BREIA or EDI programmes, e.g. 'TRIAL01', 'TRIAL02', etc. The data of such identified plates can then be easily excluded by the EQAP Co-ordinator from the evaluation of IQC data submitted by the laboratory.

(9) The implementation of a routine monitoring of the IQC data by the participating laboratories is a major objective of the EQAP. An IAEA-TECDOC has been prepared to provide guidelines to test operators to plot IQC control charts themselves in order to facilitate the recording and analysis of IQC data on a day-to day-basis [4].

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ELISA BASED TECHNIQUES FOR THE IDENTIFICATION OF FOOT-AND-MOUTH DISEASE VIRUS AND VACCINE EVALUATION IN BANGLADESH

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Abstract

ELISA BASED TECHNIQUES FOR THE IDENTIFICATION OF FOOT-AND-MOUTH DISEASE VIRUS AND VACCINE EVALUATION

Epidemiology of FMD infection was studied at farm and field levels. The rate of outbreaks increases following the monsoons and remained throughout the winter (until March). Cattle were found to be more susceptible (96.43%) than buffalo (1.01%), goat (2.27%) and sheep (0.27%). Exotic and their cross-bred animals were more susceptible than local breeds of animals (69.89% and 30.11% respectively). Dynamic FMD infection was also studied at the farm level and it was found that infection was directly related with herd immunity, season of the year and climatic conditions. Outbreaks of FMD at rural area were found to be associated with the introduction of new animals from the market in a herd (75.21%) or transportation of infected animals by road or boats. A total of 956 FMD suspected samples from 257 different field outbreaks were collected during the last five years (1995-1999). At the same time, 367 convalescent sera were collected for the analysis FMD antibodies and the virus involved in infection. FMD suspected epithelial samples were tested using indirect ELISA and 875 samples (85.36%) were found positive either against O, A or Asia I. Throughout the last five years, FMD virus type O predominated (54.07%) over FMD virus types Asia I (19%), and virus type A (16.8%) and 6.48% were found to be negative. None of the epithelial samples was found positive against FMD type C. However, five convalescence sera collected from the northern part of Bangladesh showed very strong reaction (>1:240) against FMD virus type C in LPB-ELISA. Vaccination failure was found one of the major constraints towards the control of FMD using vaccine and factors like lack of potent vaccine, inadequate vaccination coverage, poor cold chain, lack of vaccine evaluation and poor health conditions played an important role in this area. Three FMD vaccine candidates were tested using LPB-ELISA both at laboratory and field conditions. Locally produced bi-valent vaccine (O and A) developed a satisfactory levels of antibody following vaccination among the adult stock, while young calves failed to develop protective levels of antibody and disease developed following natural exposure. Two imported vaccine candidates developed protective level of antibody (>1;80) following vaccination. Ring vaccination around the infected foci was conducted and both the vaccine candidates successfully protected the vaccinated herd. Sero-conversion was studied using LBP-ELISA and 90% of the cattle developed antibody higher than 1:80. Economic losses incurred due to FMD infection were also studied. A survey was conducted on 7757 animals and losses due to loss of draft power, reduced milk production and calf mortality were estimated. Of 7757 animals examined, 4750 animals (61.23%) showed the lesions of FMD infection and loss was estimated at US $0.4 million (US $21.93 per animal). Annual loss due to FMD was estimated at about US $125 million per year. In this study, cost–benefit analysis was also made with regard to the control of FMD using vaccination programme. It was found from our study, that in an average year the loss is US $5.0 per animal (US $125 million/year) due to FMD while cost for the vaccination is not more than US $1.5 per animal (US $37.5 million/year). So the control of FMD using a vaccination programme will save at least US $87.5 million per year.

1. INTRODUCTION

Livestock plays an important and integral role in the agricultural system in Bangladesh. Cattle provide the majority of the draught power for cultivation and harvesting and the main method of transportation of agricultural goods in the rural areas. Manure from livestock is the main fertilizer for soil nutrient. Cow dung is also used as fuel from the ancient time in rural areas and recently as raw material for a biogas-plant. Cattle also provide milk and meat and cash income through trade. In Bangladesh 80% of the human population is engaged in agriculture where livestock forms a substantial component of both individual and national wealth.

During recent years the trend of raising livestock has changed quite appreciably through the establishment of mini dairy farms (5–200 cattle) with high-yielding breed of animals. More than 30 000 dairy farms have already been established throughout the country. However, the presence of FMD and its uncontrolled movement discouraged farmers to invest further in this area. Among other livestock diseases, FMD is considered to be the most serious, owing to its pathogenicity, as well as the difficulties of control. Although national economy losses clearly occur through loss of milk and meat production, abortion, infertility, mortality of young calves and incapacitation of draught power by the infected animals, no systematic study in this area has been carried out. However, general animal health reports indicate that the losses due to FMD are about US $125 million per year (US $5.0 per animal per year) [1].

An FAO/IAEA Co-ordinated Research Programme (CRP) on FMD virus Identification and Control in Southeast Asia operational since 1995 enabled samples (epithelial and blood) to be collected from different epidemics and identified using ELISA-based techniques as described in the test manual [2].

ELISA-based techniques have been shown to be both sensitive and specific for the FMD virus identification, vaccine evaluation and sero-monitoring within control programmes for FMD [3–5] and these techniques are widely used in Europe, USA, Australia and now through this CRP in Asia. The ELISA technique (an indirect method) was first used in Bangladesh in 1990 [6], however, the liquid phase blocking ELISA (LBP-ELISA) has more recently been established at the Bangladesh Livestock Research Institute (BLRI) through the support provided under the FAO/IAEA Research Contract awarded as part of the CRP.

The objectives of this Research Contract were to identify the virus strain from field outbreaks to assist selection of vaccine virus strains, and subsequently to evaluate the efficiency of vaccination as part of the national FMD control programme. In this report we have described an effort to identify FMD viruses from different epidemics, to determine their epidemiological consequences and to consider these finding in relation to vaccine needs.

2. MATERIALS AND METHODS

2.1. Disease investigation

Investigations were conducted using an appropriate questionnaire and reports of outbreaks were informed through the use of standardized postcards, which could be sent using the national postal service. Printed postcards with return ticket were distributed at Administrative Units (e.g. Upazila Veterinary Officer/Livestock Officer) with a request to immediately return the card with details to BLRI following an incidence of FMD identified by field staff. During subsequent investigations by our own staff or field veterinarians of selected areas affected by FMD, data was collected using the questionnaire and by asking individual farmers.

2.2. Sampling approach

Two types of samples were collected from the suspected FMD outbreaks. Tissues (epithelium) from tongue and inter-digital space were collected from the infected animals, preserved in 50% phospho-glycerine (v/v) and sent to BLRI virology laboratory under chilling condition. Samples were preserved at -20°C until used.

Blood was collected by jugular vein puncture using venoject needles and vacutainer tubes. The blood was left to clot overnight. Serum was decanted into sterile tubes and kept in icebox for transportation to the laboratory. In the laboratory the serum was centrifuged to remove the remaining red cells before being transferred to 2 ml cryo-vials and stored at -20°C until use.

2.3. Sample preparation

Suspected samples (at least 5 samples from each outbreak area) were prepared by grinding the frozen sample so that it produced fine granules and then making a 20% suspension in Hanks Balance Salt Solution. After centrifugation the supernatant was used as the test antigen within the antigen detection ELISA [3].

Serum was used as the test sample in the LPB ELISA tests.

2.4. ELISA methods

Samples were examined by either indirect ELISA for antigen or the LBP-ELISA for antibody. Test protocols were performed exactly as detailed in the FAO/IAEA manual supplied with the ELISA kits from Pirbight Laboratories, UK, and results were calculated using FAO/IAEA EDI software program version 2.11 [6].

3. RESULTS

During the last 5 years (1995–1999), 257 outbreaks of FMD were recorded and 243 (94.55%) were confirmed as FMD by ELISA-based techniques. The study was conducted to determine the disease epidemiology, vaccine failure, virus involvement and economic losses incurred due to diseases. Epidemiology of the disease was surveyed over 21 563 animals and 49.16% (10 601/21 563) of animals were found to be infected by FMD virus. Cattle were found to be higher susceptible (96.43%) than buffaloes (1.01%), goats (2.27%) and sheep (0.27%) (Fig. 1). Furthermore, calves were found more susceptible (35.69%) than bull/bullock (32.55%) and cows (31.44%) (Fig. 2). Exotic animals and their crosses were found more susceptible (69.89%) than the local animals (30.11%) (Fig. 3).

Outbreaks of FMD were noticed throughout the year, however, two major peaks of infection were observed. One after the rainy season and another one during winter, although outbreaks continued to occur at a significant level throughout the winter (Fig. 4).

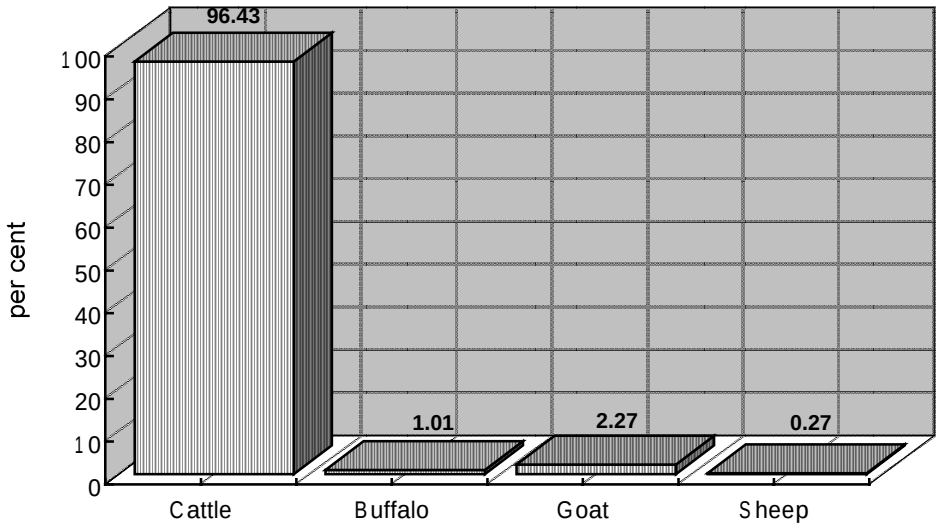


FIG. 1. Susceptibility of animals against FMD virus

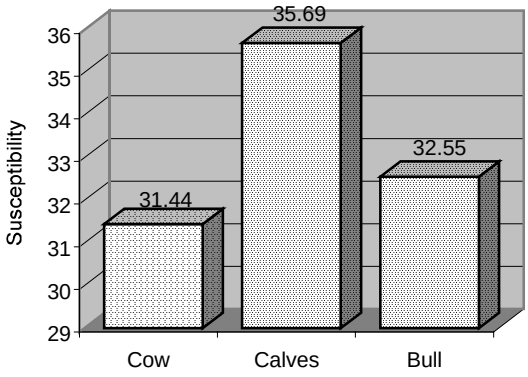


FIG. 2. Susceptibility of cattle to FMD virus based on age and sex

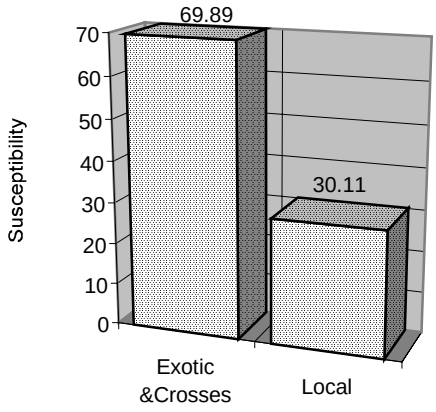


FIG. 3. Susceptibility of cattle to FMD virus based on bred of animal

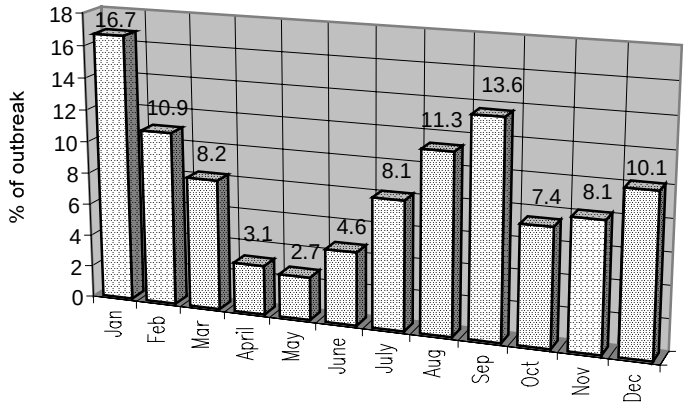
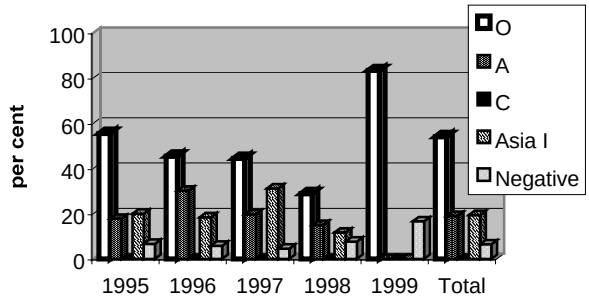


FIG. 4. Distribution of FMD outbreaks throughout the year

A total of 953 tissue samples were collected and 89.05% (884/953) were found positive for FMD virus using the indirect ELISA. Results showed that major types of FMD virus identified were O, A and Asia I. (Fig. 5) throughout the last 5 years, the involvement of FMD virus type O was always found higher than other two strains (A and Asia I). FMD virus type C was not identified from any tissue samples during the last 5 years, however, a strong antibody response was detected against FMD virus type C using LPB-ELISA in convalescent sera collected following infection from a herd where fresh tissue sample was not available during field investigation. The sensitivity of indirect ELISA was found to be higher than the complement fixation test (Fig. 6)

The dynamics of FMD infection was also studied, particularly at farm level. Two separate outbreaks of FMD were studied involving over 3000 animals. Data was collected throughout the outbreak until the last case was reported. Herd management practice, vaccination history and level of immunity against FMD following vaccination were carefully recorded and analysed. During the 30–35 days of the disease cycle, three major peaks of infection were noticed (Fig. 7) and were found to be strongly associated with sudden changes in climatic conditions at the farm such as a sudden rainfall or strong prevailing wind. Animals aged between 3 to 18 months were found to be highly susceptible and frequently related to a poor immune response to vaccination (Fig. 8).



1995	1996	1997	1998	1999	Total	Sero-type
55.55	45.49	44.49	28.93	83.33	54.07	O
17.78	30.18	19.72	15.01	0.00	19.00	A
0.00	0.00	0.00	0.00	0.00	0.00	C
20.00	18.46	31.19	11.72	0.00	19.45	Asia I
6.67	5.85	4.59	7.67	16.67	6.48	Negative

FIG. 5. FMD Sero-types identified during 1995–1999

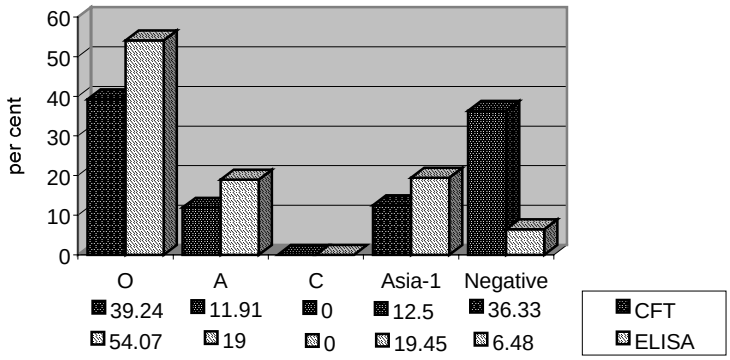
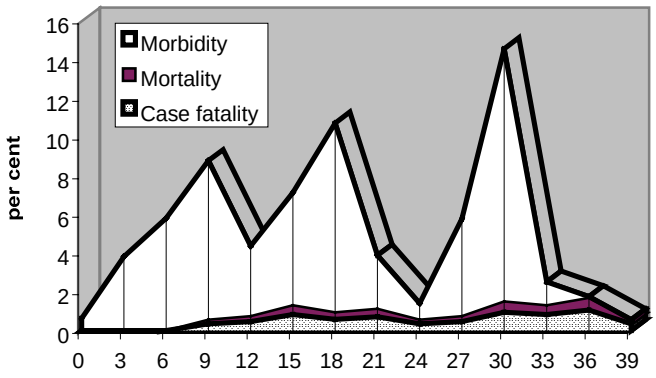


FIG. 6. Comparative level of sensitivity of CFT and ELISA



	Days post infection													
	0	3	6	9	12	15	18	21	24	27	30	33	36	39
Morbidity	0.63	3.83	5.82	8.24	3.62	5.82	9.8	2.77	0.85	5.04	13.07	1.2	0.07	0
Mortality	0	0	0	0.21	0.28	0.49	0.35	0.42	0.21	0.28	0.56	0.49	0.63	0.21
Case fatality	0	0	0	0.35	0.47	0.83	0.59	0.71	0.35	0.47	0.95	0.83	1.06	0.35

FIG. 7. Dynamics of FMD infection on a farm

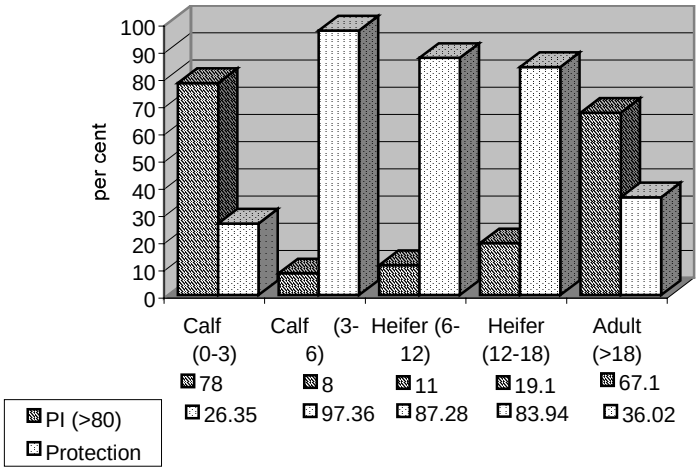
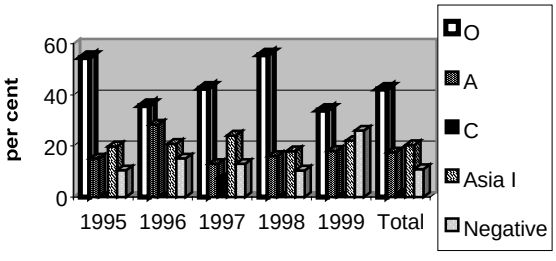


FIG. 8. Relationship between LPB-ELISA and protection against homologous FMD virus infection

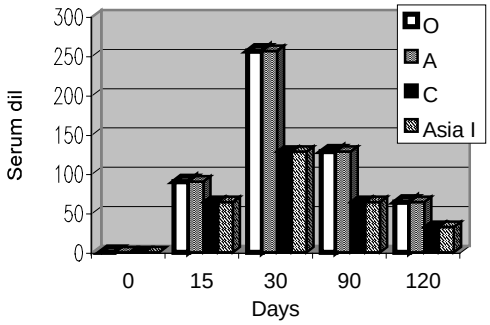
The reactivity of convalescent sera against the four major sero-types of FMD virus was studied using the LBP-ELISA (Fig. 9). The highest percentage of antibody was found against FMD virus type O, however, high levels of antibody against FMD virus type C were also identified in convalescent sera collected from a herd having moderate type of infection.

Both the locally produced and imported vaccines were tested in terms of their ability to induce an antibody response using the LBP-ELISA test. Locally produced formalin killed bivalent vaccine containing FMD virus types O and A showed higher sero-conversion among adult animals when compared to those under six months of age and significant vaccination failures (inability to induce an antibody response was recorded among this age group of animals (6–18 months). Two imported vaccines (a polyvalent vaccine containing FMD virus types O, A, C and a monovalent vaccine containing FMD virus type Asia I, BEI inactivated) induced good sero-conversion in all age group of animals and these responses were found effective against natural exposure to infection (Figs.10–12). Ring vaccination around a 5–7 km radius of a foci of FMD infection also proved to be effective with these vaccines with good protection and 90% sero-conversion (>1:80, LPB-ELISA).



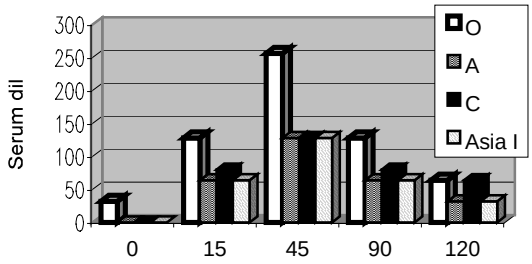
	1995	1996	1997	1998	1999	Total
O	54.65	35.85	42.6	55.66	34	42.27
A	15.11	28.3	12.96	16.03	18	17.47
C	0	0	7.4	0	0	1.14
Asia I	19.76	20.75	24.07	17.92	22	20.34
Negative	10.47	15.09	12.96	10.38	26	10.88

FIG. 9. Comparative level of FMD antibodies using LPB-ELISA



	Days				
	0	15	30	90	120
O	0	90	256	128	64
A	0	90	256	128	64
C	0	64	128	64	32
Asia I	0	64	128	64	32

FIG. 10. SN titre of anti-FMD antibodies following vaccination in goat using LBP-ELISA



	Days p/v				
	0	15	45	90	120
O	32	128	256	128	64
A	0	64	128	64	32
C	0	80	128	80	64
Asia I	0	64	128	64	32

FIG. 11. SN titre of antibodies against FMD vaccine following vaccination in cattle using LPB-ELISA

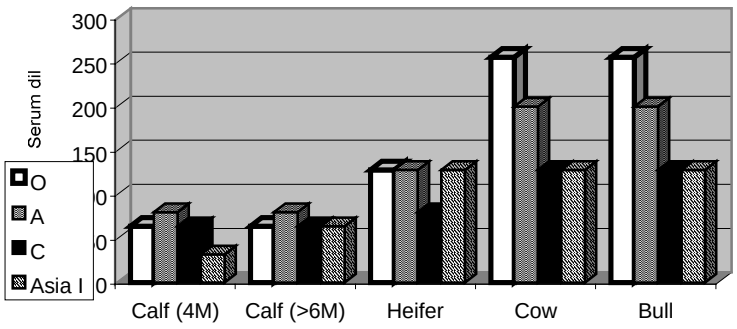


FIG. 12. Sero-conversion following FMD vaccination based on age and sex of animals (30 days p/v)

Economic analysis due to FMD infection was studied in a rural area with a cattle population of over 7000 animals. Sixty-one percent of animals (4750/7757) were found to be infected by FMD virus type O. Losses due to reduced milk production (68%), calf mortality (42.1%) and reduced draught power (53%) were analysed and found to be an average loss of US $21.93 per animal. Cost–benefit analysis was also carried out and it was determined that an average loss due to FMD was US $5.0 per animal per year (with a total cattle population of 55 million this represents losses of US $125 million throughout the country). The cost of vaccines is US $1.5 (with two doses of polyvalent vaccine) per animal, hence the net benefit is US $87.5 million per year. Clearly control of FMD using a potent vaccine is economically viable in Bangladesh.

4. CONCLUSIONS

Participation in the FAO/IAEA Co-ordinated Research Project in Bangladesh has assisted the introduction of ELISA-based techniques for the identification of FMD virus and vaccine evaluation and will have future application in the national FMD control programme. Results obtained clearly indicate that the objectives under this CRP have been achieved.

It has been shown that FMD virus types O, A and Asia I are circulating throughout the country, although FMD type O has been found to play the dominant role in FMD disease epidemiology. The Indirect ELISA has been found to be very sensitive and specific and a rapid and reliable method when compared to the previously used CFT and will now be used throughout the country for FMD virus identification. Use of LPB-ELISA for vaccine evaluation has been established for the first time in Bangladesh and has been found to be very sensitive, quick and reliable. Protective level of antibody determined by LPB-ELISA (>1:80) has been shown to be consistent with the protection against FMD natural infection.

In this study it was found that outbreaks of FMD were associated with the seasons of the year, stress factors like sudden flooding, low herd immunity and health status of animals. Cattle were found to be more susceptible then buffaloes, sheep and goat. Exotic breeds and their crosses were found to be more susceptible than local cattle. Village market and uncontrolled movement of animals played an important role in the spreading of infection. Vaccination failure in a herd has found to be associated with factors such as lack of potent vaccines, poor vaccination coverage, a poor cold chain, lack of vaccine evaluation and an inadequate public awareness of the importance of the disease. The use of formalin-killed vaccine, poor health conditions (parasitic infection, malnutrition) were also found to play an important role in vaccination failure. Economic losses due to FMD infection were estimated at an alarming level of US $125 millions per year. Cost–benefit analysis based on the use of vaccines for the control of FMD has been evaluated and shown to be effective provided that potent polyvalent, BEI inactivated vaccines are used during the national control programme of FMD in Bangladesh.

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INTRODUCTION OF ELISA TECHNIQUES FOR THE DIAGNOSIS AND EPIDEMIOLOGY OF FOOT-AND-MOUTH DISEASE IN CAMBODIA

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Abstract

INTRODUCTION OF ELISA TECHNIQUES FOR THE DIAGNOSIS AND EPIDEMIOLOGY OF FOOT-AND-MOUTH DISEASE IN CAMBODIA

Foot-and-mouth disease (FMD) is endemic in the Kingdom of Cambodia and causes major problems to farmers as well as losses in overall terms to the national economy. Losses are due to treatment of sick animal, impact on rice cultivation through the loss of ploughing capabilities, death of animals and finally, through effects on animal trade. The disease affects cattle, buffalo and pig throughout the year. In 1998, 22 cattle actually died whilst 35 000 showed clinical signs of FMD. 1400 buffalos and 102 pigs also showed signs of FMD. Virus sero-types Asia I and O were identified from cattle and virus sero-type O was found from infected pig. Tested epithelium and vesicle fluid samples from sick animals for antigen type has shown that 78% were FMD sero-type O among cattle and pigs and 21% FMD sero-type Asia I from cattle. Titration of sera from vaccinated animal against FMD after second vaccination has shown high levels of immunity. All sera tested were positive at a dilution of 1:125 for sero-types O, Asia I and A.

1. INTRODUCTION

Agriculture plays an important role in the Cambodian national economy, providing about 50 percent of the total gross domestic product with 85% of the population engaged in agriculture. Among infectious diseases, foot-and-mouth disease (FMD) is considered as the first disease priority due to the tremendous impacts both on farmers and on the national economy. One major constraint to FMD control in the Kingdom of Cambodia is the lack of capability in disease diagnosis and information on disease epidemiology. For these reasons, the National Veterinary Diagnostic Laboratory (NVDL) submitted a proposal to the Joint FAO/IAEA Division of the International Atomic Energy Agency for support in the introduction and improvement of ELISA technologies, using antigen and antibody types for FMD diagnosis as well as the study on FMD epidemiology in Cambodia under this Co-ordinated Research Project (CRP). The report below accounts the activities and outputs of the NVDL, Department of Animal Health and Production, under this CRP supported by FAO/IAEA.

2. MATERIALS AND METHODS

2.1. Outbreak investigations

Disease investigation and information on outbreaks of FMD was conducted using questionnaires and FMD monthly report forms. Standard forms were distributed to the Offices of Provincial Animal Health and Production (OAHP) throughout the country. On the basis of the information collected with these forms OAHP reports to the Central Department on the FMD situation in their respective Province. Additional disease investigation is conducted by NVDL staff to provide more details and information from these outbreaks.

2.2. Samples routinely submitted

Epithelium and vesicle fluid from animals having FMD symptoms are collected by staff of the OAHP and Non Government Organizations (NGOs), working with livestock owners in the Provinces as well as by NVDL staff during outbreaks investigations. NVDL provides special containers for preserving FMD samples to OAHP and NGOs. The containers were procured under the Research Contract from the FMD World Reference Laboratory (WRL), United Kingdom. Training on taking, preservation and dispatch of FMD specimen to NVDL has been conducted for officers of OAHP and NGOs staff, so that good quality specimen are collected and sent to NVDL or WRL for testing.

2.3. FMD efficacy trial

Three districts (Chhoung Prey, Prey Chhor and Batheay) out of sixteen districts in Kompong Cham Province were selected as sites for FMD vaccine efficacy trials. The FMD vaccine used in the

trail was imported from Rhone Poulance Company as an oil-adjuvant vaccine containing O, A and Asia I FMD virus sero-types.

Blood samples for testing for serum antibodies using the ELISA technique were collected from jugular vein of cattle and buffalo. Samples were collected from control and vaccinated animals three times during the trial.

– Before receiving FMD vaccination;
– 21 days after first animal vaccination; and
– 21 days after second animal vaccination.

Each time 500 blood samples were collected and included 100 samples from experimented animals from each selected district and 200 samples from control animals – from two out of three selected districts.

Blood samples were centrifuged, the serum decanted and this stored at -10ºC to -15ºC until tested.

2.4. FMD antigen and antibody assays

Testing for antibodies and the presence of antigens to FMD virus were carried out using ELISA kits for an indirect sandwich enzyme-linked immunosorbent assay for detection of antigens and a liquid phase blocking enzyme immunoassay for detection of antibodies of FMD virus against sero-types O, A and Asia I using bench protocols provided with the kits [1]. The basic equipment and laboratory consumables were provided under the FAO/IAEA Research Contract and through an FAO national technical co-operation project entitled "Foot-and-mouth Disease Surveillance, Control and Strategy Formulation" (TCP/RAS/6611a).

Testing for FMD virus antibody response in control and vaccinated animals was carried out using the two test protocols given below.

– A screening assay for control animals for which the sera were tested at a single dilution of 1:125
– A titration assay for vaccinated animals. A series of five-fold serum dilutions (1:125 to 1:3125) were tested in order to more accurately determine end point titres for post vaccination sera.

3. RESULTS

3.1. FMD outbreaks and their distribution

FMD outbreaks were recorded in 13 out of the 21 Provinces in the country in 1994. These include Siem Reap, Bantheay Meanchey, Battambang, Pursat, Kompong Thom, Kompong Chhnang, Kompong Speu, Kandal, Kompong Cham, Prey Veng, Svay Rieng, Kratie and Rattanakiri Provinces. The outbreaks appeared even during the dry season, which has not previously occurred in Cambodia.

In 1995, the incidence of FMD increased widely and one particular outbreak is now regarded as the largest epidemic of FMD in Cambodia during the last two decades. Nineteen out of 21 Provinces reported FMD outbreaks. Six Provinces, which were not infected by the disease in 1994, reported FMD this year. These Provinces were Koh Kong, Kompot, Kompong Soam, Takeo, Steung Treng and Mondolkiri.

In 1996, 17 FMD outbreaks were reported in 11 Provinces. The disease appeared to spread from village to village. These progressive outbreaks were considered a continuation of the outbreak in the previous year. Even though the disease occurred throughout the year, most cases were reported in July.

In 1997, 8 FMD outbreaks were recorded in 5 Provinces, namely Takeo, Kompong Cham, Siem Reap, Kompong Chhnang and Kompong Speu. Interestingly, the first cases of FMD were found in Kirivong district, which borders Viet Nam. In 1998, FMD outbreaks were reported in 12 out of 21 Provinces, i.e. Kompong Speu, Takeo, Battambang, Kompot, Kratie, Kompong Thom, Preh Vihea, Bantheay MeanChey, Siem Reap, Steung Treng, Kompong Cham and Krong Kep. All three animal species cattle, buffalo and pig were infected. Among the infected animals, 22 cattle died and 35 000 showed FMD symptoms. 1400 buffalos and 102 pigs were also reported affected. During 1997 and 1998, 47 epithelium and vesicle fluid samples from cattle and pig, having FMD lesions were collected from various parts of the country (Table I).

TABLE I. NUMBER OF EPITHELIUM AND VESICLE FLUID SAMPLES FROM ANIMALS HAVING FMD SYMPTOMS RECEIVED BY NVDL DURING 1997 AND 1998

Year	Month	Date	Location	Number of samples		
				cattle	buffalo	pig
1997	Jan.	18/01/97	Kirivong District,	2	0	0
			Tramkok District, Takeo Province	1	0	0
	Feb.	23/02/97	Kompong Siem District, Kompong Cham Province	3	0	0
	June	19/06/97	Kralang District, Siem Reap District	5	0	0
	Nov.	18/11/97	Cattle Breeding Station, Batie District, Takeo Province	1	0	0
	Dec.	12/12/97	Oral District,	2	0	0
			Somrong Tong District, Kompong Speu Province.	4	0	0
1998	Jan.	12/01/98	Borset District, Kompong Speu Province	7	0	3
	Feb.	20/02/98	Somrong District, Takeo Province	1	0	1
	Mar.	04/03/98	Tramkok District, Takeo Province	1	0	0
		21/03/98	Somrong Tong District, Kompong Speu Province	0	0	2
	Aug.	11/08/98	Kompong Svay District,	2	0	0
			Steung Sen District,	3	0	0
		24/08/98	Stong District, Kompong Thom Province	2	0	0
	Oct.	02/10/98	Pouk District, Siem Reap Province	2	0	0
	Dec.	03/12/98	Prasat Bakong District, Siem Reap Province	2	0	0
		21/12/98	Chhamkar Leu District, Kompong Cham Province.	3	0	0
Total samples received in 1997/98				41	0	6

3.2. FMD laboratory results

Epithelium and vesicle fluid samples from 18 cattle showing FMD symptoms were collected during eight different outbreaks in five Provinces in 1997. The Provinces were Takeo, Siem Reap, Kompong Cham, Kompong Chhnang and Kompong Speu. Twenty-nine FMD specimens from sick animals were submitted to the laboratory for antigen typing. These specimens were collected from the Provinces of Takeo, Kompong Speu, Kompong Thom, Siem Reap and Kompong Cham (23 specimen from cattle and 6 from pig). The results are shown in Table II. It is clear that in Cambodia FMD virus sero-types O and Asia I are commonly recovered from infected animals. FMD virus sero-type Asia I was found among cattle in Siem Reap Province, while FMD virus sero-type O was found in cattle and pigs of Kompong Cham, Takeo and Kompong Speu Provinces. Antigenic characterization conducted by WRL revealed that the FMD sero-types O from Cambodia in 1998 have greatly reduced antigenic homology with the previous recommended vaccine strain sub-type O Manisa. This sub-type O is different from those found in Thailand, Malaysia, Taiwan and the Philippines but is related to cattle and buffalo isolates of sub-type O from Viet Nam. Further studies by WRL indicate a shift towards antigenic characteristics shared by Indian/European/South American vaccine strains (sub-type O IND 53/79, O Campos and O BFS).

3.3. FMD vaccine efficacy trials

A total of 566 sera were tested for FMD antibody, using screen and titration assays. One hundred and thirty-three sera from animals after the first vaccination, 228 sera from animals after second vaccination and 200 sera from control animals.

Using the screening assay on the 200 sera from control animals revealed a low percentage with FMD antibodies (at initial test dilution). When these positive sera were re-tested they were all negative at the next dilution.

The titration assay on the 366 sera from vaccinated animals after first and second vaccination were all positive at a dilution of 1:125 against sub-types O, Asia I and A. At a serum dilution of

1:3125 (the lowest dilution at which sera were tested) the percentage positive to sero-type O was 9%, to A was 42% and to Asia I was 40%.

TABLE II. RESULT OBTAINED FROM ANTIGEN TYPING OF FMD VIRUS USING THE ELISA TECHNIQUE 1997–1998

	1997 Cattle					1998 Cattle					1998 Pig				
Province	Total tested samples	Positive			Negative	Total tested samples	Positive			Not type	Total tested samples	Positive			Not type
		Asia I	O	A			Asia I	O	A			Asia I	O	A	
Takeo	4	–	1	–	–	2	–	–	–	2	1	–	–	–	1
Kompong Cham	3	–	1	–	2	3	–	–	–	3	–	–	–	–	–
Siem Reap	5	5	–	–	–	4	–	–	–	4	–	–	–	–	–
Kompong Speu	6	–	6	–	–	7	–	7	–	–	5	–	3	–	2
Kompong Thom	–	–	–	–	–	7	–	–	–	7	–	–	–	–	–
Total	18	5	8	–	5	23	–	7	–	16	6	–	3	–	3

4. DISCUSSION

FMD virus sero-types O and Asia I are routinely isolated from pigs and cattle in Cambodia, but so far no specimen collected from buffalo has been typed. The result of an antigenic study conducted by WRL on specimen from Cambodia in 1998 revealed that type O virus antigenic characteristics are shared by Indian/European/South American vaccine strains, namely O IND 53/79, O Campos and O BFS. Further study on virus strains from Cambodia should be continued.

The screening of sera from control animals has shown some sera positive. However, when those serum samples were tested by the titration assay at a serum dilution of 1:16 the test results obtained were negative. This would indicate that such animals have very low levels of immunity to FMD virus and these could have arisen from an FMD infection in previous years.

In conclusion, although it has taken some time to introduce ELISA technology in Cambodia for both antigen typing and serology, it is now being used routinely and the results are proving critical to understanding the disease in this country and to the development of effective control strategies. ELISA for antibody detection also plays an important role for studies of the effectiveness of FMD vaccination both in the selection of vaccine types and of the best use of vaccine in the field.

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INTRODUCTION AND USE OF ELISA-BASED TECHNOLOGIES FOR THE DIAGNOSIS AND MONITORING OF FOOT-AND-MOUTH DISEASE IN HONG KONG

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Abstract

INTRODUCTION AND USE OF ELISA-BASED TECHNOLOGIES FOR THE DIAGNOSIS AND MONITORING OF FOOT-AND-MOUTH DISEASE IN HONG KONG

ELISA-based tests were introduced to assist in the diagnosis and control of foot-and-mouth disease (FMD) in Hong Kong. The tests were used to identify and type FMD viruses in clinical samples, to provide an assessment of the efficacy of vaccination programmes as practised, to train staff in ELISA technology and to strengthen quality assurance for foot-and-mouth disease and other diagnostic tests. These tests have provided the tools needed to understand why foot-and-mouth disease occurs in the face of vaccination – an essential step towards control of this disease in Hong Kong.

1. INTRODUCTION

Foot-and-mouth disease (FMD) has been diagnosed regularly in Hong Kong since records of disease occurrence were first kept. Most outbreaks of FMD occur in pigs and are due to Type O virus. Types Asia I and A FMD viruses have also been identified in ruminants, largely cattle. The last reported cases were in 1976 and 1973 respectively. Of the other viral vesicular diseases only swine vesicular disease (SVD) has been identified. This was last identified in 1989.

Most cases of foot-and-mouth disease occur during the cooler parts of the year, from November to early April, perhaps reflecting better conditions for survival and transmission of FMD virus. The disease occurs in pigs of any age, but in most outbreaks appears to spare pigs less than 3 months of age. This probably reflects the protective effect of maternally derived antibody (MDA) in piglets. Virtually all farms practice vaccination using a range of vaccination schedules, yet the disease still occurs. Not all farms experience outbreaks of this disease every year, but it remains a significant cause of economic loss for farmers through mortality, loss of production, reduced sale price for affected pigs and the on-going cost of vaccination.

Prior to the introduction of ELISA-based diagnostic tests, Hong Kong did not have the capacity to diagnosis FMD locally. Samples were sent to the FMD World Reference Laboratory (WRL) at Pirbright for testing. Serological monitoring of the response of animals to vaccination was also unavailable. This paper summarizes the results of work performed using ELISA kits supplied by the WRL as part of the IAEA/FAO Co-ordinated Research Project utilizing ELISA-based technologies to diagnose and investigate FMD.

2. ANTIGEN DETECTION ELISA

The antigen detection kit prepared for Hong Kong allowed detection of FMD virus types O, A and Asia I as well as SVD virus. The kit was used on specimens from reported cases of foot-and-mouth disease (usually on more than one sample) between 1995 and 1998. The samples comprised epithelial tissue from ruptured vesicles on the coronary band of pigs collected into FMD transport media (glycerol phosphate buffer).

In 1995, technical difficulties were experienced with the antigen kit. Internal positive control samples were within range, but we were unable to detect virus antigen in field samples that subsequently tested positive at the WRL. Samples spiked with known positive antigen could be detected, suggesting the problem lay in extracting the virus from the tissue samples.

By 1996, these technical problems had been resolved and a total of 43 clinical specimens were tested subsequently. Only Type O virus was detected. Selected samples from each FMD outbreak were forwarded to WRL for virus detection/isolation and further characterization. In all cases where WRL detected virus, tests in Hong Kong on other samples from the outbreak were also positive.

Use of this kit has allowed rapid identification of viruses in outbreaks of foot-and-mouth disease and the test will be used in the future to monitor all outbreaks of this disease.

3. ANTIBODY DETECTION

A liquid phase blocking ELISA utilising O1 Manisa antigen (as incorporated in the main vaccine used in Hong Kong at the time) was used to investigate the response of pigs to vaccination on commercial farms in Hong Kong. Two main studies were undertaken.

3.1. First Study

The first trial was performed on local pig farms to obtain preliminary information on whether vaccination, as practised in Hong Kong, stimulated an immune response. Results from this trial were used as the basis for planning of additional studies.

Samples were collected from pigs on 20 farms using a range of vaccination programmes. Usually, 10 samples were collected per age group and these were collected approximately four weeks post vaccination. On one farm, samples were collected from three different batches of pigs one, two and three months after the second dose of vaccine, given at 10 weeks of age. The results were analysed in conjunction with information provided by farmers on their vaccination programmes. This was done to assess the factors that may have contributed to the poor response to vaccination. Because of the variation in the timing of vaccination, the absence of unvaccinated control pigs and the range of vaccine formulations used, detailed statistical analysis of the data was not performed.

Follow-up investigations were conducted on farms, where the response to vaccination appeared to be inadequate. Close contact was maintained with farmers after the trial to establish whether FMD had occurred on any of the farms in the subsequent 6 months.

3.1.1. Results

The results from the trial are presented in Tables I and II. From the information provided it was apparent that many farmers did not adhere to recommended vaccination schedules. Some farmers were vaccinating only once, others were using products of dubious quality/storage history and in most cases, vaccination was being performed too early.

TABLE I. SUMMARY OF RESULTS FOR PIGS VACCINATED ONCE

Vaccine Type	Age at Vaccination (weeks)	Number positive (a)	Number negative	Total samples	% Positive	Notes (see below)
Vaccine 1	8	0	11	11	0	(b)
Vaccine 2	8.5	9	1	10	90	
	10	2	8	10	20	
	12	2	7	9	22	
[sub total–V2]		[13]	[16]	[29]	[44]	
Vaccine 3	8	1	9	10	10	
	9	13	7	20	65	
	10	15	35	50	30	
	14	9	1	10	90	
[sub-total–V3]		[38]	[52]	[90]	[42]	
Total V1–V3		51	79	130	39	

(a) A positive result was defined as a titre of 90.
(b) The antigen in this vaccine was not the same as that in the test kit.

TABLE II. SUMMARY OF RESULTS FOR PIGS VACCINATED TWICE

Vaccine Type	Age at Vaccination (weeks)	Number positive(a)	Number negative	Total samples	% Positive	Notes (see below)
Vaccine 1	5, 9	0	10	10	0	(b)
Vaccine 2	4, 7	9	1	10	90	
	4, 10	24	8	32	80	(c)
	7, 10	15	0	15	100	
	9, 12	9	1	10	90	
[sub total–V2]		[57]	[16]	[67]	[85]	
Vaccine 3	10,14	28	2	30	93	
Total — V1–3		85	22	107	79	

(a) A positive result was defined as a titre of 90.
(b) The antigen in this vaccine was not the same as that in the test kit.
(c) This batch of pigs comprised 3 separate age groups all collected on one day. These pigs had relived their second dose of vaccine 1, 2 and 3 months previously.

Less than 40% of the pigs tested appeared to respond adequately when tested 4 weeks after one dose of vaccine. This figure rose to almost 80% for pigs tested after a second dose of vaccine.

On one farm, where samples were collected from pigs one, two and three months post vaccination, the titres in the group tested three months post vaccination appeared to be lower than those in the other two groups and more pigs in this group were seronegative.

Two months after completing these tests, outbreaks of FMD occurred on some of the farms, including two on which a good response to vaccination had been demonstrated two months prior to the outbreak. Disease on these farms involved pigs older than five months of age.

On another farm, samples collected from a group of 10 week old pigs, prior to vaccination, contained low levels of antibody, presumably maternally derived.

3.1.2. Discussion

In this trial we were able to demonstrate that most pigs developed antibodies to FMD following two doses of vaccine. Nevertheless, results from one group of pigs suggested that this response might not persist through to market weight. This finding alone was not conclusive, as it was based on a 'snapshot' taken at a single point in time (i.e. three batches of pigs of different age were tested on the same day). However, when coupled with the fact that most outbreaks of FMD were occurring in older pigs, the need for further investigation of this possibility was clearly apparent.

Possible causes of the apparent vaccine failure on the two farms, where a response to vaccination had been recorded, included drops in antibody levels over time (as discussed above), changes in vaccination practice, overwhelming viral challenge, and/or antigenic variation in the virus. The two farmers involved discounted alterations in vaccination practices. They had not changed their vaccination methods nor the vaccine used after testing. Massive challenge possibly contributed to vaccination breakdown on one of these farms. It was directly adjacent to a farm housing 2000 pigs, which experienced a serious outbreak of FMD just prior to the outbreak on his farm. An antigenic variant type O strain was demonstrated in Hong Kong at the time of these outbreaks, although not specifically on these two farms. As a result of this finding, a second antigen was added to subsequent vaccines formulated for Hong Kong by one of the vaccine manufacturers.

The poor response obtained following a single dose of vaccine was not unduly surprising. It is well recognized that a priming dose of FMD vaccine does not provoke a strong response in pigs and protection depends on delivery of a second dose several weeks later. This is the recommended practice of all vaccine manufacturers. Nevertheless, in this trial a positive serological response was found in only 40% of the pigs suggesting that other factors, possibly MDA, had interfered with the response. Some farms were vaccinating as early as 4 weeks of age — a time when MDA would almost certainly be present. It has been shown by others [1], that MDA can persist as long as 10 to 12 weeks and we

also demonstrated this on one farm, where pre-vaccination samples were collected from 10 weeks old pigs.

Vaccination technique also plays a role in the magnitude of the immune response to FMD vaccine. On one farm, where the first dose of vaccine was delivered at 12 weeks of age a poor response to vaccination was noted. This was attributed, in part, to the use of short needles, which would have resulted in the deposition of vaccine in adipose tissue.

3.2. Second trial

Building on the results of the first trial a second study was undertaken that compared the serological response of two groups of pigs given either two standard 1 ml doses of vaccine or two 3 ml doses delivered at 10 and 14 weeks of age. A report of this study is included in the next chapter of this publication. Briefly, the pigs in this trial did not respond as well as expected to vaccination; persistent MDA was considered to be a key factor in causing this. Additional trials are planned to investigate this further.

4. QUALITY CONTROL AND STAFF TRAINING

A critical component of this project was implementation of quality assurance programmes. Hong Kong participated in two rounds of external quality assurance testing and successfully assayed the External Quality Assurance Programme (EQAP) panel. Laboratory technicians have continuously evaluated results of internal controls for values outside range and have implemented corrective measures for these when they occurred.

Some quality control problems were encountered, particularly when the tests were introduced. In the first antibody trial only occasional runs could be unconditionally accepted. Nevertheless, between run comparisons for results of test samples revealed little variation. Some of these problems appeared to stem from reagent instability, but these largely disappeared by the time of the second trial. A range of improvements was introduced to the laboratory during the testing programme, including new pipettes and water filtration equipment for double distilled water. Although a specific benefit relating directly to any one of these items was not demonstrated, their introduction coincided with an overall improvement in the internal quality control data.

The tests have been used to provide training in ELISA techniques for laboratory staff. The first trial in particular was used as an opportunity to introduce staff to the tests and to improve their proficiency in serological testing. Not only has this proven extremely valuable for the FMD programme, it has been beneficial in recently introduced testing programmes for avian influenza in poultry and beta-agonists in pigs. Without the skills imparted through the FMD ELISA project the testing programmes associated with these public health crises could not have been implemented as quickly as they were.

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SEROLOGICAL RESPONSE OF PIGS TO A STANDARD AND INCREASED DOSE OF FOOT-AND-MOUTH DISEASE VACCINE

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Abstract

SEROLOGICAL RESPONSE OF PIGS TO A STANDARD AND INCREASED DOSE OF FOOT-AND-MOUTH DISEASE VACCINE

Two randomly allocated age-matched groups of 17 conventionally reared pigs derived from vaccinated sows were vaccinated at 10 and 14 weeks of age with a commercially available foot-and-mouth disease vaccine, using either a 1 mL dose or a 3 mL dose. A control group of four pigs was left unvaccinated. Pigs were monitored at regular intervals from birth to 26 weeks of age for antibodies to FMD Type O virus using a liquid phase blocking ELISA. At 12 weeks post vaccination, significantly more pigs vaccinated twice with 3 mL of vaccine had developed antibodies against Type O foot-and-mouth disease virus (at an ELISA titre of 90 or greater) than those vaccinated twice with 1 mL of vaccine (chi-squared test, $p = 0.006$). Overall, the response to vaccination was poor in both groups of pigs. Four weeks after the first dose of vaccine only four pigs had detectable antibody against the virus. Twelve weeks after the second dose of vaccine only 60% of pigs given the 3 mL dose and 15% of pigs given the 1 mL dose had ELISA titres of 90 or greater. Maternal antibody is considered to have played a role in this poor response, as it was present in 27 of the 34 vaccinated pigs at the time of first vaccination. Two pigs in the unvaccinated control group developed a low level antibody response (antibody titre <90). Infection with field virus was considered a highly unlikely cause of this. These results show, that under field conditions using a widely adopted protocol not all pigs vaccinated develop antibody to foot-and-mouth disease. This, in part, may explain why vaccination programmes against this disease in Hong Kong seem to have a limited impact. The results also suggest, that an increased dose of vaccine has a positive effect on the humoral immune response against FMD virus and may improve protection against this disease. Timing of vaccination needs to be re-evaluated to reduce the impact of maternally derived antibodies.

1. INTRODUCTION

Outbreaks of foot-and-mouth disease (FMD) caused by Type O virus occur every year in pigs in Hong Kong. Most of these occur in the cooler half of the year (from November to April), presumably due to improved conditions for transmission of the virus. On most farms, vaccination is used as the key means of minimizing the impact of this disease, but is not sufficient to prevent the disease. Outbreaks of FMD still occur on farms that use vaccines manufactured and supplied by reputable firms and use the vaccine according to the manufacturer's recommendations.

In some instances outbreaks appear to be the result of antigenic variants of the virus. Such strains of type O FMD virus have been identified in Hong Kong as shown by reduced r-values when field strains are compared with vaccine strains [1]. However, not all outbreaks of disease in vaccinated herds have been due to a variant strain and the question has been raised if the antigenic payload in the vaccine may be too low to effect a protective response under field conditions.

This study was designed to assess the serological response in pigs to vaccination under field conditions when the vaccine was used according to the manufacturer's recommendation as well as at a dose three times greater than that recommended.

2. MATERIALS AND METHODS

2.1. Pigs

The pigs used in this trial were raised at the Hong Kong Government's pig breeding centre. They were derived from multiparous Landrace or Large White sows that had been vaccinated twice as grower pigs and then twice annually. FMD had not occurred on this farm since February 1993. Piglets were derived from four separate litters. Three litters were born on the same day, the fourth 3 days later. Piglets were identified with ear notches prior to the first bleed and were randomly allocated to one of three groups by drawing numbers from a hat.

After weaning at 35 days, pigs were housed in one large pen on sawdust litter for the duration of the trial and were also vaccinated against hog cholera and Aujezsky's disease as per schedule in Table I.

TABLE 1. SCHEDULE OF VACCINATION FOR PIGS

Vaccine	Age at Dose 1*	Age at Dose 2*
Foot-and-mouth disease	70 days	98 days
Hog Cholera	30 days	90 days
Aujeszky's disease	49 days	79 days

*all less 3 days for the fourth litter of piglets

2.2. Vaccine and vaccination

The FMD vaccine used was an oil-based, commercial FMD vaccine containing O1 Manisa strain (Rhone Merieux - Aftopor). The vaccine had been stored according to the manufacturer's recommendations since purchase. Pigs were vaccinated by deep intramuscular injection behind the ear by experienced farm workers under veterinary supervision using new disposable syringes fitted with 3.75 cm long 21 gauge needles.

Blood samples were collected from each pig at 7 days, 5, 8 and 10 weeks of age and then every four weeks until 26 weeks of age, when the pigs were sold. Samples were kept frozen at -20^{o}C until they were tested.

The first group of 17 pigs was given 1 mL of vaccine at 10 weeks of age and again at 14 weeks of age. The second group of 17 pigs was given 3 mL of vaccine at 10 weeks of age and again at 14 weeks of age. A third group of 4 pigs acted as control and the pigs in this group were sham vaccinated with saline.

2.3. ELISA Test

The liquid phase blocking ELISA [2] was supplied by Pirbright Laboratory and the test performed according to instructions. The antigen in the kit was the same as that used in the vaccine (i.e. Type O Manisa).

All samples were initially tested using a screening test. All samples from the 22 and 26 week old age group were retested using two-fold dilutions, including those that were negative on screening test. Samples with titres exceeding 252 were retested using higher dilutions.

2.4. Statistical analysis

In screening tests any serological response (i.e. any titre of 32 or above) was recorded as positive. Results for the 1 mL and 3 mL groups at each age group were compared using Fisher's exact test (two tail).

For the analysis of results for pigs at 26 weeks of age, for which an assessment of the magnitude of response was required, samples with a titre of 90 or more were regarded as 'positive'. Results of the two groups were compared using Chi-square analysis. Variability in the magnitude of high titre samples on retest prevented more detailed analysis of the results.

3. RESULTS

With the exception of one pig that died after week 22, all pigs remained clinically healthy for the duration of the trial. The results of screening tests on each group of pigs are summarized in Table II.

Significantly more pigs had detectable antibody at 26 weeks of age in the group of pigs vaccinated with 3 mL of vaccine than the 1 mL group.

Maternal antibody was detected in all but 7 pigs at 10 weeks of age. These sero-negative pigs were all derived from the same litter (data not shown) and although the sow was sero-positive, she was ill during lactation, leading to reduced transfer of immunoglobulins.

TABLE II. PIGS THAT GAVE A POSITIVE RESULT ON THE SCREENING TEST
(Positive pigs/total population in group that were tested)

	Wk 1	Wk 5	Wk 8	Wk 10	Wk 14	Wk 18	Wk 22	Wk 26
1 mL	16/16*	14/17	12/16*	12/17	1/17	10/16*	13/17	8/16^
3 mL	16/16*	16/17	15/17	15/17	3/17	16/17	15/17	16/17
Control	4/4	2/4	2/4	2/4	0/4	0/3*	1/4	2/4

* missing sample
^ one pig died

Only four pigs had detectable antibody at 14 weeks of age, all at low levels. In one of these pigs no antibody was detected at 10 weeks of age (the age at first vaccination) suggesting, that this was a response to vaccination. In the other three pigs, it was not established whether this was due to persistent maternal antibody or a response to vaccination.

Results for pigs at week 26 are summarized in Table III using a titre of 90 as a cut off. There is a significant difference between the 3 mL and 1 mL groups (chi-squared test, p =0.006) in terms of number of 'positive' pigs at 26 weeks of age.

TABLE III. DISTRIBUTION OF TITRES FOR PIGS AT 26 WEEKS OF AGE

	<32	≥32≤45	>45 <90	≥90<181	≥181≤256	>256	Total
1 mL	8*	5		1	1	1	16^
3 mL	1	5	1	6	2	2	17
Control	2	2					4

* one pig that gave a low positive titre (32) in the screening test, tested negative in this round of testing.
^ one pig in this group died after week 22.

4. DISCUSSION

There are a number of factors that may affect the response of pigs to vaccination. In this trial, we have demonstrated that one of these, namely the dose of vaccine used, can have a positive effect on the immune response. In comparing 1 mL and 3 mL doses of FMD vaccine administered to pigs at 10 and 14 weeks of age, the increased dose improved the humoral immune response against homologous FMD Type O virus when measured 12 weeks after the second dose.

Nevertheless, the overall response to vaccination in this trial was disappointing. In the group of pigs given 3 mL of vaccine moderate to high level titres were found in less than 60% of pigs at 26 weeks of age (12 weeks after the second dose of vaccine). This level of immunity achieved on a herd basis is unlikely to prevent the occurrence of FMD [3].

Other serological monitoring on farms where vaccination is practised has shown that vaccinated pigs do not always develop demonstrable antibody (Sims, unpublished). In addition, outbreaks of FMD in vaccinated pigs on farms in Hong Kong show that vaccination against this disease, as practised, is not preventing this disease.

Maternally derived antibody (MDA) is considered to be the most likely cause of the poor response in this trial. It was detected in 27 of the 34 vaccinated pigs just prior to inoculation with the first dose of vaccine, and probably inhibited the response to the primary vaccination. Only four pigs

had antibody against this virus four weeks post–vaccination, lending support to this proposal. MDA may also have interfered with the generation of the secondary antibody response as a result of *T. lymphocyte* immunosuppression [4].

Six pigs without detectable maternal antibody at the time of first vaccination also failed to respond serologically within 4 weeks of vaccination and, at a later stage, did not develop demonstrably higher titres than others in their group. This unresponsiveness may be due to persistence of inhibition of the immune response by MDA even when MDA is no longer detectable [5].

These results reinforce the need to deliver vaccine at an appropriate time so that MDA does not interfere with the immune response. It would appear that delaying the first dose of vaccine until at least 12 weeks of age is warranted for piglets derived from sows with good maternal immunity. This approach has been proposed by others [6], although, in using this method, one would need to take into account the variable rates of transfer of MDA, which depends in turn on the quantity and timing of the colostral intake. Vaccinating piglets at 12 weeks of age or later could provide a window of opportunity for infection in those piglets that obtained only low levels of MDA. Monitoring of MDA, either through measurement of specific FMD antibody a few days after birth in selected piglets or by using a simple indicator of colostral intake, such as a semi-quantitative zinc sulphate turbidity test on serum for immunoglobulins, would allow litters with low uptake of MDA to be identified and vaccinated earlier.

Other factors that could have resulted in the poor response to vaccination in this trial have been well thought-out but were not considered significant. The trial was conducted under close veterinary supervision. Pigs were vaccinated by experienced staff and the method of vaccination adopted should have delivered the vaccine into a deep intramuscular site. As a result, vaccination technique was not considered a likely cause.

The vaccine used in this trial had been stored properly since purchase, had not expired and was purchased from a reputable supplier. Nevertheless, antigenic degradation cannot be ruled out entirely, given that FMD antigen is labile, and the full history of the product from the time of manufacture was not available. Additional potency testing of the vaccine was not conducted.

In this trial, no attempt was made to assess whether the antibody levels achieved were protective. The cut off titre of 90 used for analysis of results in pigs 12 weeks after vaccination was based on findings in calves that those with titres below 100 became susceptible to infection [4]. Protective antibody levels for pigs under field conditions in Hong Kong have not yet been established and, in fact, it may not be possible to predict with precision the exact point at which protection occurs using serological tests [7].

The effect of vaccination on cell-mediated responses in this trial was not studied. The role of cell mediated responses in immunity to foot-and-mouth disease virus is poorly defined when compared to that of the humoral response [8] and warrants further investigation.

The apparent seroconversion (albeit at low levels) in two control pigs may be due to ‘false positive’ results. The absorbance values of these samples in all ‘positive’ wells of the ELISA plate are very close to the 50% inhibition level used as the cut-off for establishing whether a sample is positive. Low level positive reactions have been reported when using the liquid phase blocking ELISA in cattle unexposed to FMD virus [9].

The possibility of these results being due to exposure to field virus seems highly unlikely given that all the pigs were housed together, many had no detectable antibody against FMD, and no clinical disease resembling FMD occurred in these or other pigs on the farm. Misidentification of these pigs or samples cannot be ruled out entirely as a cause. Nevertheless, it is considered unlikely given that one of these animals was ‘sero-positive’ at both 22 and 26 weeks of age, and at no stage were duplicate samples found that would suggest two pigs had been identified with the same number.

The information obtained from this trial is likely to have a positive impact on the efficacy of vaccination against FMD in Hong Kong. We anticipate that increasing the dose of the vaccine given and delaying vaccination of piglets in herds, in which sows are vaccinated regularly would increase

the protection against FMD. Further studies need to be performed to ascertain whether the improved response to an increased dose of vaccine observed in this trial would still be apparent if vaccination was delayed until MDA was no longer detectable.

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FOOT-AND-MOUTH DISEASE IN LAO PDR: ESTABLISHMENT OF LABORATORY FACILITIES, OUTBREAK DIAGNOSIS AND SEROLOGICAL SURVEILLANCE

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Abstract

FOOT-AND-MOUTH DISEASE IN LAO PDR: ESTABLISHMENT OF LABORATORY FACILITIES, OUTBREAK DIAGNOSIS AND SEROLOGICAL SURVEILLANCE.

In 1997, a new foot-and-mouth disease (FMD) diagnostic laboratory was established as part of a project supported by the Australian Center for International Agricultural Research (ACIAR) in collaboration with the Department of Livestock and Fisheries (DLF), Lao PDR. The ACIAR project laboratory houses equipment and reagent supplied by the FAO/IAEA Co-ordinated Research Project (CRP) on FMD in Southeast Asia. Training has also been provided in performing FMD ELISA techniques. A serological survey to determine the sero-prevalence of FMD antibodies was conducted in Luang Prabang, Champassak and Savannakhet Provinces where a total of 1204 cattle and buffalo sera were collected from 58 villages in 13 districts. Results from the samples collected indicated that the dominant sero-type was O with a range of 16.4% in Luang Prabang to 23.4% in Champassak Province. Antibodies against sero-types A and Asia I were also detected but to a much lower level. From FMD suspected outbreaks, a total of twenty-six samples were submitted for FMD diagnosis between December 1997 and December 1998 of which ten where typed as O, three were typed as Asia I and thirteen were negative. The economic impact of FMD in Lao PDR is also discussed.

1. INTRODUCTION

In May 1997, a project sponsored by the Australian Center for International Agricultural Research (ACIAR) in collaboration with the Department of Livestock and Fisheries (DLF), Lao PDR commenced and focused on two major livestock diseases, foot-and-mouth Disease (FMD) and classical swine fever. The first objective of the project was to establish a laboratory facility in Vientiane for the diagnosis of the target diseases and to provide training to DLF staff. Prior to the commencement of the project, the FAO/IAEA Co-ordinated Research Project (CRP) on FMD in Southeast Asia had supplied reagents and equipment to facilitate the diagnosis of FMD outbreaks and serological surveys. In December 1997, the ACIAR project laboratory was commissioned, the reagents and equipment relocated and DLF staff trained in FMD ELISA techniques.

During 1997, a serological survey of FMD sero-prevalence was undertaken in Luang Prabang, Champassak and Savanakhet Provinces as part of FAO/TCP/6611 and the FAO/IAEA Research Contract awarded under the CRP to this laboratory. One thousand two hundred and four sera were collected from cattle and buffaloes from 58 villages. These samples were subsequently assayed at the laboratory and the results are presented in this paper. Additionally, results of routine detection and sero-typing from suspected-FMD outbreaks are also presented.

2. MATERIALS AND METHODS

2.1. ELISA technologies

The routine detection and sero-type identification of FMD was accomplished by the use the FMD antigen typing ELISA (AT-ELISA) which was supplied in kit form by the Institute for Animal Health (IAH), Pirbright. The methodology as prescribed by IAH was used for the AT-ELISA.

Serological determination of FMD sero-types was undertaken using the FMD liquid phase blocking ELISA (LPB-ELISA) for the detection of serum antibodies, which was also supplied in kit form by the IAH, Pirbright. The methodology as prescribed by IAH was used for the LPB-ELISA with the exception that sera were screened at a final 1:40 dilution and positive sera were titrated in the two-fold dilution series 1:40–1:320 to attempt to determine the end point.

2.2. FMD outbreak investigations

Provincial or district livestock officers submitted samples for FMD diagnosis and sero-typing from suspected FMD cases to the project laboratory. To enable the safe transit of epithelial specimens, samples were placed in a transport medium (50% glycerol + 50% PBS) that in turn was placed inside locally produced transport containers constructed from PVC water pipe. Samples were submitted to the laboratory via the local postal service or by bus.

2.3. FMD sero-prevalence studies

Three Provinces, Luang Prabang, Savanakhet and Champassak were chosen for FMD sero-prevalence studies. Fifty-eight villages from thirteen districts were included in the survey (see Table I and Fig. 1).

TABLE I. LOCATION AND NUMBER OF SAMPLES TAKEN DURING FMD SEROPREVALENCE SURVEY

Province	Districts	No. of Villages sampled	Total samples
Luang Prabang	Luang Prabang	5	100
	Pak-ou	5	102
	Xieng Ngeun	5	100
	Nambark	4	100
Savanakhet	Chantabuly	3	57
	Xaiphouthong	2	32
	Songkhone	5	102
	Champhone	5	100
	Outhaumphone	5	101
Champassak	Paksong	5	97
	Phaethong	5	115
	Sakhuma	3	100
	Bachiengcharoensouk	6	98
Total	13 Districts	58	1204

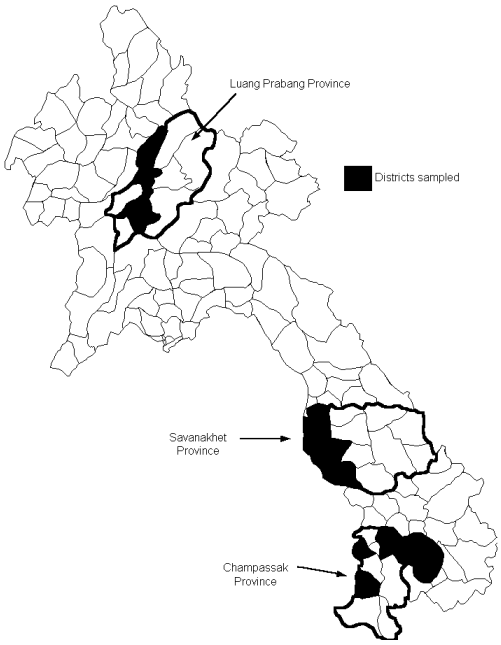

FIG. 1. Location of districts providing samples during FMD seroprevalence survey

During the visit to each village, an interview with the livestock holders was held usually in the village headman's house to collect livestock statistics thereby enabling a sampling frame to be constructed for randomization of animals to be sampled. The survey team aimed to sample at least 20 cattle or buffaloes from each village although, due to a shortfall in animals in some villages this was not always possible. 1204 sera were collected in total.

2.4. Economic impact of FMD

An estimate of the economic impact of FMD in village livestock production systems was calculated using anecdotal evidence based on discussions with experienced field staff within DLF.

3. RESULTS AND DISCUSSION

3.1. Implementation of FMD ELISA technologies

The implementation of the FMD AT-ELISA and FMD LPB-ELISA in the first instance was facilitated by ELISA kits supplied by IAH, Pirbright as part of the FAO/IAEA CRP on FMD in Southeast Asia in conjunction with FAO Technical Co-operation Project (TCP) RAS/6611. With the assistance of these two projects and the ACIAR project the FMD ELISAs were implemented within a two week period and found to perform reliably on initial and subsequent use. Staff training to ensure familiarization with ELISA concepts and specific test procedures was also undertaken over a two-month period. Quality control procedures as described by Blacksell et al [1] using the QCEL program was implemented to ensure confidence in the test results. In our experience we have found that the following is essential to good ELISA performance in our laboratory:

– good quality laboratory water (in our case we use a commercial deionisation unit)

– thorough washing of all glassware and plastic ware

– adequate refrigeration for the storage of reagents.

3.2. FMD outbreak investigations

FMD is endemic in Lao PDR with wide spread outbreaks reported since 1980. The level of FMD incidence in Lao PDR is influenced to a large extent by the demands of illegal international animal trade, as the country is an established thoroughfare to major livestock markets in neighbouring countries.

Samples from FMD suspected outbreaks in the Vientiane Municipality submitted to the DLF in 1996 prior to the establishment of the project laboratory were retrospectively sero-typed as type Asia I. Overall, a total of twenty-six samples were submitted for diagnosis from December 1997 to December 1998 of which ten where typed as O, three were typed as Asia I and thirteen were negative.

In 1998, FMD outbreaks were reported at the beginning of the year in Vangvieng district of Vientiane Province that was sero-typed as Asia I. In September, a larger FMD outbreak sero-typed as type O was reported in Samakysay district of Attapeu Province that subsequently spread to another four districts of the Province and Paksong district of the ajoining Champassak Province. It would appear, that the FMD outbreak spread from Attapau to Champassak following the movement of affected animals along Highway 10 connecting the two Provinces. The number of large animals in the Attapau/Champassak type O outbreak estimated to be affected by FMD was 5810 out of total population of 27 600 animals (21%). Epithelial samples were collected from acutely affected animals and sent to the FMD World Reference Laboratory, Pirbright, United Kingdom where detailed characterization of the samples is currently underway. The origin of the outbreak in Attapeu and Champassak Provinces has yet to be determined. A summary of positive sample details is presented in Table II and the geographical location of positive samples is presented in figure 2.

TABLE II. SUMMARY OF FMD POSITIVE SAMPLES SUBMITTED FROM DECEMBER 1997 TO DECEMBER 1998

Date	Province	District	Species	Sero-type
January 1998	Vientiane	Vangvieng	Cattle	Asia I
September 1998	Attapeu	Samakysai	Buffalo	O
October 1998	Attapeu	Saysettha	Buffalo	O
November 1998	Attapeu	Phouvong	Cattle	O
December 1998	Champassak	Paksong	Cattle	O
December 1998	Champassak	Khong	Buffalo	O

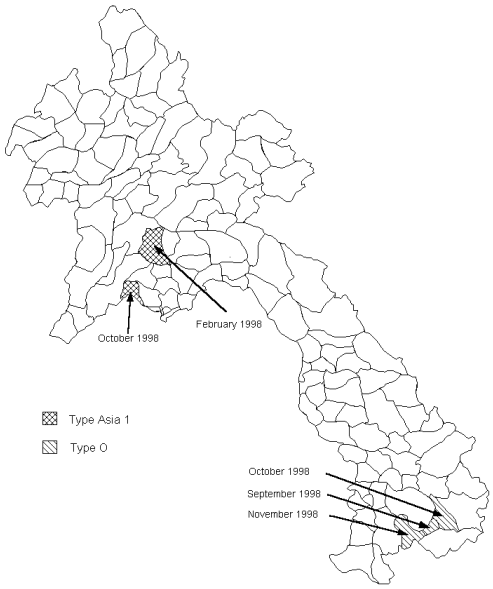

FIG. 2. Geographical location and time of FMD positive sample submission

3.3. FMD sero-prevalence studies

Results of FMD sero-prevalence studies are presented in Table III. It is evident, that the dominate sero-type antibodies are type O with a range of 16.4% in Luang Prabang to 23.4% in Champassak Province. Champassak was the only Province to show antibodies for type Asia I greater than 2% (12.6%). Type A results remained reasonably consistent across the provincial data at approximately 5% although a type A virus has never been detected from an FMD outbreak in Lao PDR.

Further FMD sero-prevalence studies are ongoing in other Provinces as part of the ACIAR project to further assess the level of FMD antibody prevalence in Lao PDR.

TABLE III. OVERALL RESULTS OF PERCENTAGE FMD SERO-PREVALENCE. RESULTS PRESENTED IN PARENTHESIS ARE THE 95% CONFIDENCE INTERVALS FOR EACH PERCENTAGE RESULT.

Province	Type O	Type A	Type Asia I	Indeterminate	Negative
Luang Prabang	16.4 (6.1–26.7)	5.7 (1.0–10.5)	0.2 (-0.2–0.7)	0.2	77.4
Savanakhet	20.4 (10.9–29.7)	5.4 (2.2–8.5)	1.5 (0.5–2.5)	1.0	71.7
Champassak	23.4 (12.2–34.8)	3.9 (1.8–6.0)	12.6 (4.7–20.5)	4.1	56.3

3.4. Economic impact of FMD

In recent times, FMD has mainly affected cattle and buffalo in Lao PDR. The affected animals lose condition quickly and become lame. In some cases this results in permanent disability due to secondary infections of the hoof. The primary impact of FMD outbreaks is on available ploughing and draught power resources, which can cause delays or an inability to plant rice crops. Smallholders resort to hiring of buffaloes from non-affected villages, which probably exacerbates the spread of disease. Constraints on the hiring of draught power are the limited financial means of most farmers and the limited availability of animals for hire. With the exception of major urban centres such as Vientiane, motorized ploughing machinery is generally unavailable to most of the affected villages. Therefore, a major impact of FMD in Lao PDR is on food security.

While it is difficult to accurately assess the true economic impact of FMD in Lao PDR, an attempt was made using available resources. Given that there are no official exports of livestock from Lao PDR to neighbouring countries and dairy farming is in its infancy, the main impact of FMD is on village agricultural production systems. It was estimated, that in the case of a draught animal being infected with FMD during peak production periods the cost of replacement draught power and treatment is US $50.00 per animal. More research in this area is required to increase the accuracy of this value.

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INTRODUCTION AND USE OF ELISA BASED TECHNOLOGIES FOR THE DIAGNOSIS AND MONITORING OF FOOT-AND-MOUTH DISEASE IN MALAYSIA

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Abstract

INTRODUCTION AND USE OF ELISA BASED TECHNOLOGIES FOR THE DIAGNOSIS AND MONITORING OF FOOT-AND-MOUTH DISEASE IN MALAYSIA

Continued outbreaks of foot-and-mouth disease (FMD) in northern Malaysia drove the decision to establish a diagnostic surveillance capability at the regional laboratory in Kota Bharu. Based on using ELISA based diagnostic systems the laboratory was equipped for the detection of both the conservative virus and a serological response in animals. Considerable detail was given on the subsequent testing that was carried out clearly demonstrating the value both of the ELISA technology but also of what can be achieved at reasonable costs for conducting routine surveillance of FMD.

1. INTRODUCTION

Malaysia has encountered sporadic outbreaks of foot-and-mouth disease (FMD) and a 'stamping out' strategy had been the method of choice since the first FMD outbreak in 1973. However, the policy had to be reviewed in the 1980's due to continued introduction of the disease from the neighbouring country of Thailand and in many instances the extensive nature of these outbreaks. Both factors contributed to making it impractical to continue the original stamping out policy. As of 1980 stamping out was only carried out when the number of animals involved was small and outbreak confined. Strategic vaccination and this 'modified stamping out ' method effectively controlled the disease until 1991. However, the disease scenario has continued to change and most importantly FMD has continued to persist in the Border States despite intensive efforts of control by the Department of Veterinary Services, Malaysia (DVS). As part of a renewed effort to resolve the problem the DVS felt a strong need to establish diagnostic and monitoring facilities in the Regional Veterinary Laboratory at Kota Bharu (RVLKB), Kelantan, in the hope that would assist in more effective control and potentially lead to eradication of FMD. This paper is a summary of the work that has been carried out under a Research Contract awarded under the FAO/IAEA Co-ordinated Research Project on 'The use of ELISA based technology in the diagnosis and monitoring of FMD'.

2. COST OF THE DISEASE

It has always been known that FMD has been a disease of high cost to the Malaysian livestock Industry, particularly the export market. It has been estimated that the cost is around RM 5 million annually in materials and logistics for control [1]. On the average about 300 000 doses of FMD vaccine are purchased annually costing about RM 1 million /year (Table I). The cost of vaccinating per cattle in the field ranges from RM 7.50 to RM 10.00 per dose, taking into consideration related cost such as staff salary. Malaysia exports about RM 500 million/annum worth of livestock and livestock products. Effective control of FMD in the border districts is crucial to maintaining this export trade by preventing any possible spread of the disease to the southern part of the peninsular. Illegal movements of cattle in 1995 spread the disease to the southern States of Negri Sembilan, Malacca and Johore, which resulted in considerable losses to export revenue. Therefore, effective control and eradication of FMD from peninsular Malaysia is vital for the continued development of the livestock industry in the country.

3. CO-ORDINATED RESEARCH PROJECT UNDER IAEA

The CRP "Introduction and use of ELISA based Technologies for the Diagnosis and Monitoring of FMD" officially commenced on 15 Sept. 1994. The first Research Co-ordination Meeting (RCM) of the FAO/IAEA was held on 17 February 1995 in Bangkok. The meeting discussed the specific diagnostic needs of the country, equipment, reagents, ELISA kits and manpower training. A Computer system with software to assist the ELISA kit and an ELISA reader was provided in July 1995 followed by FMD ELISA kit with reagents in August 1995. A special room was renovated in the RVLKB for this FMD work and additional equipment were purchased through special funds allocated by the DVS for this project. The Laboratory facilities for the ELISA test were completed by December 1995 followed by work on the ELISA test kit in 1996. The training programme sponsored by IAEA in

March 1996 at Ho Chih Minh City helped much to resolve many of the technical problems encountered with the kit.

TABLE I. FMD VACCINE PURCHASE & VACCINATION IN PENINSULAR MALAYSIA 1992–1998

Year	FMD Vaccine Purchase (Doses)	Cost of Purchase	No. of Vaccinations (Doses)
1992	260,700	–	139,993
1993	246,100	–	186,394
1994	258,300	–	322,822
1995	550,000	–	309,813
1996	450,000	RM1,075,050	249,217
1997	250,000	RM850,000	207,264
1998	100,00	RM370,000?	136,527

* FMD Vaccination only in Border States except in 1995 – Vaccination in outbreak areas of Southern States of Peninsular Malaysia. Cost of vaccine/dose for 1997 & 1998 was RM3.40 & RM3.70 respectively

A paper entitle “The Use of ELISA and PCR Technique in the Diagnosis of Foot-and-mouth Disease in Peninsular Malaysia” was presented at the 8th Scientific Congress of the Veterinary Association Malaysia in August 1996. This paper highlighted on the establishment of the FMD diagnostic Unit in Kota Bharu and the future role in the control of FMD in Peninsular Malaysia. The preliminary results of the Antigen and Antibody Detection ELISA Kits received under the FAO/IAEA Research Contract was presented at the congress.

The year 1997 was a landmark for the RVLKB as the ELISA kits performed well in terms of internal quality control values (IQC) with much help and advice from World Reference Laboratory for FMD (WRL). Virus typing (antigen detection) service for sero-types O, A, C and Asia I and antibody monitoring services for sero-types O, A and Asia I was provided by RVLKB. Two additional antibody detection kits at a cost of about RM 36 000 were purchased by DVS from WRL as the ELISA kit provided through the Research Contract by IAEA was used up by April 1997.

In August 1997, a national serological survey was carried out to establish immunity status to FMD in the Border States of Peninsular Malaysia. The findings of the survey were presented in the 9th Veterinary Scientific Congress in October 1997 held in Penang. The paper is attached in next chapter of this technical document.

In 1998, work on virus typing was minimal, as there was no new FMD outbreak after May. However, the antibody monitoring work continued. New antigen detection and antibody kit was received from FAO/IAEA towards the end of the year. Two additional antibody kits were purchased to cater for the monitoring work and a vaccine trial that is underway. It is also anticipated that there will be a serious budgetary constraint this year and this additional kits would be able to cater for some of the work this year.

4. TRAINING

To further strengthen our diagnostic capability, two of our laboratory staff, a veterinary officer and a technician underwent a four-month training at WRL in FMD diagnosis using ELISA and PCR techniques. The training was fully sponsored by the Malaysian Government. The laboratory personnel successfully completed the training and returned in February 1998 to continue their work in the FMD unit at the RVLKB.

Subsequent to this our laboratory staff also attended a workshop on ‘External Quality Assurance for Diagnostic ELISA’ in Bamako, Mali, in June 1998. This workshop was sponsored by the IAEA and gave special emphasis on establishment and implementation of a quality laboratory system in the

FMD diagnostic work. The preparation of a quality manual is underway and IQC charts are used on routine basis to evaluate validity of the ELISA test plates and also to identify any abnormal trends. This training was important, as the RVLKB is committed to obtain accreditation status through active participation in the External Quality Assurance Programme (EQAP) conducted by FAO/IAEA.

5. NEW FMD LABORATORY

The construction of a new FMD diagnostic block at a cost of RM 300 000 (Table II) started in August 1998 and is to be completed by April 1999. The block has 6 laboratory sized rooms with special design for ELISA and PCR work. Minimum security features had also been incorporated in the design and all the FMD diagnostic work will be carried out in this building.

6. EXTERNAL QUALITY ASSURANCE PROGRAMME (EQAP)

As required under the FAO/IAEA Research Contract, the RVLKB took part in two EQAP rounds conducted by the IAEA. The results of the 1st test samples in 1996 identified a problem in IQC values not being within the established limits of the antibody ELISA test due to many technical problems. The water quality posed some problems. The dilution of the control antigens had to be lowered to retain the IQC values within the lower and upper limits. Since August 1997, many of the technical problems were resolved and the antibody detection kit performed well with good IQC values. Again this was achieved by lowering the dilution rate of the control antigens. The 2nd EQAP results were submitted in January 1999 for the appraisal of the EQAP technical committee of IAEA.

7. FMD DIAGNOSTIC SERVICE AT RVLKB

7.1. Virus typing using antigen detection kit

A total of 142 epithelial tissue samples were received from six states in Peninsular Malaysia from November 1995 to December 1998 (Table III). The State of Kelantan contributed 79 (55.6%) samples as more active collection of samples was carried out by the RVLKB followed by the State of Perlis 32 (22.5%) samples, mainly from the quarantine station. Samples were also received from some suspected cases in the States of Pahang and Negri Sembilan, but no clinical outbreak was involved. Virus type O was detected in 33 cases followed by 26 cases of virus type A and 16 of Asia I. Virus typing (antigen detection) was successful in 52.8% of 142 tissue samples received. The unsuccessful detection was mainly due to poor quality specimens received at the laboratory. Tissue samples were also collected from some suspected cases with mucosal ulceration and salivation. Eleven cases from Pahang and Negeri Sembilan were of that nature and no clinical disease was involved. This lowered our diagnostic success, but nevertheless gave useful information and confidence in diagnosis to the field personnel involved in the disease control programme. Tissue samples were also sent to WRL for confirmatory diagnosis, whenever it was deemed necessary, especially at an outbreak in new area or at detection of a new virus strain. RVLKB earned a distinction when it correctly and promptly identified the virus type A in Pasir Puteh in August 1997, which was confirmed by WRL. Further sub-typing by WRL found the strain to be antigenically different from existing sub-types. In general, there was an increasing trend in the number of tissues samples tested. From 5 samples in 1995 the number increased to 83 samples in 1997, when there was wide spread outbreak in the Border States. However, the number was down to 27 samples in 1998 and no new cases of FMD were reported since May 1998 (Table IV).

7.2. Antibody detection and monitoring

A total of 6317 serum samples wer tested by antibody detection screening assay. The results of the testing are given in Table V. There was a marked increase in the number of samples tested. From about 1000 samples in 1995/96 it increased to 2749 in 1997 followed by 2269 samples in 1998. Forty-eight percent of the samples tested were from quarantine stations followed by the State Veterinary Departments. Random testing is carried out on imported cattle at quarantine stations to assess the immunity status and to monitor compliance of import protocol. Antibody titration assay was carried out on 1119 samples (Table VI) that warranted information on protective level of antibody in the cattle

tested. Eight hundred (71.5%) samples tested were from the national serological survey carried out in August 1997.

TABLE II. COSTING FOR THE ESTABLISHMENT OF FMD DIAGNOSTIC FACILITY IN THE REGIONAL VETERINARY LABORATORY, KOTA BHARU

Item	Cost of Item (RM)	Remark
1995/96		
1. Computer System with software to run ELISA kit		Funded by IAEA
2. ELISA Reader (Immunoskan Plus)		
3. FMD ELISA kit, Plats & Reagents		
4. Water distiller & Deionizer	3,500	
5. Orbital Shaker (Certomat)	2,150	
6. Balance (Sartorius)	4,300	
7. Incubator (Memmert)	3,720	
8. Fridge (Dewpoint)	2,800	
9. Deep Freezer ⁻85°C (Nuaire)	18,600	
10. Autoclave (Hirayama)	14,000	
11. Micropippetes	2,200	
12. Generator	19,000	
13. Cryo-preservation Vials/Racks	5,000	
14. Computer Printer (EPSON)	1,500	
15. Renovations/working – benches & Furniture	8,500	
1997		
1. Microplate Autowasher (BioTek Instruments)	16,000	
2. FMDV ELISA kit & Reagent	36,000	
1998		
1. 1 set Finnpipette (Labsystems)	3600	
2. Finntip (Labsystems)	3600	
3. Hop Plate (Stuart Scientific)	3,200	
4. FMDV ELISA kit & Reagent	39,000	
Total:	RM 186,670	

TABLE III. FMD VIRUS TYPING USING ANTIGEN DETECTION ELISA BY STATES IN PENINSULAR MALAYSIA (NOVEMBER 1995 – DECEMBER 1998)

State /Province	No. of Tissue Samples	Virus Type Detected				Positive Diagnosis	FMD Diagnosis Confirmation
	(Epithelium)	O	A	C	Asia I	(% detection)	by WRL
Kelantan	79	17	18	–	4	49.4%	12
Perlis/Padang Besar	32	11	8	–	–	59.4%	2
Terengganu	13	–	–	–	12	92.3%	9
Negeri Sembilan	8*	–	–	–	–	0%	–
Kedah	7	5	–	–	–	71.4%	–
Pahang	3*	–	–-	–	–	0%	–
Total:	142	33	26	–	16	52.8%	22

* Suspected cases – No outbreak involved

TABLE IV. VIRUS TYPING FOR FMD USING ANTIGEN DETECTION ELISA ACCORDING TO STATES IN PENINSULAR MALAYSIA AND TIME (NOVEMBER 1995 – DECEMBER 1998)

State	Location Sample	Tissue Sample	Time Month/yr	Result Positive to Virus Type				WRL Confirmation
				O	A	C	Asia I	
Kelantan	Bachok	4	11/95	–	–	–	4	6
		5	1/97				2	
			12/97		3			
		5	3/98		2			
	Gua Musang	4	1/96	2				2
	Pasir Putih	11	12/96	1				
			9/97	1			3	
			8/97		1			
			2/98					
	Kota Bharu	11	6/96	1				1
			3/97	2				
			11/97		4			2
			12/97		4			
	Pasir Mas	5						1O,1A
	Machang	9	9/97	2				
			5/97			2		2
	Tanah Merah	11	3/97	5				
			8/97				3	2
	Tumpat	8	11/97		2			
	Quarantin R.Panjang	5	1/97	5				2
Terengganu	Batu Rakit	4	3/96				4	2
	Manir	5	11/95				4	
			2/96				1	3
	Ulu Terengganu	4	2/96				3	2
Perlis	Padang Besar	33	8/96	1				
			10/96	1				
			3/97	4				
			3/98	6	2			
			9/97		4			
			10/97		2			2
			11/97					
Kedah	Jitra	5	9/96	4				
	Padang Terap	2	3/97	1				
Pahang	Kuantan	3						
N.Sembilan	Jelebu	1						
	Jempul	7						

TABLE V. ANTIBODY DETECTION FOR FMD BY ELISA SCREENING ASSAY (NOVEMBER 1995 – DECEMBER 1998)

Submitter of Serum Samples	Serum Tested	% of Samples Positive			Remarks
		Type O	Type A	Asia I	
Quarantine Station, Rantau Panjang	2791	92.0	90.0	88.1	Vaccinated (Importation)(PI)
SVD Kedah	969	87.8	93.1	87.9	Vaccinated
RVL Kota Bharu	596	75.0	76.3	73.7	Vaccinated
RVL Bukit Tengah	528	72.3	60.9	65.7	
SVD Kelantan	502	61.0	66.7	62.0	Monitoring
Quarantine Station, Padang Besar, Perlis	267	93.6	95.5	94.8	Vaccinated (Importation) (PI)
SVD Terengganu	241	96.6	100.0	94.5	Vaccinated
Veterinary Research Institute, Ipoh	149	91.3	91.9	88.6	Recovered
RVL Alor Setar	114	44.7	63.4	35.1	Suspected Case
SVD Perlis	77	45.5	37.7	44.2	–
RVL Kuantan	46	97.8	97.8	95.7	Vaccinated
RVL Petaling Jaya	37	81.1	100.0	78.4	Vaccinated
Total:	6317	84.5	84.0	81.8	

RVL – Regional Veterinary Laboratory
SVD – State Veterinary Department

TABLE VI. ANTIBODY DETECTION FOR FMD BY ELISA: TITRATION ASSAY (JANUARY 1996 – DECEMBER, 1998)

Submitter of Serum Samples	No. of Serum SamplesTested	% Of Samples Positive			Remarks
		Type O	Type A	Type Asia I	
RVL, Kota Bharu	628	42.7	40.8	54.0	
RVL, Petaling Jaya	340	48.8	22.9	43.8	
Quarantine Station Rantau Panjang	90	81.1	70.0	75.5	Vaccinated (Importation)
RVL, Bukit Tengah	36	44.4	–	80.6	
SVD, Kelantan	25	76.0	68.0	60.0	
Total:	1119	48.4	38.2	53.6	

RVL – Regional Veterinary Laboratory
SVD – State Veterinary Department

8. CONSTRAINS/LIMITATIONS

Some level of technical difficulty was encountered in managing the internal quality control of the ELISA test. There was a marked decline in the concentration/strength of antigens provided for the ELISA kits. The dilution of the control antigen has to be decreased drastically to meet the internal quality control specifications. As such, this resulted in a much fewer number of samples that could be tested especially for the antibody ELISA kit. The high cost of the ELISA kits again forbids much wider usage in the antibody monitoring of vaccination programmes. With the current economical crisis in this region, funding to continue this FMD work is encountering serious constraints. It is imperative that a continued support in providing the FMD ELISA kits is needed to sustain good work in the region.

9. CONCLUSION

The FAO/IAEA Research Contract has been instrumental in the establishment of the FMD diagnostic facility in Peninsular Malaysia. The FMD diagnostic capability of the RVLKB has progressed remarkably well in the last few years. The commitment and support of the DVS in this project had been phenomenal. Special funds of substantial amount had been channelled for training, equipment and infrastructure establishment. The highlight of this is the construction of a special FMD block for this work. The laboratory has been engaged actively in the FMD control programme in the country. Prompt diagnosis in virus typing and antibody monitoring services has given much support to the field operations of the control programme. With the continued support of the FAO/IAEA and WRL, it is envisaged that the RVLKB would play a crucial role in the control of FMD in the country and the region as a whole.

ACKNOWLEDGEMENT

On behalf of the Department of Veterinary Services, Malaysia and RVLKB, we wish to extend our sincere appreciation and thanks to the Joint FAO/IAEA Division, International Atomic Energy Agency (IAEA) for their financial assistance and technical support in this Project and the World Reference Laboratory for FMD (WRL) for their expertise and guidelines on the ELISA kits, including training for our staff. We specially thank the Director General of DVS for his support in this work.

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IMMUNITY STATUS OF FOOT-AND-MOUTH DISEASE IN THE BORDER DISTRICTS OF PENINSULAR MALAYSIA

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Abstract

IMMUNITY STATUS OF FOOT-AND-MOUTH DISEASE IN THE BORDER DISTRICTS OF PENINSULAR MALAYSIA

A serological survey for the prevalence of protective level of antibody to Foot-and-mouth disease (FMD) was carried out in 10 border districts in Peninsular Malaysia. A liquid phase blocking ELISA kit prepared and standardized by World Reference Laboratory (WRL) for FMD was used for the testing. A total of 800 serum samples collected by a random process were tested for protective level of antibody for virus types O, A and Asia I. An overall mean prevalence for antibody to FMD in the 'immune-belt' region was found to be 51.0%, 37.3%, 53.6% for virus types Q, A, and Asia I respectively and 28.9% for all the three sero-types. The percentage of cattle population having protective level of antibody was too low to prevent active spread of FMD infection. There was also substantial variation in the prevalence of antibody detected at the district level and varied from a low mean of 18.8% for the State of Kedah and a high of 67.5% for the district of Besut. More than 70% of the population need to have protective level of antibody to effectively prevent disease spread. The States of Kedah and Kelantan had variable levels of vaccination coverage from 1994 and had less than 45% coverage for the year 1996. A coverage of more than 90% would be essential to maintain high herd immunity and the current high variability in the vaccination coverage at the district level will only favour a higher infection on rate in the field.

1. INTRODUCTION

Peninsular Malaysia has faced many outbreaks of foot-and-mouth disease (FMD) since 1973 [1]. These outbreaks have been effectively controlled through vaccination and a modified stamping out method. However, the scenario has changed since 1992 whereby sporadic outbreaks continue to emerge in the border districts. Many reasons have been attributed to this problem including limited success in movement control at the border region and low herd immunity in the 'buffer zone'. Low vaccination coverage has been incriminated as the main reason for this low herd immunity. Mass vaccination is used as a key strategy of control in the Border States of Kelantan, Terengganu, Perlis, Kedah and Perak. Annual vaccination of cattle and buffaloes using a trivalent vaccine is mandatory in these States. However, the vaccination coverage varied widely in these States with the exception of the State of Terengganu. Higher coverage of up to 80% is usually achieved subsequent to an outbreak. Though vaccination has been deployed as a key strategy of control in the 'immune-belt region' the assessment of immunity status in animals in these areas has not been possible (no antibody monitoring capability) and has not been carried out so far. An epidemiological investigation to evaluate the immunity status in this region would be vital for an effective control and eradication programme. The immunity status of the animals in these strategic border districts would reveal valuable information as to the cause of the persistent infection in this region. This could also outline the future strategy of control and eradication.

The objective of this study is to:

– Assess the immunity status to FMD in the high-risk border districts of Peninsular Malaysia.
– Determine correlation between vaccination coverage and antibody prevalence.

This paper was presented at the Nineth Veterinary Association Malalysia Scientific Congress, 3–5 October 1997, Penang, Malaysia

2. MATERIALS AND METHODS

2.1. Sampling Procedures

Serum samples were collected from cattle in the border and FMD high-risk districts in the States of Kelantan (4), Terengganu (1), Perlis (1), and Kedah (4). Ten districts were picked for this survey based on a high risk of having FMD and being in a strategic location. About 80 sera per district were determined using computer software EPINFO, based on a population size of about 20 000 at 95% confidence interval and 10% accuracy and using an estimated antibody prevalence of 30%. Longitude/latitude gridlines were drawn on the district maps at an interval of one minute (2 km in distance) and each square was numbered. Eight squares from each district were selected at random lot and the exact position of these squares was determined by using a Global Positioning System (GPS) — GARMIN GPS II. Serum samples were collected from cattle within one kilometer radius from the position located by the GPS system. The serum samples were tested using ELISA kits provided by the Joint FAO/IAEA Division, which have been prepared and standardized by FMD World Reference Laboratory (WRL) at Pirbright, United Kingdom. The antibody detection kit is a liquid phase blocking ELISA technique for the detection of FMD virus antibodies in serum as described in the literature [2,3]. The assay was used as a single dilution screening assay and as a quantitative titration assay, resulting in an end-point titre determination for each serum. The kit detected antibody for FMD virus types O, A and Asia I. The assay protocols followed are as described by FAO/IAEA Bench Protocol June 1995 and June 1997.

2.2. Assay interpretation

The diagnostic threshold for the screening assay was set at 50% percentage inhibition (50 PI) at serum dilution of 1:32. Serum samples positive to the test (PI $\geq$ 50) were further tested by titration assay to assess 'protective level' of antibody. The serum samples were tested at two fold dilutions of 1:64 and 1:128 to determine protective level of antibody. An antibody titre of $\geq$ 90 is considered protective, which indicates that the animal, at the time of bleeding, was protected against infection from homologous antigen of the particular FMD virus sero-type. Antibody prevalence for all three sero-types was calculated based on the presence of protective level of antibody in all three sero-types.

A spreadsheet was used to tabulate the results and plot the charts. The vaccination coverage from August 1996 to July 1997 of each sub-district where samples were collected was analysed for correlation with immunity status. Correlation analysis was done using a statistical program SPSS.

3. RESULTS AND DISCUSSION

Table I shows the prevalence of cattle with protective level of antibody for FMD. The State of Kelantan had a mean antibody prevalence of 49.7%, 32.8%, 59.7% for virus types O, A and Asia I respectively and 25.9% for all three sero-types. Kedah had a mean antibody level of 44.1%, 30.3%, 40.9% for virus types O, A, Asia I and 18.8% for all sero-types. The State of Perlis had 63.8%, 47.5%, 56.3% for virus types O, A, Asia I respectively and 42.5% for all sero-types. The district of Besut had a level of 71.3%, 72.5%, 77.5% for virus types O, A, Asia I, respectively and 67.5% for all sero-types. The overall mean prevalence of protective level of antibody for the districts in the immune belt region was found to be 51.0%, 37.3%, 53.6% for virus types O, A, Asia I, respectively and 28.9% for all sero-types.

The antibody prevalence for virus types O, A and Asia I varied widely in this survey. The trivalent vaccine used in the national FMD control programme had been purchased from a reputable supplier and induced good immune response to all three sero-types (Palanisamy – unpublished data). Antibody monitoring programme of imported cattle and buffalo at quarantine stations also conforms to this finding (Palanisamy – unpublished data). Immunity due to natural infection could be the main cause for the wide variation between the sero-types. The incidence of FMD outbreak due to virus type A had been rare, restricted to Selama Perak and Kedah in 1995 and 1996. As such, percentage of animals detected with antibody for virus type A and antibody for all three sero-types together is more likely to be of vaccine origin rather than from natural infection in the field. Generally, higher antibody prevalence has been observed for sero-types Asia I and O compared to type A. The districts of Pasir

Puteh, and Bachok, in the State of Kelantan had much higher prevalence for Asia I due to widespread infection with this virus type in 1995 and 1996. The districts of Kubang pasu, Kota Setar and Padang Terap however, had much higher prevalence of antibody for virus type O. This could be due to the protracted outbreak with virus type O since 1995 and 1996.

TABLE I. CATTLE WITH PROTECTIVE LEVEL OF ANTIBODY TO FMD IN THE BORDER DISTRICTS OF PENINSULAR MALAYSIA – AUGUST 1997

	Sample Size	Type O %	Type A %	Type Asia I %	All Sero-type %
KELANTAN					
TUMPAT	80	56.3%	28.8%	50.0%	22.5%
PASIR MAS	80	37.5%	31.3%	47.5%	18.8%
BACHOK	80	46.3%	36.3%	67.5%	33.8%
PASIR PUTEH	80	58.8%	35.0%	73.8%	28.8%
Mean for State		49.7%	32.8%	59.7%	25.9%
TERENGGANU					
BESUT	80	71.3%	72.5%	77.5%	67.5%
PERLIS					
PERLIS	80	63.8%	47.5%	56.3%	42.5%
KEDAH					
KUBANG PASU	80	53.8%	30.0%	45.0%	13.8%
KOTA SETAR	80	31.3%	11.3%	20.0%	10.0%
PADANG TERAP	80	65.0%	43.8%	52.5%	35.0%
PENDANG	80	26.3%	36.3%	46.3%	16.3%
Mean for State		44.1%	30.3%	40.9%	18.8%
Overall Mean		51.0%	37.3%	53.6%	28.9%

There is substantial variation in the prevalence of antibody to FMD in the Districts surveyed and varied from a low mean of 18.8% for the State of Kedah and a high of 67.5% for Besut, Terengganu. The overall mean for the districts in the 'buffer zone' was found to be only 28.9%. Too low a level to prevent an active spread of the disease in the cattle population as substantial numbers of susceptible animals are maintained for infection to persist. Gleeson et al, (1993) [4] have Stated that in order to prevent an epidemic 70% of the population need to be protected, but to absolutely prevent an outbreak on a herd basis 95% protection is required [5].

Poor vaccination coverage is the main factor for the low protective level of antibody in the border districts. This is evident from Table II. The district of Besut in Terengganu has had maintained a high coverage of more than 90% for the last 3 years, hence a good protective level of antibody of >70% for the three homologous sero-types. However, there is substantial variation in the coverage for the districts in the States of Kelantan and Kedah. Coverage of less than 45% for 1996 for the above States warrants a serious look at the problem. Work in Thailand indicates that vaccinating 70% of the village cattle and buffaloes twice a year is unlikely to produce a level of herd immunity sufficient to

prevent spread of FMD virus. Reasons for this low level of immunity includes poor response to initial vaccination, decline in titres between vaccination and increase in susceptible population through birth and this requires coverage of nearly 100% to be effective [6]. Vaccination coverage of >80% is crucial for an effective control of the disease.

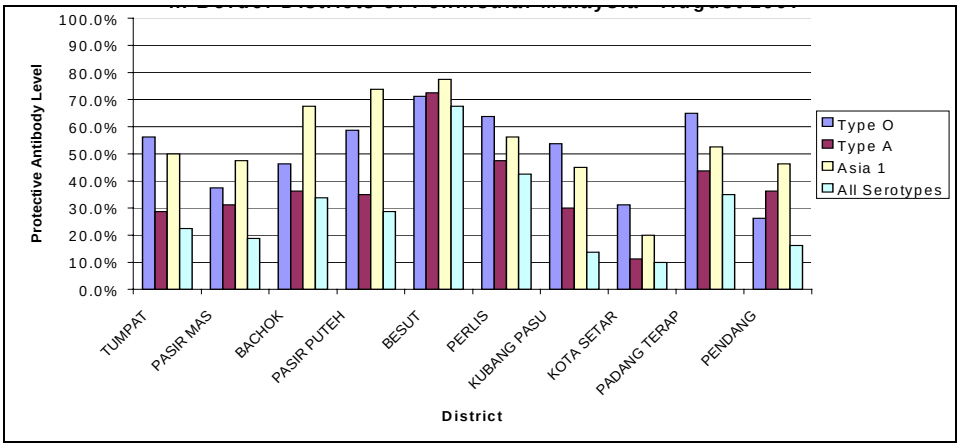

FIG. 1. Cattle with protective level of antibody to different virus types of FMD in border districts of peninsular Malaysia – August 1997

The reported vaccination figures (Table II) do not truly reflect the immune status of the population, as the percentage of animals truly protected would be much lower. The protection is dependent on the potency of the vaccine (the correct strain and optimum antigenic content), maintenance of 'cold-chain' and vaccine delivery to the animal. The current vaccination coverage is too low to prevent any active spread of the disease in the Border States.

TABLE II. FMD VACCINATION COVERAGE (%) IN STRATEGIC DISTRICTS OF PENINSULAR MALAYSIA 1992–1996

	Years				
State	1992	1993	1994	1995	1996
PERLIS	94.3	71.9	86.2	30.5	92.0
KEDAH					
K.Setar	–	–	61.5	2.1	52.6
Pendang	–	–	71.6	17.6	23.4
K.Pasu	–	–	84.5	18.7	27.9
P.Terap	–	–	–	14.0	32.2
Mean	–	–	72.5	13.1	34.0
TERENGGANU					
Besut	72.9	86.9	94.7	90.3	97.6
KELANTAN					
Tumpat	24.8	–	80.1	79.2	52.1
P.Mas	40.5	–	47.0	–	37.5
K.Bharu	33.5	–	33.8	68.7	26.1
Bachok	27.9	–	24.4	79.5	50.4
P.Puteh	40.5	–	75.7	27.4	41.1
Mean	33.4	–	52.2	51.0	41.4

There was poor correlation between vaccination coverage at sub-district and antibody prevalence for the three sero-types which was found to be 0.1216, 0.1553 and 0.1396 for sero-types O, A and Asia I respectively. This could have been influenced by faulty animal census figures at sub-District level. This is reflected by the poor correlation coefficient and many outliers with way-off values of more than 100% for the coverage. However, a good correlation of 0.7813 and 0.6101 was found for vaccination coverage (Fig. 2) at district level with that of antibody prevalence for all three sero-types and type A respectively. This supports the view of high vaccination coverage resulting in high prevalence for protective level of antibody in the cattle population.

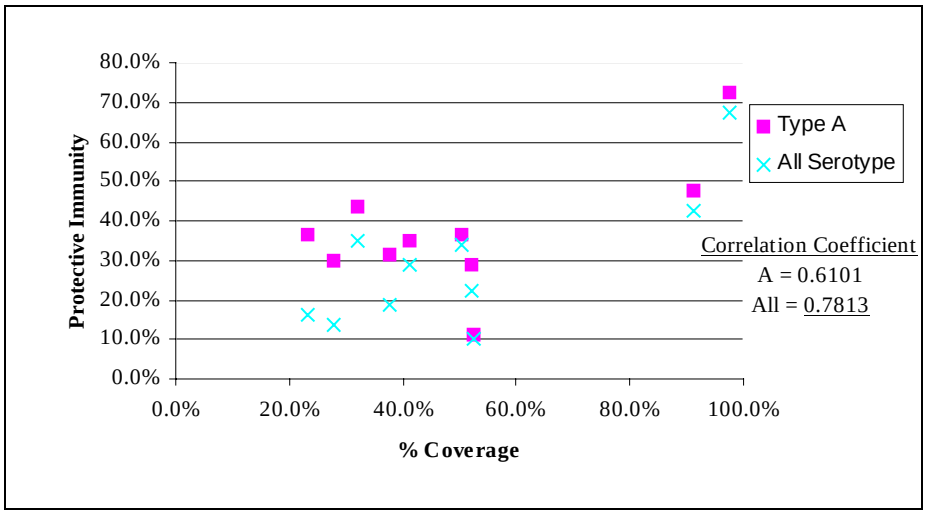

FIG. 2. Correlation plot of vaccination coverage (%) and antibody level

FMD detection in about 8 consignments of animals at the checkpoints and quarantine stations in 1996 reflects the constant threat of disease introduction into the country. Numerous illegal movement routes and increased smuggling activity during festive seasons have contributed to many outbreaks. As such, FMD control and eradication strategy have to focus on mass vaccination to maintain a high herd immunity of more than 80% to prevent future outbreaks. This could be achieved by maintaining a vaccination coverage of >90% in all the districts with minimal variability in the coverage at the village level.

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PREVALENCE OF ANTIBODY TO FOOT-AND-MOUTH DISEASE IN CATTLE AND BUFFALO IN MYANMAR

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Abstract

PREVALENCE OF ANTIBODY TO FOOT-AND-MOUTH DISEASE IN CATTLE AND BUFFALO IN MYANMAR

A serological survey for the prevalence of antibody to foot-and-mouth disease (FMD) was performed in six Divisions and three States in Myanmar. A liquid phase blocking ELISA prepared and standardized by World Reference Laboratory (WRL) for FMD was used for this study. A total of 831 serum samples from cattle and buffalo were collected by a random process and assayed for antibody against FMD virus types O, A, C and Asia I. Positive reactions to FMD virus O, A, C, and Asia I sero-types were detected. Even in the free zone area, (Ngape township) and the buffer zone (Minbu township) serum samples showed positive reactions. Ten percent of the sera tested showed positive reactions to all sero-types within the free zone and buffer zone. The majority of cattle and buffaloes, except those in the FMD free and buffer zones, were not vaccinated against FMD. The percentage of positive sera in each State and Divisions varied from 16 to 90 for at least one sero-type. More epithelial specimens from FMD outbreaks should be submitted for investigation and further nation-wide serological surveys for FMD should be carried out if a national policy for FMD control and eradication is to be effective and enforceable.

1. INTRODUCTION

Myanmar is primarily an agricultural country and is undertaking measures not only to produce more food for domestic consumption but also for export. Measures undertaken to enhance surplus of production include expansion of cropping areas, optimum utilization of land, increasing per acre yield and improving the quality of crops harvested. Plans have been adopted to put 16 million acres under monsoon and summer paddy with increased paddy output in the near future.

The economy of Myanmar depends mainly on agriculture with 65% of the total working population engaged in this sector. Livestock form an integral part of rural economy and 85% of cultivation is carried out by animal draft power. Livestock ownership is distributed mainly among the smallholder farmers.

In 1998/99, cattle and buffalo population were recorded as 10.49 million and 2.33 million respectively. It is estimated that 55% of the total population of cattle and buffalo is used for draft power. Thus, draft animals are given high priority and are a major socio-economic component in the national agricultural scene. Clearly the income from animal production is an important factor in rural areas.

According to the reports and epidemiological data, in Myanmar four important infectious diseases occur in cattle and buffalo. These are foot-and-mouth disease (FMD), Haemorrhagic Septicaemia (HS), Black leg and Anthrax. Among these, foot-and-mouth disease is the only major economic disease in Myanmar that has never been brought under systematic control.

FMD has been known to occur in Myanmar since 1887. FMD is considered to be the most significant epizootic virus condition of livestock in Myanmar. The disease is highly prevalent in valuable livestock species especially cattle, buffalo and pigs. About 55% of the buffalo and cattle provide 85% of draft power in Myanmar. The incidence of FMD fluctuates throughout the year. The highest incidence of disease usually occurs during the onset of the monsoon season (May to August) and again at the end of the year (October to December). The first episode in any one year is usually observed as progressive outbreaks of FMD involving large numbers of animals through a series of villages. FMD outbreaks appear to coincide with crop plantation and harvesting. Hence the FMD virus is thought to be spread through the use of draught cattle and buffalo and/or the increase in trade of animals just prior to the monsoon season. The disease is often considered to be mild, of short duration, and of low mortality. However, importantly, a large number of animals are infected in any one outbreak. It is therefore difficult to estimate the total annual economic losses to Myanmar. It should be noted that FMD is the only major economic disease in Myanmar that has never been brought under systematic control. Among the seven States and seven Divisions in Myanmar, FMD outbreaks are

common in those areas where there is a large concentration of livestock, as found in the low-lying plains from the central part of Myanmar to the Delta coast. These regions include Yangon, Ayeyarwady, Bago, Magway, Mandalay, Sagaing Divisions and Mon State. An epidemiological investigation to evaluate the immune status of animals in these regions would be an essential precursor to an effective control and eradication programme for FMD. The immune status of the animals in this State and these Divisions would not only reveal valuable information as to the distribution of this disease in Myanmar but would form the basis for a FMD control and eradication strategy.

1.1. Background for FMD virus typing and serology in Myanmar

Field staff of the Livestock Breeding and Veterinary Department routinely reacts promptly to FMD outbreaks and visit the area to assist in the control of the disease and its spread to surrounding areas. Notification, isolation, zoo-sanitary measures, and animal movement control are carried out in co-operation with the local authorities. Specimens from diseased animals were collected and submitted to the FMD laboratory, Yangon, for the identification of sero-types. Serum samples from convalescent animals were also sent for serological examination.

Plan for surveillance of FMD and investigation of sero-types was commenced in 1977 through support provided under an FAO/UNDP Co-operation Project. This Project was first and foremost for FMD control in Myanmar. However, its longer term objectives were to promote the standard of disease control measures for FMD and hence to increase the healthy stock contributory towards the development on livestock products.

The short-term objectives of the project focused on creating an ability to perform virus typing by serological techniques and to study the types of FMD virus in the country. It lasted three years starting from 1977/78 to 1979/80. The serological method used in differentiation of FMD virus was complement fixation test (CFT). According to the results obtained, three sero-types O, A and Asia I viruses were identified. This was carried out using specific anti-serum supplied by the Animal Virus Research Institute, Pirbright, UK and Plum Island Laboratory, USA. This was the first identification of FMD virus type A in the country.

1.2. Establishment of an ELISA unit for FMD

In June 1995, Mr. N.P Ferris of the World Reference Laboratory (WRL), UK, visited Mynamar as an IAEA expert supported under a IAEA Technical Co-operation Project (MYA/5/007). He provided practical training in the ELISA both for FMD antigen and antibody detection. Following this further FMD virus typing and serology was carried out both under this Technical Co-operation Project and the FAO/IAEA Research Contract (8046/RB). ELISA kits for both FMD antigen and antibody detection as well as ELISA equipment including an ELISA reader linked to a computer system were supplied. As a result, the traditional method CFT was substituted with the more effective ELISA technology.

The objectives of the studies reported in this paper were to determine the distribution and prevalence of antibody to FMD virus in cattle and buffalo in Myanmar and to advise on a national policy for the control and eradication of FMD in Myanmar.

2. MATERIALS AND METHODS

2.1. Sampling frame for survey

A total of 816 serum samples from cattle and buffaloes were collected in 1997 from densely populated and FMD endemic areas in the State of Mon (10), in the Divisions of Bago (12), Ayeyarwady (2), Sagaing (5), Magway (13), Mandalay (14), and Yangon (1). Nineteen townships were chosen for this survey, based on previous knowledge of high risk areas for FMD. Fifty-eight samples were collected in 1998 from Shan State (7) and Rakhine State (3). Serum samples were collected by the of the FMD laboratory, Livestock Breeding and Veterinary Department (LBVD), Ministry of Livestock and Fisheries (MLF), except those from Shan and Rakhine States. The serum samples were collected randomly from 2 to 5 townships in each State or Divisions.

2.2. Sample collection and submission

Blood samples were collected by vein puncture using 10 ml non-pyogenic disposable syringes disposable needles (0.80 x 38 mm 21G) and dispensed into sterile collection bottles. The decanted serum samples were collected in sterile Wheaton serum bottles. Each serum sample was kept in small amounts using 2 ml cryovials and stored at -20ºC. The serum samples from Rakhine and Shan States were provided by the Diagnostic Laboratory, LBVD, Insein, Yangon, Myanmar.

2.3. Antibody detection

Eight hundred and thirty-one serum samples were tested for antibody to FMD sero-types O, A, C and Asia I. Liquid phase blocking ELISA kits provided through the FAO/IAEA Research Contract under the Co-ordinated Research Project prepared and standardized by the WRL were used to assay all samples for FMD virus antibody [1–4]. Sera were assayed at a single dilution of 1:32 using the ELISA as a rapid, qualitative screening method. The assay procedures followed were those described in the IAEA/WRL Bench Protocol of July 1997 and September 1997. Sera were considered positive if one or more wells at the 1:32 dilution showed more than 50% inhibition as compared to the antigen control. The prevalence of animals sero-positive for each sero-type was calculated for each State and Division.

The total number of samples tested between July 1997 and December 1998 is shown in Table I with the overall serological results shown in Table II.

TABLE I. FMD VIRUS TYPING BY ELISA IN MYANMAR (JULY 1997 TO DECEMBER 1998)

SR. NO.	Description	Number of samples	No virus detected	FMDV Sero-types				Remark
				O	A	C	AsiaI.	
1	Field outbreak samples	125	43	79	–	–	3	
2	Seed virus samples	89	2	36	–	–	51	
3	EQAP samples	6	4	–	1	1	–	
	Total	220	49	115	1	1	54	

TABLE II. FMD ANTIBODY DETECTION BY ELISA SCREENING TEST IN MYANMAR (JULY 1997 TO DECEMBER 1998)

Description	Serum samples from cattle and buffalo	Serum samples from goats
Total number of samples	118	225
Number of samples negative	244	151
Samples positive FMD sero-type(s)		
Sub-type O	151	23
Sub-type A	77	6
Sub-type C	8	-
Sub-type Asia I	42	6
Sub-types O and A	119	–
Sub-types O and C	18	–
Sub-types O and Asia I	74	15
Sub-types A and C	11	–
Sub-types A and Asia I	24	–
Sub-types C and Asia I	3	–
Sub-types A, C and Asia I	11	–
Sub-types O, A and C	53	–
Sub-types O, A and Asia I	95	1
Sub-types O, C and Asia I	22	8
Sub-types O,A, C and Asia I	234	15

2.4. Antigen Detection

FMD antigen detection is based on a standard indirect sandwich ELISA technology to determine the presence of FMD virus antigens in specimens from FMD outbreaks in Myanmar and FMD viral antigens used in formulation of locally produced FMD monovalent vaccine (seed virus). Kits were provided through the Research Contract from the WRL and were able to detect FMD sero-types O, A, C and Asia I. The bench protocol issued by WRL was strictly followed to ensure a standard level of assay performance.

3. RESULTS

The States and Divisions sampled and tested are densely populated and report many cases of suspected FMD each year. Small numbers of cattle and buffalo, excluding those in the FMD buffer zone, are vaccinated with locally produced FMD monovalent vaccine from the FMD laboratory, Yangon (FMD sub-types O and Asia I). Table III shows the numbers of cattle and buffalo vaccinated in these States and Divisions. Table IV shows the percentage of sera collected from the townships in the selected States and Divisions. Table V shows the percentage of serum collected for assay from each State or Division. Table VI shows the prevalence of antibody to FMD virus sero-types O, A, C and Asia I. Antibodies against FMD virus sero-type O, were the most prevalent in all States or Divisions examined except in Rakhine where sero-type A was the most prevalent. The prevalence of antibodies to sero-type C was similar to that of sero-type O in Rakhine.

TABLE III. FMD VACCINATION COVERAGE IN MYANMAR (1996–1998)

	Number of cattle and buffaloes vaccinated	
State /Division	1996/97	1997/98
Bago	1 300	550
Magway	29 131	35 489
Rakhine	4 496	5 222
Yangon	–	2 050
Total	34 927	43 311

FMD monovalent vaccines for sero-type 0 & Asia I

TABLE IV. PERCENTAGE OF SERUM SAMPLE COLLECTION IN SELECTED TOWNSHIPS IN MYANMAR

Township	Cattle and buffalo	Sample size	% of collection
Mudon	36 179	50	0.138
Kyaikmayaw	60 704	30	0.049
Mawlamyine	10 673	27	0.252
Pyu	72 776	80	0.109
Pyay	45 202	49	0.108
Mawlamyinegyunn	79 025	60	0.075
Yegyi	48 099.	48	0.099
Sagaing	90 839	24	0.026
Tabayin	64 552	22	0.034
Ye-U	52 027	20	0.038
Monywa	50 516	20	0.039
Chaung-0o	39 229	18	0.045
Ninbu	60 656	60	0.098
Ngape	20 765	64	0.308
Pyin-Oo-Lwin	28 536	77	0.26P
Meiktila	111 008	47	0.042
Hmawbi	47 350	30	0.063
Hlegu	43 819	53	0.120
Kyauktan	80 490	37	0.045
Mungdaw	91 063	5	0.005
Buthidaung	47 955	19	0.039
Mu-se	32 403	17	0.052
Lashio	49 640	17	0.034
Total	1 263 506	874	0.069

TABLE V. PERCENTAGE OF SERUM SAMPLE COLLECTION IN STATES AND DIVISIONS IN MYANMAR

State/Divisions	Cattle & buffalos	Sample size	% of collection
Mon	371 810	107	0.028
Bago	1 311 106	129	0.009
Ayeyarwady	1 309 802	108	0.008
Sagaing	2 041,898	104	0.005
Magway	1 523 820	124	0.008
Mandalay	1 623 348	124	0.007
Yangon	528 213	120	0.022
Rakhine	806 395	24	0.003
Shan	1 473 392	34	0.002
Total	10 989 784	874	0.008

TABLE VI. CATTLE WITH ANITBODY POSITIVE TO FMD IN DENSELY ANIMAL POPULATED STATE AND DIVISIONS OF MYANMAR (1997–1998)

	Sample size %	Type O %	Type A %	Type C %	Type Asia I %	All sero-types %
Mon State						
Mudon	47	72.34	55.31	44.68	53.19	25.53
Kyaikmayaw	41	65.85	68.29	51.21	51.21	29.26
Mawlamyine	15	73.33	73.33	13.33	53.33	13.33
Mean for State		70.50	65.64	36.40	52.57	22.70
Bago Division						
PYU	76	93.42	90.78	57.89	68.42	50.00
Pyay	44	84.09	59.09	27.27	27.27	15.90
Mean for Division		88.75	74.93	42.58	47.84	32.95
Ayeyarwady Division						
Mawlamyinegyunn	40	35.00	2.50	45.00	-	-
Yegyi	40	35.00	70.00	27.50	35.00	7.50
Mean for Division		35.00	36.25	36.25	17.50	3.75
Sagaing Division						
Sagaing	24	66.66	79.16	33.33	54.16	29.16
Tabayin	22	63.63	77.27	40.90	54.54	36.36
Ye-U	20	80.00	60.00	70.00	75.00	55.00
Monywa	20	60.00	40.00	40.00	35.00	25.00
Chaung-Oo	16	93.75	62.50	68.75	62.50	50.00
Mean for Division		72.80	63.78	50.59	56.24	39.10
Magway Division						
Minbu	77	59.74	49.35	32.46	35.06	9.09
Ngape	47	74.46	42.55	14.89	57.44	10.63
Mean for Division		67.10	45.95	23.67	46.25	9.86
Mandalay Division						
Pyin-Oo-Lwin	60	36.66	46.66	16.66	45.00	10.00
Meiktila	64	50.00	42.18	15.62	54.68	12.5
Mean for Division		43.33	44.42	16.14	49.84	11.25
Yangon Division						
Hmawbi	30	70.00	23.33	13.33	6.66	3.33
Hlegu	53	60.37	30.18	9.43	32.07	5.66
Kyauktan	37	81.08	78.37	24.32	27.02	13.51
Mean for Division		70.48	43.96	15.69	21.91	7.50
Rakhine State						
Maungdaw	5	60.00	80.00	40.00	20.00	20.00
Buthidaung	19	89.47	100.00	94.73	78.94	73.68
Mean for State		74.73	90.00	67.36	49.47	46.84
Shan State						
Mu-se	17	52.94	82.35	41.17	64.70	29.41
Lashio	17	58.82	88.23	52.94	64.70	47.05
Mean for State		55.88	85.29	47.05	64.70	38.23
Overall Mean		65.93	60.93	38.06	45.82	24.86

Generally, antibodies to sero-type A were more prevalent than those to sero-type Asia I, the latter being a virus sero-type, which is currently present in Myanmar. Antibodies to sero-type C were less common in all Regions. In order to clarify these results, a selection of 80 sera from Ayeyarwady, Bago, Mandalay, Magway, Mon, Shan, Sagaing and Yangon Regions was examined at the WRL by titration in the ELISA and the virus neutralization test. Furthermore, antibody to the non-structural (NS) protein 3ABC was measured as an indicator of previous infection.

Table VII summarizes the results by Region, the results generated to date in terms of serological evidence for infection with each of the sero-types examined. In most Regions there was evidence of infection with both types O and Asia I. In Yangon and Ayeyarwady Divisions, the results suggested that only type O was circulating. More sera from each Region need to be examined by titration before definitive conclusions can be drawn regarding the likely sero-types of virus present in each Region.

TABLE VII. PRELIMINARY SEROLOGICAL EVIDENCE OF INFECTION WITH FMD VIRUSES IN MYANMAR

Division/State	Sero-type of FMDV implicated				Comment
Mon	Y	N	N	Y	Uncertain whether or not type 0 infection present
Bago	?	N	N	Y	
Ayeryarwady	Y	N	N	N	
Sagaing	Y	N	N	Y	
Magway	Y	N	N	Y	
Mandalay	Y	N	N	Y	
Yangon	Y	N	N	N	
Ralchine	nd	nd	nd	nd	
Shan	?	?	N	N	Single animal positive against type A (NS antibody negative)

Virus typing results, from specimens submitted by local veterinary staff of LBVD show that only FMD virus sero-type O and Asia I have been identified in Myanmar since 1981. Before that time, virus sero-types O, A and Asia I, were also present. A total of 125 specimens from FMD outbreaks, 89 for seed virus and 6 EQAP samples were tested by using ELISA technique for the project period (from July 1997 to December 1998). Among the field outbreak specimens, 79 samples were positive for virus type O, and only 3 for virus type Asia I during the past 18 months. The others have shown negative results for FMD virus sero-types (Table VIII).

TABLE VIII. FMD VIRUS TYPING BY ELISA IN MYANMAR (JULY 1997 TO DECEMBER 1998)

Description	No. of samples	No. virus detected	FMD virus sero-types			
			O	A	C	Asia I
Field outbreak samples	125	43	79	–	–	3
Seed virus samples	89	2	36	–	–	51
EQAP samples	6	4	–	1	1	–
Total	220	49	115	1	1	54

4. DISCUSSION

The number of animals vaccinated (cattle and buffalo) from 1996 to 1998 is shown in Table III. It is obvious from a comparison with the total cattle and buffalo population of each region (Table IV) that only a very small proportion of the susceptible stock is potentially protected by vaccination and even this in a limited number of regions.

In the Minbu township (buffer zone) where cattle and buffalo had been vaccinated against sero-type O and Asia I, antibodies to all the virus sero-types assayed were prevalent and rates of sero-prevalence to type O, the sero-type used in the vaccine, were no higher in these townships than in

other townships where vaccination was not carried out. Ngape township, the FMD free zone, also recorded a high prevalence of antibody to FMD virus sero-type O and Asia I.

Undoubtedly the high prevalence of antibody to FMDV sero-types O and Asia I is due to the annual occurrence of these viruses in Myanmar. It is possible that, because very few clinical samples from suspect cases of FMD virus are submitted for identification. FMD virus sero-type A is still present in Myanmar albeit at a low level. The presence of antibodies to sero-type C is confusing and requires further investigation.

This survey has clearly shown that FMD virus sero-types O and Asia I are circulating widely in many Regions (States and Divisions) of Myanmar. The situation with type A and C is less clear and will be the subject of a subsequent survey. When surveying for evidence of infection, it is clear that screening at a dilution of 1:32 provides little information on the sero-types of virus responsible for sero-positivity due to the high degree of cross reactivity between sero-types at this low serum dilution. Only by titration can more specific information on the sero-type involved be derived. In future surveys, sera will be examined at a higher dilution (1:100) and will be titrated against any virus for which they are positive. This will increase the specificity of the testing procedure and will have little negative effect on the sensitivity as nearly all infected animals have titres considerably greater than 1:100 against the sero-type involved.

Whilst sero-surveillance is undoubtedly useful, primary diagnosis of FMD should always be confirmed through either detection of FMD antigens by ELISA or preferably isolation of the virus responsible. Great efforts, therefore, need to be made to increase the number and geographical range of samples collected in Myanmar for FMD antigen detection.

Further work to be carried out includes:

- titration of positive sera to determine the significance of the antibodies to each sero-type
- monitoring of vaccination programmes (pre and post vaccination sera investigation including titration)
- studies of age distribution relative to immune status in each State/ Division.

Effective control and the possible eradication of FMD from Myanmar will require the co-ordinated support of local owners, veterinary services, the Governments of Myanmar and bordering countries and International Organizations. The following approaches and strategies are considered key to success:

- the vaccination of at least 70% of the susceptible animals [5]
- the enforced obligatory reporting of FMD
- the control on animal movement and disinfecting of personnel and vehicles on and off infected premises
- the complete ring vaccination around infected premises
- the continued nation-wide sero-surveillance for FMD
- the prevention of unauthorised movement of animals in and out of the country
- the adequate monitoring of vaccination (safety, efficacy, inocuity, quality assurance)
- the submission of samples for virus typing wherever an FMD outbreak occurs
- the provision of sufficient vaccine for all sero-types present in Myanmar.

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THE USE OF ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) FOR THE DIAGNOSIS AND MONITORING OF FOOT-AND-MOUTH DISEASE IN THE PHILIPPINES

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Abstract

THE USE OF ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) FOR THE DIAGNOSIS AND MONITORING OF FOOD-AND-MOUTH DISEASE IN THE PHILIPPINES

The establishment and use of the indirect sandwich ELISA for the detection of foot-and-mouth disease (FMD) virus antigen sero-types O, A and C and the liquid phase blocking ELISA (LPB-ELISA) for antibody levels against similar FMD sero-types has been adopted for routine diagnosis at the FMD diagnostic laboratory, PAHC. A total of 552 epithelial samples and 4401 serum samples were tested starting 1995 to 1998. Out of 552, 84 (17.9%) were found negative and 468 (84.78%) diagnosed as positive for sero-types O and C (42% of the total positives). Within 4 years, 62 representative samples were sent to WRL for FMD for confirmation diagnosis. From 62 samples sent 54 (87%) were diagnosed as positive and 8 (12.9%) were negative. Serum samples received were either for diagnosis (71 samples), surveillance (3002 serum), post vaccination titre (1303 serum) and for the FAO/IAEA external quality assurance programme (25 samples) by the FAO/IAEA Co-ordinated Research Project on FMD. The assay has been a useful tool in the fast diagnosis and confirmation of FMD suspect cases and in the measurement of antibodies against FMDV in serum samples from all animals either vaccinated or infected. In the future, the assay will be use for potency testing of imported vaccines and for monitoring and surveillance purposes to show freedom from disease for the support documentation from OIE.

1. INTRODUCTION

Foot-and-mouth disease (FMD) has seven sero-types namely O, A, C, SAT 1, 2, 3 and Asia I. Each sero-type is clinically indistinguishable from the other and cannot be differentiated clinically from other vesicular diseases (vesicular exanthema, vesicular stomatitis and swine vesicular disease) [1]. Laboratory confirmation is therefore necessary and requires a highly sensitive and specific test, to distinguish it from other vesicular diseases and be able to identify the specific sero-type of FMD virus (FMDV).

In the Philippines only three sero-types, namely O, A and C, have been diagnosed, since the first reported case in June 1902. Sero-type O was first confirmed in 1959 followed by sero-type A in 1975 and sero-type C in 1976. From then on, the 3 sero-types have appeared alternately from one outbreak to another. From 1990 to 1993 sporadic cases due to sero-type C were reported. In August 1994, a massive outbreak due to sero-type O started with the island of Luzon mainly affected. Based on sequencing data from the World Reference Laboratory (WRL) for FMD, Pirbright, UK sero-type O from 1994 up to the present have 18 percentage nucleotide difference from the previous O type isolates (prior to 1994) but were found to be genetically similar to O1 Hongkong. This would indicate that the present type O is a recent introduction.

With the Government FMD Control and Eradication Programme now in place, and for proper implementation of the programme, the FMD National Task Force has categorised the country into four different zones based on the current disease situation. The first zone comprises 15 Provinces within five regions and is the FMD Control Zone (endemic area). Second is the FMD-Free Buffer Zone with the southern part of region 4 and the entire region 5. Activities within this area involve no vaccination plus active disease monitoring and surveillance by serology. Third is the FMD-Free Zone, which comprises the entire Visayas and Mindanao. Strict quarantine and surveillance by serology is implemented in this zone. The area has been free from the disease for more than 10 years now except Leyte in Visayas, which had an isolated case in 1996. The fourth zone is the FMD-Free Protected Zone, which comprises three regions namely region 1 except the Province of Pangasinan, region 2 and CAR except the Province of Benguet (Fig. 1).

The FMD laboratory supports the FMD National Task Force with diagnosis and research. In order for the laboratory to be more effective and reliable, the Bureau of Animal Industry (BAI) in 1995, signed a Research Contract with the FAO/IAEA on the use of ELISA technology for FMD diagnosis. This paper describes the use, adaptation and evaluation of the assay in order to assess its

performance and to ensure that the system is sufficiently robust for use as the standard diagnostic assay for FMD in the Philippines.

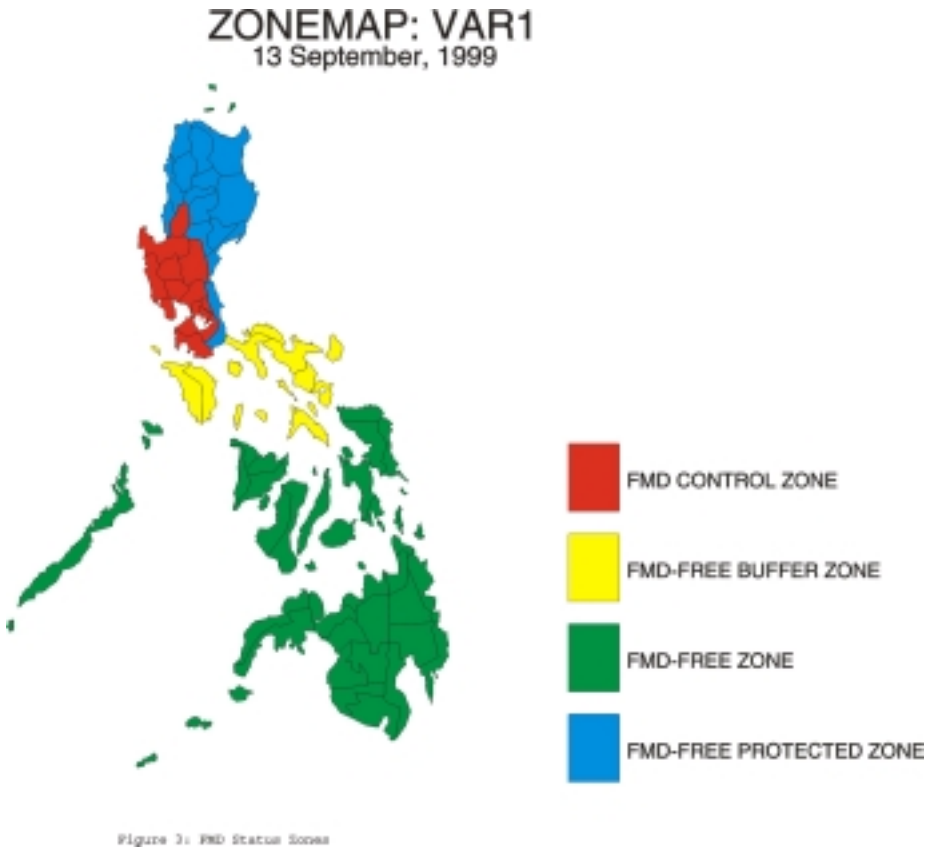


FIG. 1. Zonemap

2. MATERIALS AND METHODS

2.1. Vesicular epithelium and fluid samples

All samples were from field cases and tested by making a 10% suspension (vesicular epithelium) or as undiluted vesicular fluid. Suspensions or fluids were tested for the presence of FMDV antigen sero-types O1 Manisa, A24 Cruzeiro and C3 Resende using the indirect sandwich ELISA as described by Roeder and Le Blanc Smith in 1987 [2], Ferris and Dawson in 1988 [3].

2.2. Blood serum samples

Blood samples were allowed to clot and the serum tested according to N.Ferris [4]. A total of 4401 serum samples were tested, 25 of which were for the FAO/IAEA External Quality Assurance Programme (EQAP), 71 for diagnosis, 1303 for post vaccination titre and 3003 for surveillance purposes.

2.3. Assay reagents

Reference antigen, antisera (rabbit and guinea pig sources), 21 days post vaccinal sera, conjugate and substrate were provided by the World Reference Laboratory (WRL) for FMD, Institute for Animal Health, Pirbright Laboratory, UK.

2.4. Indirect sandwich ELISA for FMDV antigen detection

All samples from FMD suspect animals (vesicular epithelium or fluid) were tested using the Indirect Sandwich ELISA as described by Roeder et al [2] for the presence of FMDV antigen sero-types O1 Manisa, A24 Cruzeiro, C3 Resende and occasionally Asia I. The test is interpreted by the

colour development which is measured and interpreted with respect to the antigen content of the test sample [5].

2.5. Liquid Phase Blocking ELISA for detection of antibodies of FMDV

Blood serum samples coming from previously infected and non infected animals were tested for the presence of antibody against FMDV sero-types O1 Manisa, A24 Cruzeiro and C3 Resende. The liquid phase blocking ELISA (LPB-ELISA) was performed as described by Hamblin et al [6-8]. The test is interpreted by percentage inhibition or the reduction of colour development on test samples as compared to the controls containing the antigen only.

2.6. ELISA Data Interchange (EDI)

EDI is an FAO/IAEA computer program, which automates the reading and calculation of the test results of the LPB-ELISA used for FMDV antibody detection. The software was provided by the Systems Development Section of the International Atomic Energy Agency (IAEA), Vienna, Austria.

3. RESULTS

3.1. Antigen capture ELISA

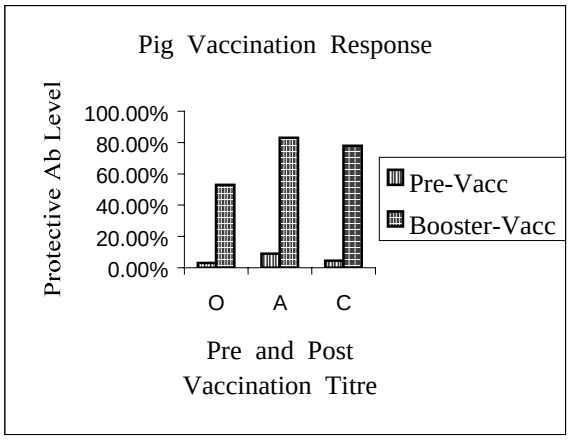
Results of samples tested by the laboratory and WRL for the presence of FMDV antigen sero-types O, A and C are shown in Table I. In 1995, out of the 161 positive samples diagnosed 17 samples (10.5%) were sent to WRL for confirmation of results. Virus were isolated from only 13 samples but all 14 were positive for FMDV antigen sero-type O. Representative samples sent to WRL for FMD for the 1996, 1997 and 1998 were 9.4%, 15% and 16.6% respectively of the total samples received. All were positive for FMDV antigen sero-type O. A total of 45 samples were sent to WRL from 1996 to 1998 and were all positive for FMDV antigen sero-type O, with an 8.8% negative for FMDV isolation. Positive samples from buffaloes represent 1.9% and cattle 0.6% of the total positive cases.

TABLE I. ANTIGEN CAPTURE ELISA RESULTS FROM THE FMD LABORATORY, PAHC (PHILIPPINES) AND WRL 1995–1998

Samples Tested by FMD Laboratory, Philippines																
	1995				1996				1997				1998			
FMD sero-type	O	A	C	-ve	O	A	C	-ve	O	A	C	-ve	O	A	C	-ve
Pigs	156	0	0	27	72	0	0	5	124	0	0	25	104	0	0	13
Cattle	0	0	0	3	0	0	0	2	0	0	0	2	1	0	0	0
Buffaloes	3	0	0	4	0	0	0	2	3	0	0	0	3	0	0	0
Sheep/goats	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Samples Tested by World Reference Laboratory for FMD, UK																
Pigs	14	0	0	3	6	0	0	1	17	0	0	0	16	0	0	0
Cattle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Buffaloes	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1
Sheep/goats	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

3.2. Antibody detection ELISA

One of the major uses of this assay has been to assess vaccine efficacy. Figure 2 shows an example of the serological response to vaccination as measured by ELISA on carabaos using two different types of vaccine and on pigs using one type of vaccine. Pre-vaccination protective antibody levels in pigs against FMD type O recorded by ELISA at 2.98%, 8.9% for type A and 4.47% for type C. These antibody titres were considered maternal antibodies as all experimental animals came from vaccinated sows. One month following primary vaccination, animals were sampled and the protective antibody levels increased significantly to 53% for type O, 83% for type A and 78% for type C.



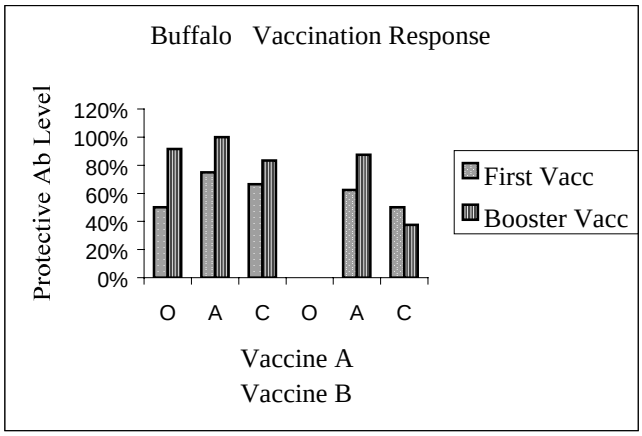


FIG. 2. Pig and buffalo antibody response after primary and secondary vaccination against FMD

Protective antibody levels presented in percentage were recorded by ELISA in carabaos sera following collection six months after the primary vaccination and one month after secondary vaccination. Two different types of vaccines were used. Based on recorded antibody titres, between the 2 groups of experimental animals, levels of protective antibodies vary significantly among the three sero-types of FMDV. The levels of protective antibodies did not change significantly after secondary vaccination. Similar experiments were also conducted at different locations and different groups of animals. Results based on ELISA did not differ significantly.

Table II shows the results of a comparative study of herd immunity between the three different commercial vaccines used by the FMD control programme. Results showed that commercial brand F gives statistically significant lower levels of protection compared to commercial brand E and G. This study was conducted on pigs in 13 different farms from 12 areas.

TABLE II. COMPARATIVE STUDY BETWEEN THREE VACCINE BRANDS ON THE ASSESSMENT OF HERD IMMUNITY IN PIGS

Vaccine brand	Sample size	FMD Antibody type O	FMD Antibody type A	FMD Antibody type C
E	351	121 (34%*)	125 (36%)	187 (53%)
F	289	55 (19%)	73 (25%)	75 (26%)
G	39	12 (31%)	14 (36%)	17 (44%)

* percent protected

Tables III and IV show the results on the vaccine trials conducted in cattle and carabaos using both oil and aqueous vaccines. A further study was undertaken on the protective responses using different needle sizes to demonstrate to field personnel the significance of using the correct needle size in order to achieve the desired immunity level when animals are vaccinated (Table V).

TABLE III. VACCINE TRIALS IN CATTLE USING OIL AND AQUEOUS VACCINES

Vaccine	Pre trial			Post Vaccination			Post Booster 1		
	O	A	C	O	A	C	O	A	C
Oil	60%*	30%	40%	100%	90%	90%	100%	100%	100%
Aqueous	75%	50%	75%	100%	100%	100%	100%	100%	100%

*antibodies detected against FMDV types

TABLE IV. VACCINE TRIAL IN CARABAO USING OIL AND AQUEOUS VACCINES

Vaccine	Pre trial			Post Vac			Post Booster 1			Post Booster 2			Post Booster 3			Post Booster 4			Post Booster 5		
	O	A	C	O	A	C	O	A	C	O	A	C	O	A	C	O	A	C	O	A	C
Oil	50%	77%	59%	85%	100%	85%	100%	100%	100%	85%	100%	100%	85%	100%	100%	62%	92%	77%	62%	100%	92%
Aqueous	0%	47%	43%	0%	86%	43%	57%	86%	86%	43%	86%	43%	29%	100%	43%	0%	29%	29%	14%	71%	0%

TABLE V. PROTECTIVE RESPONSES USING DIFFERENT NEEDLE SIZES.

Length	Pre Vac			Post Vac			PB1			PB2		
	O	A	C	O	A	C	O	A	C	O	A	C
3/4 inch	0.00	18.50	0.00	48.14	62.90	62.90	85.18	85.18	85.18	87.50	75.0	87.5
1 inch	3.50	28.57	3.50	46.42	64.28	71.43	89.20	89.28	89.80	77.77	55.5	77.77
1.5 inches	0.00	13.60	0.00	54.54	86.36	90.90	100.0	95.40	100.0	100.0	100.0	100.0

4. DISCUSSION

FMD diagnosis in the Philippines started in 1975 with the use of the conventional Complement Fixation test (CFT) and the Mouse Inoculation test (MIT). Laboratory tests were then confined to the antigen detection of FMD sero-types O, A & C.

Serological tests were carried out for the Philippines by the WRL for FMD but these were very costly for the Government and seldom done. In 1991, the FMD laboratory made a study on the comparison between CFT and ELISA for FMD diagnosis. Results showed that ELISA was far more sensitive and specific than CFT confirming the claims of previous authors who had made similar studies several years earlier.

It was only in 1995 when the Philippine Animal Health Center (PAHC), Bureau of Animal Industry (BAI) signed a collaborative research contract with FAO/IAEA on the use of ELISA technology for FMD diagnosis, that the assay became routinely used for both diagnosis and serology for FMD.

During the past 5 years, the laboratory has tested 552 samples for virus antigen detection and 4401 serum samples for serology. Serology testing has been carried out for routine diagnosis, for sero-monitoring and for sero-surveillance. Sera have also been tested as part of the FAO/IAEA EQAP. For confirmatory diagnosis, serological results (based on the use of the ELISA for antibody detection) may not give an unequivocal answer, but such data has been proven useful in cases where good quality epithelial or fluid sample are not available for testing because of a delay in reporting of outbreaks. Serology is also particularly useful in assessing the effectiveness of the vaccination programmes.

It is of course very important to routine quality assure the assay and Figure 2 shows one example of the internal quality control charts that are routinely plotted to ensure that the assay is performed within the limits set by IAEA.

In conclusion, the introduction and use of the ELISA for FMD monitoring, surveillance and for evaluating the effectiveness of FMD vaccination programmes has proved extremely valuable and a robust system. It is concluded, that this assay should now be the standard for FMD diagnosis in the Philippines. To ensure that the assay is performing within acceptable limits it is recommended, that the laboratory performs its own routine fully documented internal quality control (particularly with regard to serology) and that it continues to participate in the FAO/IAEA EQAP at least once in a year.

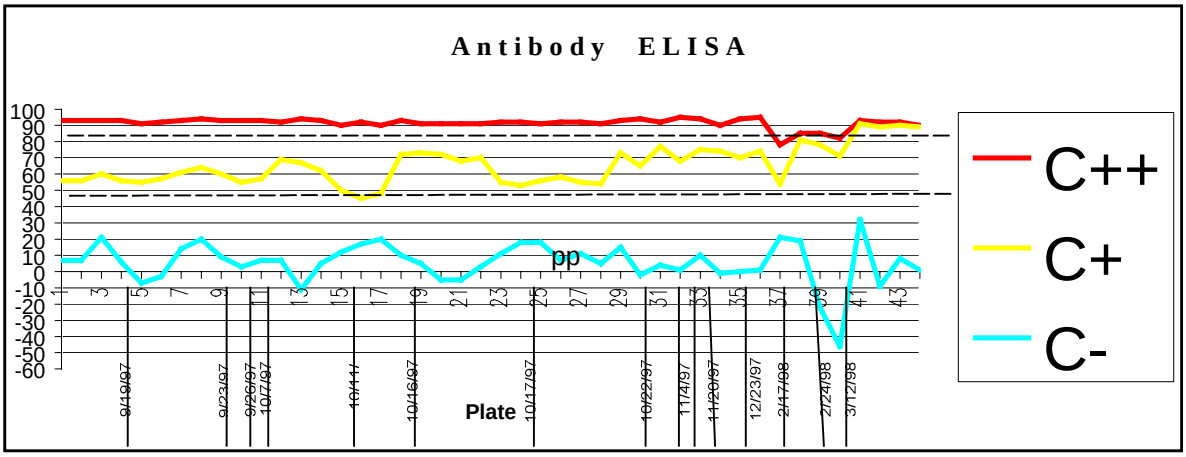


FIG. 3. Example of the FMD laboratory internal quality control of LPB-ELISA performance

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ANTIGENIC RELATIONSHIP OF FOOT-AND-MOUTH DISEASE VIRUSES FIELD OUTBREAK IN THAILAND

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Abstract

ANTIGENIC RELATIONSHIP OF FOOT-AND-MOUTH DISEASE VIRUSES FIELD OUTBREAK IN THAILAND

The antibody titre against FMD type O, A and Asia I was measured in 125 serum samples submitted to the laboratory. The antibody titre was estimated by a duplicate well two-fold dilution series using the liquid phase (LPB) ELISA systems from the World Reference Laboratory (WRL), Pirbright, United Kingdom (supplied by FAO/IAEA) and Pakchong, FMD Center, Thailand. The titres expressed as log to base compared by linear regression. The linear regression equation coefficient (R) between the antibody titre from IAEA and Pakchong systems were R=0.80 for type O, R=0.73 for type A and R=0.80 for type Asia I respectively. In addition the antigenic relationship to the current vaccine strains of type O, A and Asia I FMD field viruses isolated during 1994–1998 was investigated by duplicate well two-fold dilution series LPB ELISA method using Pakchong reagents. The serological relationship (r-value) of 111 field isolate viruses, type O = 74 samples, type A = 2 samples and type Asia I = 35 samples have been studied and described. Most of the field isolates type O showed r-value greater 0.40 = 97.30%, r-value range 0.2–0.39 = 2.70%, and r-value <0.19 = 0 %, indicating that vaccine strain O/Udornthani/87 should be protected the field virus strains as well as the r- value of type Asia I field isolates were greater 0.40 = 97.14% and r-value range 0.20 – 0.39 = 2.86 which indicated that the recent vaccine strains Asia I/Petchburi/85 could protect against all field strains. While the r-value of type A field isolate virus in 1997 showed the antigenic diverge from A/Nakornpatom/87 recent vaccine strain, r-value = 0.125.

1. INTRODUCTION

Foot-and-mouth disease (FMD), a highly contagious disease of cloven-hoofed animals, is an important disease of livestock in Thailand. Of the 7 sero-types, three (sero-types O, A and Asia I) are considered endemic in Thailand. Serological tests such as virus neutralization (VN) test and the liquid phase blocking ELISA (LPB ELISA) have been used to study the antigenic relationships of FMD field isolate viruses to vaccine strains [1,2]. Antigenic variation of FMD occurs very often under field conditions. Therefore, it is important to investigate the appearance and spread of variant viruses in the field and to select new vaccine strains, when necessary, to maintain the effectiveness of the FMD control programme. In addition, these assays have been used to monitor the effectiveness of the immunoprophylaxis programmes by measurement of antibody titres in field vaccinated animals.

In Thailand endemic FMD in domestic animals causes economic loss because of lower production and trade restrictions. It is estimated the economic loss is over 10 000 million Baht a year in export of animals. The Department of Livestock Development (DLD) has invested over 650 million Baht to increase annual vaccine production from 33 million to 40 million doses of trivalent vaccine (types O, A, Asia I) to provide 80% herd immunity in the animal population over the country. DLD has established the grant project on National FMD eradication programme to strengthen the vaccination campaign and to restrict animal movement in order to control and eradicate FMD from Thailand and neighbouring countries.

The FMD Center of the DLD takes responsibility for producing FMD trivalent vaccines mentioned above and also producing the diagnostic reagents for FMD diagnosis such as rabbit trapping antibodies, guinea pig detecting antibodies for ELISA test and VIA antigen for AGID test. These reagents are supplied to Regional Veterinary Research and Diagnostic Centers over the country. Therefore, as the part of the IAEA Co-ordinated Research Project (CRP), antibody titres against FMDV were measured by LPB-ELISA using the IAEA reagents kit and Pakchong reagents. The results were studied and compared to the IAEA test in order to establish the local diagnostic assay to an international standard level.

The objectives of this investigation were:

– To compare the 2 assay systems of IAEA reagent kit and Pakchong reagent used in liquid phase blocking ELISA (LPB-ELISA) for antibodies measurement.

– To study antigenic relationship of FMD type O, A and Asia I field isolate viruses in Thailand during 1994–1998 for epidemiological studies of distribution and antigenic character of field viruses.

2. MATERIAL AND METHODS

2.1. Serum samples

Serum samples from cattle vaccinated with DLD trivalent FMD vaccine were sent from field veterinarians for antibody measurement to monitor vaccine responses. One hundred and twenty-five sera were tested by duplicate well two-fold dilution series LPB-ELISA method as described by Hamblin et al [3] using the IAEA ELISA reagent kit supplied from WRL Pirbright Laboratory, United Kingdom, and the Pakchong reagents produced at FMD Center Pakchong, Nakhonratchasima, Thailand.

2.2. Viruses and field isolate samples

Reference viruses type O, A and Asia I were obtained from current vaccine seed strains O/Udornthani/87 (O/UDN/87), A/Nakornpathom/87 (A/NPT/87) and Asia I/Petchaburi/85 (As1/PBR/85). The field viruses were isolated in primary lamb kidney cell or the BHK-21 cell line. Isolates were usually passaged twice in the cell line of isolation, then a further 4 or 5 times in BHK-21 cells. The sero-type of the cell culture supernatant fluids was again confirmed by antigen ELISA before using in the LPB-ELISA.

2.3. Bovine antisera

The reference sera used in LPB-ELISA, bovine anti-O/UDN/87, A/NPT /87 and Asia I/PBR/85 were prepared in experimental cattle vaccinated and then challenged at 21 days post vaccination with homologous virus. Animals were bled at 7 days post challenge for immune sera.

Rabbit and guinea pig antisera were produced by intramuscular injection with purified 146S antigen of type O, A and Asia I mixed with complete Freund adjuvant. Rabbits were boosted with antigen in incomplete Freund adjuvant at 28 days after the first injection. The serum was collected 10 days after the booster injection. For guinea pig, sera were produced by single intramuscular injection of purified 146S antigen mixed with complete Freund adjuvant. Sera were collected at 28 days after injection.

2.4. Liquid phase blocking ELISA (LPB ELISA)

The LPB-ELISA for measurement of antibodies to FMD was performed by duplicate well two-fold dilution series method as described by Hamblin et al [4] with the modification that the substrate used was tetramethylbenzidine (TMB) rather than orthophenyldiamine (OPD) in the Pakchong system. The 125 serum samples submitted from field were tested in both assay systems. Each sample was tested at least twice and the titre used for the regression analysis was the mean of the estimates. The antibody titres from both ELISA systems were compared by the linear regression equation (R).

2.5. The serological relationship (r-value)

The reference virus and field isolate viruses were titrated by indirect sandwich ELISA method as described by Hamblin et al [5] and Kitching et al [6]. A fixed concentration of reference and field isolate viruses giving an optical density (OD) in the range of 1.0 to 1.5 were reacted with bovine post vaccination and challenged serum with homologous virus, O/UDN/87, A/NPT/87 and As1/PBR/85 respectively. The serological relationships (r-value) [7] were calculated as this ratio:

$$\text{r-value} = \frac{\text{serum titre against heterologous field strain}}{\text{serum titre against homologous vaccine strain}}$$

TABLE I. ELISA REAGENTS USED IN ANTIBODIES TEST RECEIVED FROM IAEA AND PAKCHONG.

Reagent	IAEA system	Pakchong system
Trapping antibody	Rabbit α O1 Manisa Rabbit α A22 Iraq Rabbit α Asia I Israel	Rabbit α O vaccine strain Rabbit α A22 vaccine strain Rabbit α Asia I vaccine strain
Detecting antibody	Guinea pig α O1 Manisa Guinea pig α A22 Iraq Guinea pig α Asia I Israel	Guinea pig α O vaccine strain Guinea pig α A22 vaccine strain Guinea pig α A22 vaccine strain
Antigen	O1 Manisa (inactivated) A22 Iraq (inactivated) Asia I Israel (inactivated)	O vaccine strain A22 vaccine strain Asia I vaccine strain
Conjugate	Rabbit α GP IgG-HRP	Rabbit α GP IgG-HRP
Substrate	OPD	TMB
Diluent buffer	PBST+5% skimmed milk powder	PBST+10% NBS+5%NRS
OD reading	Wavelength 492 nm	Wavelength 450 nm

The guideline suggestion for r-value obtained by LPB-ELISA and the criteria of interpretation have been proposed by Samuel et al [8] and Doughty et al [9] as this following:

R = 0 – 0.19 highly significant serological variation from the reference strain.
R = 0.2 – 0.39 significant difference from the reference strain, but protection may be satisfactory if using a sufficiently potent vaccine.
R = 0.4 – 1.0 not significantly different from vaccine strain.

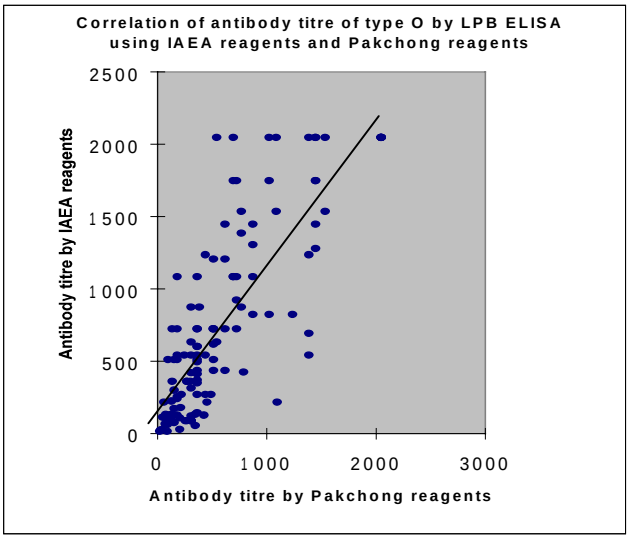
3. RESULTS

Liquid phase blocking ELISA (LPB ELISA)

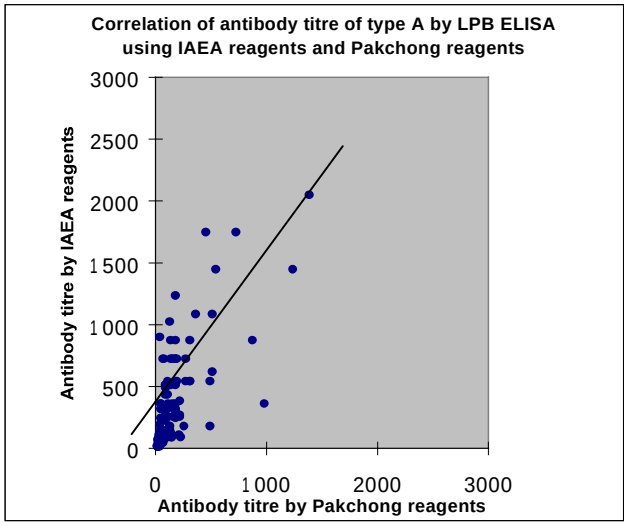
Comparison of the titres obtained in the two assay systems gave a correlation linear regression line (R) of type O = 0.80, type A = 0.73 and type Asia I = 0.80 respectively. The results are shown in Fig. 1.

The FMD field outbreak viruses type O and Asia I isolated in Thailand during 1994–1998 have been tested by LPB-ELISA method using Pakchong reagents in order to study serological relationships (r-value) between virus vaccine strain and field strain.

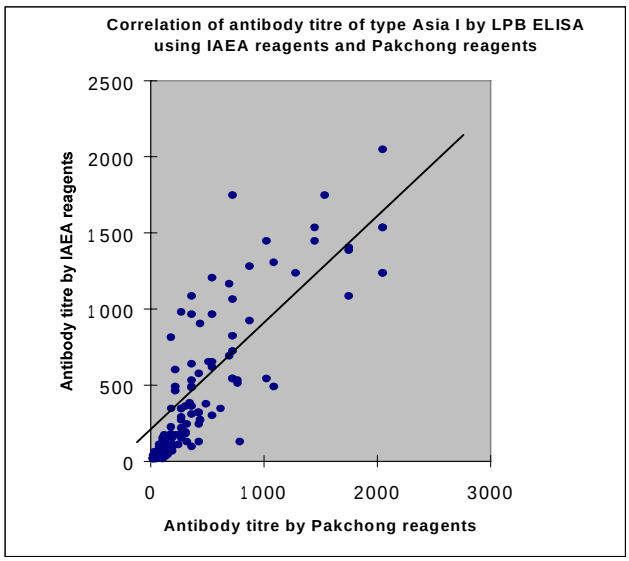
The r-value of 111 field isolate viruses, type O = 74 samples, type A = 2 samples and type Asia I = 35 samples have been investigated and r-value were calculated as described above. The results are shown in Table II and Figs. 2 and 3 respectively.



Y = 1.51224 * X – 128.495
R = 0.79792
N = 125



Y = 1.84 * X + 15.1517
R = 0.72846
N = 125



Y = 1.15079 * X – 103.88
R = 0.79747
N = 125

FIG. 1. Correlation of antibody titre of FMDV by LPB ELISA using IAEA and Pakchong reagent

Total Samples	Range of r-value	No. of samples	Percentage (%)
74	0.4–1.0	72	97.30
	0.20–0.39	2	2.70
	0–0.9	0	0

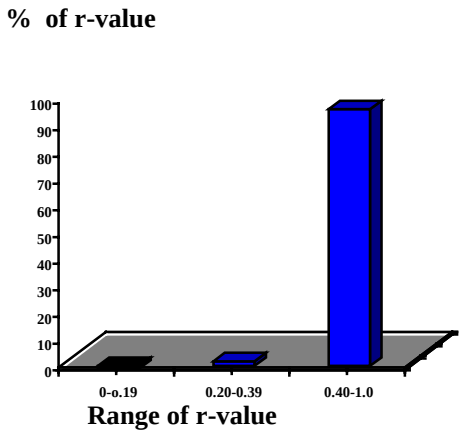


FIG. 2. The distribution of r- value of FMDV Type O field isolates in Thailand during 1994–1998

Total Samples	Range of r-value	No. of samples	Percentage (%)
35	0.4–1.0	34	97.14
	0.20–0.39	1	2.86
	0–0.9	0	0

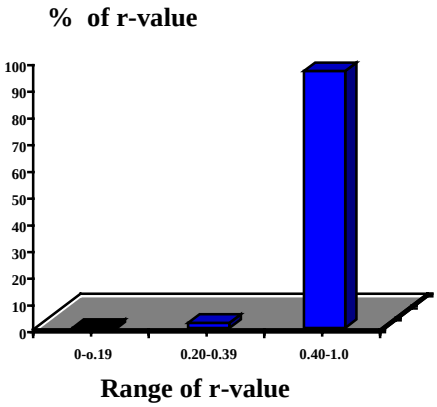


FIG. 3. The distribution of r- value of FMDV type Asia I field isolates in Thailand during 1995–1997

TABLE II. THE RESULT OF R-VALUE OF FMDV TYPE A NEW OUTBREAK IN THAILAND IN 1997

Virus	A/Udorn/87	A 40/97 (Sakol/97)
A132/87 (Udorn/87)	1.0	< 0.1
A 40/97 (Sakol/97)	<0.1	1.0
A 28/97 (Nakornsawan/97)	0.125	1.0

4. CONCLUSION AND DISCUSSION

The antibody titres of 125 field serum samples were compared by antibody measurement against FMD virus type O, A and Asia I using IAEA and Pakchong reagent systems. The linear regression coefficient (R) from both systems showed that the titres determined with these two systems were highly correlated (type O = 0.80, type A = 0.73 and type Asia I = 0.80 respectively). The results indicated that the Pakchong reagents can be used to replace IAEA reagent in ELISA antibody

measurement assay system. It was observed that the antibody titre against type A was higher when using the IAEA kit, whereas the Pakchong reagents gave higher titres for the O and Asia I systems. However, at the time the work was carried out the titre of the locally produced antigens for sero-type A was not stable on storage, compared to the IAEA kits. It is possible that as the antigen concentrations were increased to adjust the OD back to 1.5 units, some reduction in titre occurred in the Pakchong system. Results for the more stable Asia I antigen gave the best correlation in the comparison. But in majority, the antibody titre from the Pakchong system was highly correlated to the IAEA system for significant OD value at range 1.0–1.5.

The FMD Center also takes responsibility to supply the Regional Veterinary Research and Diagnostic Center over the country with diagnostic reagents such as ELISA reagents [10] and VIA antigen [11] for FMD diagnosis. Therefore, it is important that the standardization of our diagnostic reagents are based on the international standard level in order to establish the standard diagnosis laboratory for Regional Reference Laboratory (RRL) for FMD in Southeast Asia countries. The result from this investigation showed the significant correlation between the two systems of Pakchong reagents and the IAEA standard reagent kit.

The serological relationship between virus vaccine and field outbreak strain of FMDV types O, A and Asia I has been studied. A total of 111 field isolate viruses collected during 1994–1998 (type O = 74 samples, type A = 2 samples, type Asia I = 35 samples) were tested. The results were expressed as r-value (2,6). For the FMDV type O field strains studied 97.30% had values greater than 0.4, and were defined as O/UDN /87 related. This finding indicated that the virus vaccine strain O/UDN/87 would protect against the majority of field outbreak strains of sero-type O recently circulating in Thailand. The remaining 2.70% of field outbreak gave an r-value <0.39 indicating some antigenic divergence from the vaccine strain O/UDN/87. It is not uncommon to find some field viruses divergent from the main population, but at this time most field isolates would be covered by the vaccine strains.

For FMDV type Asia I 97.14% of the field strains studied had r-value greater than 0.40, and so were defined as related to the reference vaccine strain Asia I/PBR/85. This indicated, that there was no significant change in the antigenic characteristics among field outbreak and vaccine strain during the years 1995–1997. Previous studies on serological relationship of type Asia I field isolates during the past five years by VN test and two-dimensional LPB-ELISA [2,12] also demonstrated no significant antigenically change of those field isolated viruses from vaccine strain.

These investigations confirm recent studies on the serological relationship between type O and Asia I virus vaccine and field outbreak strains and indicate a relative stability in the endemic virus situation. It is therefore not necessary to consider selection of new vaccine viruses for these two sero-types.

The situation with type A was somewhat different. The serological relationship of type A was not investigated during 1993–1996 because there were no outbreaks of FMDV type A at that period of time. Unfortunately, an unexpected outbreak of a new type A was found in Thailand by the end of the year 1997. It was very interesting that the two cases of new type A outbreak, A/Sakolnakorn/97 (A/Sakol/97) and A/Nakornsawan/97 gave an r-value <0.19 when compared to the vaccine strain A/NPT/87. When the two new viruses were compared using the A/Sakol/97 system as a reference virus the r-value was 1.0 (Table II). However, in this system A/NPT/87 gave the r-value of <0.1, indicating the serological antigenic divergence was in both directions. The conclusion was that this new virus was distinctly different from the recent seed vaccine strain A/NPT/87, which has been classified as A22 virus group by WRL.

Subsequenly a rabbit trapping and guinea pig detecting antibodiy against the new type A outbreak strain were prepared for subtyping investigation. In addition, an emergency vaccine of the new type A has been prepared as a monovalent vaccine as well as bovine serum three weeks post vaccination for use in the study. The serological relationship of type As which are classified as A22 and the new A new outbreak strains were examined and compared. The result in Table II demonstrated that the antigenically characteristic of the new type A outbreak was diverged from recent seed vaccine strain of A22 group This result correlates with those from the World Reference Laboratory (WRL)

who studied the same panel of new type A outbreak viruses from Thailand and other countries. (See report by Dr. A. Donaldson in this publication).

The number of field outbreaks of FMD in Thailand has dramatically decreased during the past two years. This demonstrates that the efforts of the National FMD Control and Eradication programme have been highly successful and closesly linked to this success has been the use of appropriate vaccine for controlling and eradicating the disease outbreak. All this would indicate that final success with eradication of FMD, the target set by the DLD, will be achieved in the near future.

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THE USE OF MOLECULAR BIOLOGY TECHNIQUES FOR THE DIAGNOSIS AND EPIDEMIOLOGICAL STUDY OF FOOT-AND-MOUTH DISEASE VIRUS IN THAILAND

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Abstract

THE USE OF MOLECULAR BIOLOGY TECHNIQUES FOR THE DIAGNOSIS AND EPIDEMIOLOGICAL STUDY OF FOOT-AND-MOUTH DISEASE VIRUS IN THAILAND

The detection of foot-and-mouth disease (FMD) virus from various kinds of field samples (tissue extract and cell culture isolate) was studied using the polymerase chain reaction (PCR) technique. The gene selected for diagnosis was the polymerase gene and an amplification target product of 454 bp in length was produced using AP5/AP6 primer sets. The PCR product was further examined by NcoI endonuclease digestion. The presence of the internal restriction site was confirmed by demonstration of two small fragments of 330 bp and 124 bp in length. Forty-nine samples that gave positive and negative results by ELISA typing and were positive by the PCR test were tested by NcoI digestion to confirm the results. About 10% of PCR products could not be confirmed by the method. Furthermore the FMD RNA polymerase gene could be detected by the PCR method in samples negative in both ELISA typing and the virus isolation test. A total of 23 samples were examined and compared after each stage of the testing process. At the end of the extraction for ELISA the amplification product band at 454 bp was detected in 74% of the negative tissue extract samples, and in 48% at the end of the virus isolation procedure. The PCR technique was shown to rapidly and sensitively detect FMD viral genome, when compared with virus titration by tissue culture infectious dose 50% ($TCID_{50}$) method. The PCR was about 10 times more sensitive than the virus titration technique in detection of virus. Therefore, the PCR technique can be used in conjunction with current procedures for FMD diagnosis, to support the routine standard ELISA typing and virus isolation test on clinical samples. The first step of the nucleotide sequencing technique was introduced with a view to study genomic differentiation of FMD outbreak viruses. The appropriate primer sets for each of the three endemic sero-types were optimized and used to detect the PCR products from field isolate viruses. The PCR products of FMDV type O, A and Asia I showed a clear band at 720 bp, 814 bp and 914 bp respectively. This work showed that the technique could be introduced to perform the nucleotide sequencing to support epidemiological investigations.

1. INTRODUCTION

The current diagnostic methods for foot-and-mouth disease virus (FMDV) such as antigen-capture enzyme immunosorbent assay (ELISA) [1], virus isolation, liquid phase blocking ELISA [2] and VIA-agar gel immunodiffusion (AGID) test [3] have been used for routine diagnosis at the FMD Center, Pakchong, Thailand for many years. The virus isolation test using primary cell culture of bovine thyroid gland [4] or primary lamb kidney [5] have been demonstrated to be the most sensitive for detection of virus in clinical samples and oesophageal-pharyngeal fluid or probang samples. Nevertheless, virus isolation procedure is slow, labor intensive and expensive. Recently, the molecular techniques polymerase chain reaction (PCR) and nucleotide sequencing have been published for detection of FMDV from animal tissue [6].

The oligonucleotide primers were selected for the conserved genomic sequences of the viral RNA polymerase (3D) gene [7] ideal for detection of all sero-types of FMDV. This technique may provide a rapid and sensitive laboratory diagnostic test. Rapid laboratory diagnosis and epidemiological investigation by molecular characterization of virus isolates from field are very important requirements in control and eradication of FMD in Thailand. The nucleotide sequencing at the VP1 FMDV genome, provides the most detailed information about an isolate. In this region sero-type O [8], sero-type A [9] and sero-type Asia I [10] are present. Nucleotide sequencing will be useful for tracing back to the origin of any virus causing outbreak in the country.

The aims of this study are:

(1) To established the PCR technology for detection of FMDV in various samples such as tissue samples and cell culture isolate samples in order to improve and develop the diagnostic technique.

(2) To establish the nucleotide sequencing of FMDV outbreak in Thailand to provide a basic molecular epidemiology information in order to trace back to the origin of virus causing outbreaks.

2. MATERIALS AND METHOD

2.1. Test samples

Routine diagnostic samples from cattle, buffaloes and pigs were submitted to the laboratory for FMD serotyping by ELISA test. The clinical samples collected from tongue or interdigital epithelial tissue were extracted by grinding with sand and prepared 10% suspension in phosphate buffer solution. The virus isolates were passaged three times in either primary lamb kidney cells or BHK-21 cells before use. Sample negative in initial typing test was inoculated onto tissue culture for attempted virus isolation and typing. The present study, the above samples were used to amplify viral RNA by PCR and nucleotide sequencing.

2.2. Viral RNA preparation

Viral RNA was extracted from test samples using TRIzol ™ (GIBCO, UK) according to the manufacturer's protocol.

2.3. Oligonucleotide primers

Oligonucleotide primers were synthesized as a single-stranded DNA using an automated synthesizer (Applied Biosytems, Foster City,USA) and the commercial random hexamer (Promega, USA) was also used for first strand synthesis in reverse transcription step and then two specific primer sets were used in the PCR amplification step, which based on sequences from highly conserved region within VP1,VP3 and 3D of FMD viral genome [7,11]. The primer sequences used in RT-PCR and sequencing are shown in Table I.

TABLE I. THE OLIGONUCLEOTIDE PRIMERS USED FOR RT-PCR AND SEQUENCING OF FOOT-AND-MOUTH DISEASE VIRUSES

Primer Designation	Primer sequence 5'→ 3'	Location	Product length (bp)
NK61	GACATGTCCTCCTGCATCTG	2B	Universal primer
O 1D - ROD1	TGTTGAAAACTACGGTGGTGA	1D	700 – 720
A 1C – 612	TAGCGCCGGCAAAGACTTTGA	1C	813 – 816
As1 1C- 505	TACACTGCTTCTGACGTGGC	1C	908 – 914
AP 5	AGGACAAAGCGCTGTTCCGC	3D	454
AP 6	TCAGGGTTGCAACCGACCGC	3D	Antisense primer

2.4. Reverse transcription

The viral RNA was reverse transcribed in 25 µl reaction mix cosisting of 5 µl of sample (extraction RNA suspension), 2.5 µl of 10 mM dNTP's, 5ul of 5X buffer, 1ul of MoMLV (200 U/ul, Promega, USA), 0.5 µl of 0.1M DTT, 1 µl of Random Hexamer or 25 pmol/1µl of NK61 primer. The reaction mixture was run in thermal cycler (Omnigene, Hybaid, UK) for 60 minutes at 42°C.

2.5. PCR amplification

The specific primer set of AP5/AP6, NK61/ID-ROD1, NK61/1C-612 and NK61/1C-505 were used for amplification of cDNA at each target gene of FMD polymerase (3D) segment and FMDV sero-type O, A and Asia I respectively.

The PCR mixture contained of 5 µl of cDNA, 5 µl of 10X Taq buffer, 1 µl of 1 mM dNTP's, 0.5 µl of Taq polymerase (Promega, USA), 3 µl of 25mM $MgCl_2$, 1 µl of 25 pmol Primer 1 and Primer 2 corresponding to the sero-type as described above and volume was made up to 50 µl with DEPC treated H_2O. The mixture was over lid with 20 µl of mineral oil. The PCR reaction was run in the thermal cycling block following the thermal profile: 94ºC for 1 minute, 55°C for 1 minute, 72ºC for 1.30 minutes for 30 cycles, final extension at 72ºC for 5 minutes. After completion 5 µl of PCR products were analyzed on 1.5% agarose gel run at 100V for 30 minutes and stained with ethidium bromide (1 µl/ml) for 5 minutes then destained with distilled water for 10–15 minutes. The product was readily visible under the ultraviolet (UV) transilluminator.

2.6. Restriction enzyme analysis

A PCR product of 454 bp in length was presumed as positive for FMDV from the original sample. The PCR product was purified by using a Wizard Prep DNA purification kit (QIAGEN, Germany) according to the manufacturer's protocol. Confirmation of a specific PCR product was performed by using restriction enzyme digestion with NcoI endonuclease which digest the PCR product into 330 bp and 124 bp as described by Meyer et al [7].

3. RESULTS

The establishment of PCR technology for detection of FMD viral genome in infected tissue sample and cell culture isolate was successful. Field samples from cattle, buffaloes and pigs were used for amplification of viral RNA by PCR technique after those samples have been tested by ELISA typing and virus isolation.

The result in Fig. 1 shows the analysis of PCR product from field sample using appropriate primer sets of AP5/AP6 which complementary to the antigenomic sense at RNA polymerase gene position. The PCR product produced was the estimated length at 454 bp. The confirmation of the PCR product using restriction enzyme analysis of NcoI endonuclease was shown a clear digested fragment at 330 bp and 124 bp in length.

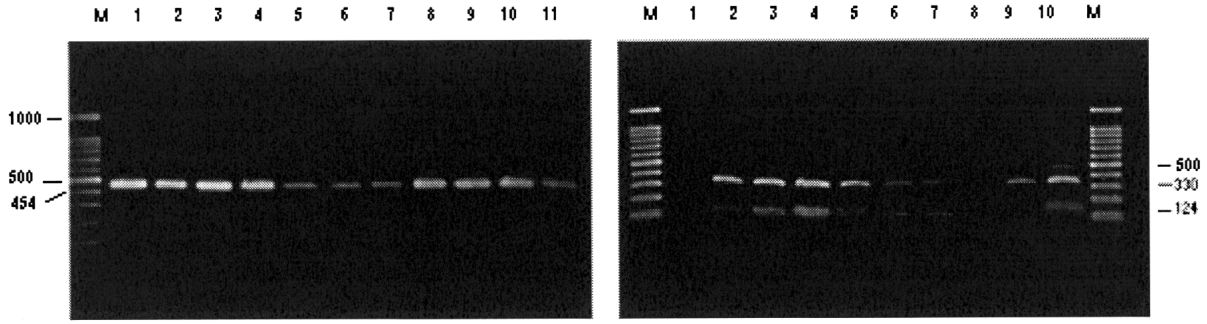

FIG. 1. Analysis of PCR product from field sample using 3D gene specific primer and confirmation of 454 bp band using NcoI enzyme digestion.

M = 100 bp Ladder DNA marker (Promega, USA),

Left gel: no. 1–10 = PCR product using AP5/AP6 primer set giving product band at 454 bp, No.11 = positive cont rol sample

Right gel: no. 1–10 = NcoI enzyme digest into 2 fragments of 330 bp and 124 bp.

By routine standard ELISA typing and virus isolation test of all field samples, twenty three samples, which were classified as negative following initial ELISA typing test and a subsequent virus isolation procedure, were tested by PCR to detect FMD RNA polymerase gene. The initial tissue sample extract was tested along with the matching passage 3 virus isolation tissue culture fluid. The result is shown in Table II.

In addition, a number of specimens were tested to examine the specificity of the PCR technique method. To confirm the PCR product, NcoI enzyme digestion was used. The samples selected were either original tissue sample extracts or virus isolation cell culture fluids. In Table III forty-nine samples regarded as ELISA positive and negative came from these sources. The result showed that 89.80% of the samples could be digested into small fragments of 330 bp and 124 bp by NcoI enzyme reaction. This demonstrates the consisting of the restriction site of NcoI enzyme in most of positive samples and remaining only 10.20% was not digested by NcoI enzyme reaction. The sensitivity of detection of FMD antigen by virus titration using tissue culture infectious dose 50% ($TCID_{50}$) method and PCR technique was studied and compared. The result in Table IV shows that the PCR technique was $1\log_{10}$ more sensitive than the tissue culture method.

TABLE II. PCR RESULT BY DETECTING 3D GENE OF FMDV FROM BOTH TISSUE EXTRACT AND VIRUS ISOLATION SAMPLES WHICH GIVING ALL NEGATIVE RESULT BY ELISA TYPING TEST.

Total sample	Source of sample	PCR test using primer sets of AP5/AP6	
		Positive	Negative
23	Tissue extract fluid	17 (73.91%)	6 (26.09%)
23	Cell culture isolate fluid	11 (47.82%)	12 (52.18%)

Positive = PCR positive result and the 454 bp of PCR product band was detected.
Negative = PCR negative result and the 454 bp of PCR product band was not detected.

TABLE III. COMPARISON OF ENZYME DIGESTION ANALYSIS OF PCR PRODUCT BAND AT 454 BP FROM VARIOUS TISSUE EXTRACTS AND CELL CULTURE ISOLATION FLUIDS WHICH GAVE POSITIVE AND NEGATIVE RESULT BY STANDARD ELISA TYPING.

Type of Sample	Total Samples	Positive PCR giving product Band at 454 bp	NcoI Digestion giving product band at 330bp and 124bp	
			Positive	Negative
Positive by ELISA typing	31	31	30	1
Negative by ELISA typing	18	18	14	4
Total sample	49	49	44 (89.80%)	5 (10.20%)

TABLE IV. COMPARISON OF PCR BAND AND VIRUS TITRATION FOR DETECTION OF FMDV TYPE A.

FMDV type A $10^{7.5}$ $TCID_{50}$ / 0.1ml	Cytopathic effect (CPE) from virus titration	PCR result
Undiluted	+	+ (Strong band)
10^{-1}	+	+ (Strong band)
10^{-2}	+	+ (Strong band)
10^{-3}	+	+ (Strong band)
10^{-4}	+	+ (Strong band)
10^{-5}	+	+ (Strong band)
10^{-6}	+	+ (Weak band)
10^{-7}	–	+ (Weak band)
10^{-8}	–	–

+ = Positive result, CPE occurred in virus titration method or PCR product band detected in PCR method
– = Negative result, No CPE occurred in virus titration method or no PCR product detected in PCR method

The use of PCR technology for epidemiological studies of FMD field isolate viruses type O, A and Asia I was introduced. This initial work was to establish the basis for nucleotide sequencing in order to study the genomic differentiation of FMD field outbreak viruses in Thailand. The appropriate primer set of NK61/1D-ROD1, NK61/1C-612 and NK61/1C-505 were used to amplify the VP1 and VP3 target gene of FMDV type O, A and Asia I, which giving product length at 720 bp, 814 bp and 914 bp respectively. The result is shown in Fig. 2.

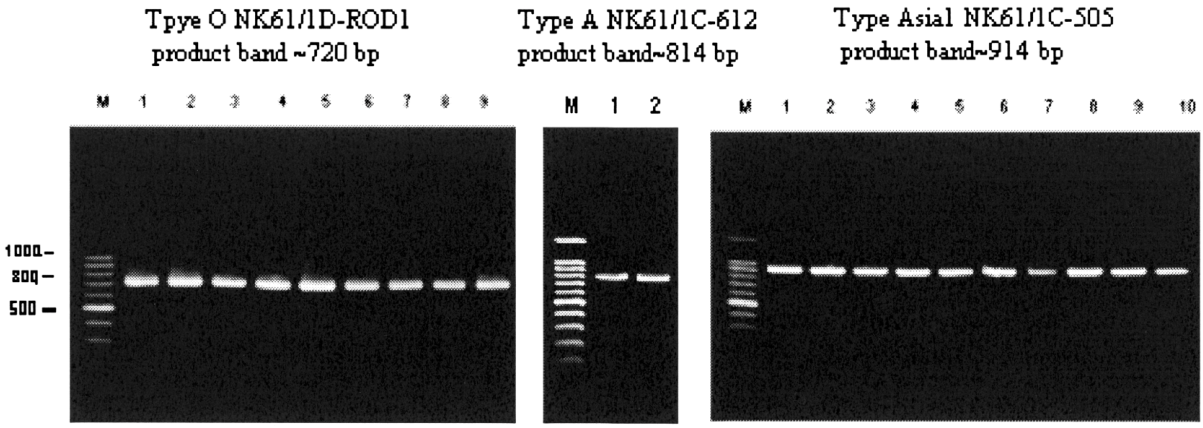

FIG. 2. Analysis of FMD field isolate virus type O, A and Asia I giving PCR product band at 720 bp, 814 bp and 914 bp respectively which were used for nucleotide sequencing.

M = 100 bp ladder DNA marker (Promega, USA)

4. DISCUSSION

PCR technology was successful in amplification of a portion of the FMDV RNA polymerase gene in various types of samples, tissue extracts and cell culture isolation supernatant fluids. The PCR primer set of AP5/AP6 was designed from a highly conserved region of FMDV sequence at 3D region [12] and allowed the production of a PCR product of 454 bp for all sero-types. The enzymatic RNA digestion of the polymerase gene product using NcoI was demonstrated to be a highly specific and sensitive method to confirm the identity of the PCR of 454 bp product band into 330 bp and 124 bp [7]. This sequence of the RNA polymerase gene contained a unique restriction site for NcoI endonuclease producing 330 bp and 124 bp products.

An interesting result observed was that virus was detected by PCR amplification of cDNA in 74% of the original 'negative' tissue extracts, and was still detected in 48% of the same group of samples after 3 serial passages in tissue culture (Table II). This suggests that either viral RNA persisted during the tissue culture isolation procedure or that there was a low level of virus replication for some specimens without observable sample cytopathic effects.

The PCR technique when compared with the tissue culture $TCID_{50}$ method for virus infectivity was shown to be a highly sensitive detecting method [13]. In this study a comparison of the two methods was made using a type A vaccine strain. The PCR method detected virus about 10-fold more dilute than the cell culture titration, i.e. the difference in sensitivity is about 10 TCID50 in 0.1 ml.

The current procedure of rapid and accurate diagnosis of FMDV outbreak is very important for the control and eradication of FMD programmes in the field. In addition, it is important to monitor outbreak viruses to ensure the continued suitability of vaccine viruses. The PCR technique can be used in conjunction with current procedures for FMD diagnosis to support the routine standard ELISA typing and virus isolation test in clinical specimens that may have a low level of virus. Poor quality samples often create a problem in the routine standard ELISA typing test because of undetectable levels of virus. If the PCR could be incorporated into routine diagnosis this would increase the overall sensitivity of virus detection procedures. However there would also be a need to introduce sero-type specific primer sets for sero-type identification [14,15]. Nested PCR would be needed to increase the specificity and sensitivity of the PCR, but the rink of cross contamination would be increased.

The first steps of the nucleotide sequencing technique were introduced at the FMD laboratory with a view to future study of the molecular epidemiology of FMDV field isolates in Thailand and the region. The primer sets of NK61/1D-ROD1, NK61/1C-612, and NK61/1C-505 were optimized and used to produce the PCR products from a collection of field isolate viruses. The PCR products of FMDV type O, A and Asia I were shown clearly as bands at 720 bp, 814 bp and 914 bp respectively. In the future we hope to apply nucleotide sequencing to investigate the epidemiology of FMD to assist the control and eradication efforts in Thailand and our neighbouing countries [11].

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FOOT-AND-MOUTH DISEASE VIRUS TYPING FROM FOOT-AND-MOUTH OUTBREAKS IN THE CENTRAL PROVINCES OF VIET NAM

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Abstract

FOOT-AND-MOUTH DISEASE VIRUS TYPING FROM FOOT-AND-MOUTH OUTBREAKS IN THE CENTRAL PROVINCES OF VIET NAM

A total of 167 tissue samples were collected from Foot-and-mouth disease (FMD) infected animals from 57 FMD outbreaks to detect the sero-type of the FMD virus by the ELISA technique. The ELISA kit has been prepared and standardised by the World Reference Laboratory (WRL), UK and supplied under a Research Contract as part of an FAO/IAEA Co-ordinated Research Project. Eight tissue samples from cattle and one tissue sample from pig were sent to WRL for further study on the sero-type and to characterize the FMD viruses present in Viet Nam. The study was carried out from March 1996 to May 1998 in the central region of Viet Nam and the FMD type O virus was detected in these outbreaks only. The FMD type O virus from cattle and the FMD type O virus from pig are two distinct FMD type O viruses in Viet Nam.

1. INTRODUCTION

FMD outbreaks have occurred in many central Provinces of Viet Nam. These outbreaks have been effectively controlled by 'ring vaccination' and animal movement control for many years [1]. The FMD vaccine that has been used to control FMD outbreak in Viet Nam is a trivalent vaccine that contains the sero-types O1, A22 and Asia I. In 1997, there were many FMD outbreaks in cattle which could not be effectively controlled by that vaccine. To date, there is not enough information about the FMD virus sero-type of the FMD virus which caused FMD outbreaks in the central region of Viet Nam.

The objective of this study was to:

- Detect the sero-types of FMD virus that caused the FMD outbreaks and find out the distribution of these sero-types of FMD virus in the central Provinces of Viet Nam.
- Select appropriate samples for sending to WRL, Pirbright, UK to further characterize the FMD virus.

The duration of this study was from March 1996 to May 1998.

2. MATERIALS AND METHODS

2.1. Sample collection

Two to three tongue tissue samples were collected from infected animals at the beginning of each outbreak. Samples were kept in 0.4 M phosphate buffer with 50% glycerine and sent within 72 hours on ice to the Veterinary Laboratory of the Regional Animal Health Centre, Ho Chi Minh City.

2.2. ELISA Kit

The samples were tested using the ELISA kit prepared and standardized by FMD World Reference Laboratory (WRL) at Pirbright, United Kingdom and provided by the Joint FAO/IAEA Division through a Research Contract awarded under an FAO/IAEA Co-ordinated Research Project. This FMD virus antigen detection kit uses an indirect sandwich ELISA to detect four possible sero-types: O, A, C and Asia I, of FMD virus in tissue samples. The assay protocols followed are described by IAEA/WRL Bench Protocol [2].

2.3. Characterization of FMD virus

This was carried out at the WRL, Pirbright, England. Details are described below.

2.3.1. The serological relationship (r-value)

The reference virus and field isolate viruses were titrated by indirect sandwich ELISA method as described by Hamblin et al [3] and Kitching et al [4]. A fixed concentration of reference and field isolate viruses giving an optical density (OD) of 1.5 was reacted with bovine post vaccination and challenged serum with homologous virus. The serological relationship (r-value) [5] was calculated as this ratio:

$$\text{r-value} = \frac{\text{serum titre against heterologous field strain}}{\text{serum titre against homologous vaccine strain}}$$

The guideline suggestion for r-value obtained by LPB-ELISA and the criteria of interpretation have been proposed by Samuel et al [6] and Doughty et al [7] as follows:

– when r = 0 – 0.19 this is highly significant serological variation from the reference strain.
– when r = 0.2 – 0.39 this represents a significant difference from the reference strain, but protection may be satisfactory if using a sufficiently potent vaccine.
– when r = 0.4 – 1.0 there is no significantly difference from vaccine strain.

2.3.2. The molecular relationship

Field virus isolates were sequenced and the sequence date compared through the production of an dendogram which compares degree of sequence homology for characterization purposes [7] (see also paper by A. Donaldson in this publication).

3. RESULTS

The results of FMD virus typing are shown in Tables I, II and III for the years 1996, 1997 and 1998 respectively. In 1996 39 samples from 12 outbreaks were tested, in 1997 103 samples from 31 outbreaks were tested and in 1998 25 samples from 14 outbreaks were tested. In all cases only FMD type O virus was detected.

Nine tissue samples were sent to WRL, for further sero-typing and the results are shown in Table IV.

The relationship of the two distinct FMD type O viruses isolated from cattle and pig in Viet Nam and other type O viruses is shown through the comparable r-values (Table V).

TABLE I. THE RESULT OF FMD VIRUS TYPING FROM MARCH 1996 TO DECEMBER 1996.

Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Nr of outbreaks	–	–	1	4	2	3	0	0	0	0	2	0
Nr. of samples	–	–	4	12	7	9	0	0	0	0	7	0
Result sero-type	–	–	O	O	O	O	–	–	–	–	O	–

TABLE II. THE RESULT OF FMD VIRUS TYPING FROM JANUARY TO DECEMBER 1997

Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Nr of outbreaks	0	4	7	2	2	4	4	3	1	1	2	1
Nr. of samples	0	13	22	7	8	13	11	10	4	4	7	4
Result sero-type	–	O	O	O	O	O	O	O	O	O	O	O

TABLE III. THE RESULT OF FMD VIRUS TYPING FROM JANUARY TO MAY 1998

Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Nr. of outbreaks	5	3	4	1	1	0	0	0	0	0	0	0
Nr. of samples	7	6	8	3	2	0	0	0	0	0	0	0
Result sero-type	O	O	O	O	O	–	–	–	–	–	–	–

TABLE IV. SERO-TYPING RESULTS FROM WRL, UK COMPARED TO THE RESULTS OBTAINED IN VIET NAM

Species of animal	Number of samples	FMDV sero-typing result from Regional Animal Health Centre HCMC, Viet Nam	FMDV sero-typing result from WRL, Pirbright, England
Cattle	8	Sero-type O, only	Sero-type O, only
Pig	1	Sero-type O	Sero-type O

TABLE V. THE R-RESULTS MEAN BETWEEN THE FMD TYPE O VIRUSES FROM VIET NAM AND OTHER FMD TYPE O VACCINE VIRUSES

Virus Serum	O Vietnam 1/97	O Vietnam 2/97	O Vietnam 3/97	O Vietnam 4/97	O Vietnam 7/97	O Vietnam 9/97
O-'3039' BVS 'r'	0.33	0.25	0.79	0.22	0.23	0.32
O-MANISA BVS 'r'	0.53	0.50	0.70	0.29	0.17	0.30
O-PHILIPPINES BVS 'r'	0.50	0.50	1.00	0.53	0.49	0.45
O-BFS 68 BVS 'r'	0.39	0.20	0.23	0.30	0.30	0.30

BVS : Bovine Vaccinated Sera – 21 days.
O VIET NAM 3/97: Field isolated FMD type O virus from pig.
O VIET NAM 1/97, 2/97, 4/97, 7/97 and 9/97: Field isolated FMD type O viruses from cattle.

4. DISCUSSION

The study was carried out in eight central Provinces of Viet Nam, two of them having a border with Campuchia. From March 1996 to May 1998, 67 tissue samples were collected from 57 FMD outbreaks. The FMD type O virus was detected in these samples only. The results of FMDV typing from the laboratory of Regional Animal Health Center HCMC and WRL are the same, with only FMD type O virus being detected by both laboratories.

The FMD type O virus from cattle and FMD type O virus from pig in Viet Nam are two distinct FMD type O viruses. The FMD type O virus from pig is closely related to FMD type O virus from Taiwan, Hong Kong and Philippines, whereas the FMD type O virus from cattle is closely related to FMD type O virus from Thailand and Malaysia.

The FMD type O virus from pig has an r-value of 0.79, 0.70, 1.00 and 0.23 against the FMD vaccine virus strains O-'3039', O-Manisa, O-Philippines and O-BFS 68 respectively. According to this result, the FMD type O virus from pig in Viet Nam can be effectively controlled by the vaccine containing any of the strains O-'3039', O-Manisa and O-Philippines. In the field, all pig FMD outbreaks have been effectively controlled by a mono-valent vaccine containing strain O-Manisa only.

The r-values between FMD type O viruses from cattle and the FMD strain O-Manisa vaccine virus are very low (max: 0.53 and min: 0.17), therefore many cattle FMD outbreaks that were caused by FMD type O viruses could not effectively be controlled by trivalent vaccine containing strains O-Manisa, A 22 and Asia. To improve the effectiveness of the national FMD control programme, the Department of Animal Health has chosen the FMD vaccine containing strains A 22, Asia I and three strains O: O-Manisa; O-'3039' and O-Philippines for controlling cattle FMD outbreaks. This has clearly been successful and resulted in the reduction of FMD outbreaks in 1998 (compare Tables I, II and III).

In the future, more effort will be made to fully characterize isolates of FMD virus from outbreaks in Viet Nam and in particular their relationship to FMD virus vaccine strains being used in the National Control Programme.

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DIAGNOSIS AND CONTROL OF FOOT-AND-MOUTH DISEASE IN SRI LANKA USING ELISA-BASED TECHNOLOGIES — ASSESSMENT OF IMMUNE RESPONSE TO VACCINATION AGAINST FMD USING ELISA

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Abstract

DIAGNOSIS AND CONTROL OF FOOT-AND-MOUTH DISEASE IN SRI LANKA USING ELISA-BASED TECHNOLOGIES — ASSESSMENT OF IMMUNE RESPONSE TO VACCINATION AGAINST FMD USING ELISA

The policy for control of FMD since 1964 in Sri Lanka has been the vaccination of high quality stock in government farms and in places where stock improvement was in progress once a year. From 1993, a supplementary vaccination during February to March was adopted to cover the young stock in addition to the annual vaccination programme. However in the field this was not successful due to the shortage of vaccines and less co-operation from farmers. The focus of this study was to study the effectiveness of the national immunisation programme carefully and develop strategies to get the maximum benefit from limited resources. Vaccination coverage during 1995, 1996 and 1997 in SP was low (3.4%, 4.45% and 3.5% respectively). However, during the outbreak of the disease at Kalutara district in WP, vaccination was adopted in border areas to have a buffer zone to prevent the leak of FMD to SP. The mean protective antibody level in the whole district of Galle was found to be 42.4%. FMD control and eradication strategy in Sri Lanka no doubt has to focus on preventing the free movement of animals without Health Certificate, on continuous mass vaccination in areas bordering the endemic Provinces NWP, NCP and EP to maintain a high herd immunity of more than 80% to prevent future outbreaks and also to protect the improved breed in the field and in State farms. This study shows that this is yet to be achieved.

1. INTRODUCTION

Sri Lanka is a tropical Island in the Indian Ocean with an area of 65 610 km^2. The greatest length of the Island is 432 km (270 miles) while the greatest breadth is 224 km (140 miles). The country is divided into three zones, i.e. lowland; midland and upland based on elevation demarcated at 300 m to 1000 m above mean sea level. The country experiences two monsoons annually, the South-West monsoon (May to August) and the North-East monsoon (November to February). On the basis of rainfall, the country is divided into three zones: Wet zone (>2500 mm); Intermediate zone (2000–2500 mm) and Dry zone (< 2000 mm). Thus the South-West of the island experiences an annual rainfall of 2500 mm to over 5000 mm while North-West and South-East of the Island experience a rainfall of less than 1250 mm. Sri Lanka is administratively divided into 9 Provinces with 25 districts consisting of 256 Divisional Secretarial divisions. It has a livestock population of 1 583 600 cattle, 715 200 buffaloes, 519 300 goats, 11 800 sheep and 76 300 pigs.

1.1. Foot-and-Mouth Disease

Foot-and-mouth disease (FMD) has been officially reported in the island in 1902. This disease had assumed epidemic proportions in 4 to 6 years. Out of the seven sero-types of FMD virus, only one sero-type, type O is been continuosly reported in Sri Lanka. Sero-type C was introduced to Sri Lanka through cattle imported from India in 1970. Disease due to type C has been identified up to 1978 and then again appeared only in 1984 causing outbreaks in Northern Province (Kaluwanchikudy veterinary range), Western Province (Kadawatha) and Uva Province (Badulla). FMD due to sero-type C has not been reported thereafter. FMD has been commonly reported among cattle and buffaloes. Disease among the porcine population which remains unvaccinated was first detected in 1982 in Western Province (Welisara and Horana veterinary ranges).

The outbreak of disease in epidemic form in1987 resulted in 86,000 cases, the highest number recorded so far. Cases among goats and sheep were reported for the first time during this outbreak. This outbreak continued till 1994. In 1995, two outbreaks in the month of June were suspected for FMD. In the first suspected outbreak, only one isolated case was reported. This was subsequently refuted to be FMD after investigation.

The second incidence was reported in Uva Province involving 50 local cattle in one village. The epidemiological feature was not supportive to consider it as FMD. This disease was not confirmed by laboratory. Thus the country was free of the disease for nearly two years during 1995 and 1996.

However in early January 1997, FMD was reported in a village close to a national sanctuary in the North-Western Province (NWP). This outbreak was confirmed to be type O by World Reference Laboratory (WRL), UK. Due to the ongoing civil disturbance in the North and East Provinces, the border areas have not been vaccinated against FMD since 1984. The disease was later introduced to other Provinces (Table I) mainly by unauthorized transport of animals. Animal movements have been recognized as the major method of spread in endemic regions [1,2]. Most common mechanism of spread of FMD in Sri Lanka is by the movement of infected and disease incubating animals. Indirect transmission of through contaminated products and formites are also seen. In 1997, cases were reported from NWP, North-Central (NCP), Western (WP), North-East (EP), Central (CP) and Sabaragamuwa (Sa) Provinces. Outbreak of FMD in the CP was as a result of introduction of 13 animals that have been salvaged from slaughter from a cattle holding place in Colombo without proper health certificates. The disease in CP spread rapidly since the climatic condition was favourable for wind borne spread. Sri Lanka being a tropical country, wind borne infection was considered to be rare or non existence. But during the recent outbreak in 1997, FMD spread rapidly in Kandy district through wind. The climate was very gloomy and windy. Disease was not reported from Southern (SP) and Uva (U) Provinces during 1997.

FMD continued in 1998 in the NWP and CP. Six veterinary ranges (Ibbagamuwa, Kurunegala, Narammala, Polpithigama, Ridigama, Wariyapola) in Kurunegala district in NWP and three veterinary ranges (Galewela, Naula, Udunuwara) in Kandy and Matale districts in the CP reported continuation of 1997 outbreak till January/February 1998. Fresh outbreaks for 1998 were reported from NCP, NWP, NEP, WP, Sa and also in SP. The outbreak of disease in SP was also a result of introducing animals from infected areas in the NCP and Eastern Provinces (EP) under a development programme without informing the authorities.

FMD continued in 1999, recording 12 outbreaks affecting 3,692 cattle/buffalo with 16 deaths up to mid February. Disease has been reported from five Provinces (Table II). Uva Province still remain free. With the introduction of artificial insemination (AI), we have replaced gradually the more tolerant indigenous cattle and buffaloes in our country with more susceptible improved breeds. The incidence of FMD has become more in the recent past and is expected to increase in the future since the emphasis during the past few years and even at present is on AI.

1.2. Control strategies

The accepted control strategies available for FMD are, stamping out; tracing the outbreaks; legislation; quarantine; movement control; vaccination; import/export regulations and zoo-sanitary measures. The strategies we follow now to some extent are tracing the outbreaks, movement control, vaccination and zoo-sanitary measures. Although vaccination against FMD has been carried out for more than three decades, the low vaccination coverage has made this disease to rise to epidemic proportions once in 4 to 6 years.

The main constraint has been the limitation of funds to purchase adequate quantities of good quality vaccine, maintenance of cold chain up to the point of inoculating the vaccine and motivating the farmers to get their cattle immunized against FMD despite of giving the vaccine free of charge. The enthusiasm of veterinarians in vaccinating cattle against FMD is also low, mainly due to the fact that administration of vaccine by S/C is difficult under field conditions and requires repeated vaccination to evoke protective immunity even with the superior quality vaccine. Further, the immunity developed is short, 3–6 months.

The policy for control of FMD since 1964 was the vaccination of high quality stock in government farms and in places where stock improvement was in progress once a year. From 1993, a supplementary vaccination during February to March was adopted to cover the young stock in addition to the annual vaccination programme. However in the field this was not successful due to the shortage of vaccines and less co-operation from farmers.

TABLE I. FMD VACCINATION FROM 1995–1998 (BY PROVINCE)

PROVINCE	TARGET	IMMUNZATION	YEAR
West	26,000	37,463	1998
	11,200	56,185	1997
	10,300	5,763	1996
	12,250	7,178	1995
Central	30,000	21,354	1998
	31,600	51,345	1997
	30,500	28,110	1996
	26,700	23,687	1995
Southern	15,000	11,732	1998
	8,400	7,739	1997
	11,500	9,945	1996
	28,000	7,567	1995
North central	220,000	66,519	1998
	45,000	195,257	1997
	43,750	26,600	1996
	47,000	22,280	1995
North western	123,000	73,380	1998
	6,800	123,624	1997
	11,600	2,400	1996
	38,350	10,101	1995
North & East	10,000	10,438	1998
	27,600	12,945	1997
	13,500	49	1996
	–	10,235	1995
Uva	45,000	4,698	1998
	64,300	20,942	1997
	45,500	36,184	1996
	49,000	31,036	1995
Sabaragamuwa	6,000	7,395	1998
	4,400	6,501	1997
	4,800	1,613	1996
	9,600	2,953	1995

The FMD vaccine was produced locally up to 1993. The production had to be stopped due to technical difficulties and as of 1994 vaccine was imported. Limitations of funds did not permit to procure adequate amount of vaccine. The number of doses of vaccine imported was not sufficient to have a satisfactory coverage.

Prophylactic vaccination programmes should be strengthened by strict control over the health regulations on animal movement including disinfection of vehicles and quarantine measures. Vaccination programmes should be carried out extensively in accessible areas using a good quality vaccine maintaining the cold chain. Appropriate schedules need to be developed for localities individually. Quarantine zones have to be developed since contact with live animals is the most significant means of spread of this disease. Thus, a long-term commitment towards the control of FMD including adequate budgetary allocation for the control programme could reduce the intensity of infection and spread of the disease to free areas.

The immunisation strategy planned for 1999 has taken into account the naturally aquired immune status of bovine population soon after the recent outbreak of disease. A total of 925,000 FMD vaccinations were to be carried out in 1999. But the financial allocation provided for this year would permit only 500,000 doses to be procured.

In 1997, the coverage was higher since more animals had to be vaccinated due to outbreaks of the disease, which occurred in all the provinces except South and Uva. Additional vaccines were procured from funds obtained through transfers. During this year 19,478 cattle and buffaloes were affected. These outbreaks could have been averted if sufficient vaccination coverage would have been adopted in previous years, so that a minimum protective level of immunity could have been maintained.

The economic losses due to FMD in cattle and buffaloes could be categorized as direct loss of milk production ranging from 25% to 45%, indirect losses due to increased calving interval, loss or premature disposal of adults and calves, loss due to abortions, draught loss due to permanent or temporary disability and loss due to delayed maturity. The loss of milk production alone for the 1997 outbreak has been calculated to be over Rs. 75 million and the total economic loss in the region Rs.100 million. If we continue to have a low vaccination coverage, the disease could well assume higher proportions thereby resulting in enormous economic losses at a time when we are taking all efforts to increase milk production in the country by introducing improved breeds through imports and artificial inseminations.

The cost of vaccination against FMD for the years 1997 and 1998 were analysed taking into consideration the cost of imported vaccine and the expenditure incurred in carrying out the field immunisation programme. In 1997, a sum of US $232,600 was spent to immunize 509,446 bovine incurring US $0.45 per animal whilst in 1998 US $125,000 has been spent to vaccinate 232,976 bovine incurring US $0.53 per animal.

Thus it appears to be more appropriate to study the effectiveness of the national immunisation programme carefully and develop strategies to get the maximum benefit from limited resources.

2. MATERIALS AND METHODS

2.1. Sampling framework

Seventeen districts in seven provinces (NWP, NCP,CP,WP,SP, Sabaragamuwa and UVA) having a total bovine population of 1,733,400 based on the 1996 report of the Department of Census and Statistics were included in this study. Sample collection in North and East Provinces were not attempted due to continued civil disturbances in these Provinces. The number of sites per district was based on the bovine population in a given district. Sampling sites were selected by a random procedure using map co-ordinates to give a 95% confidence limit [3]. A total of 142 sites, 8–10 sites per district were identified to include 1770 cattle and buffaloes.

2.2. Sample Collection

Blood samples were collected from 12–15 cattle/buffaloes above six months of age within 5-kilometer radius of a given site. Sera were separated at the respective Veterinary Investigation Centre, labelled to identify the district/site/ animal and stored at -10ºC until transported to the testing laboratory. At the receiving laboratory, sample identification numbers checked and stored at -20ºC until serological testing was carried out. These samples were collected from April 1997 to May 1998.

2.3. Liquid Phase blocking ELISA to detect antibodies for FMD virus

Serum samples were tested by ELISA kit provided by the International Atomic Energy Agency (IAEA) under a FAO/IAEA Research Contract. This kit has been prepared and standardized by the World Reference Laboratory, Pirbright, United Kingdom. The antibody detection kit is a liquid phase blocking ELISA for the detection of antibodies for FMD virus in serum [4,5]. This assay was used as a single dilution screening assay and as a titration assay to determine the protective antibody titre for FMD virus type O. The IAEA/WRL Bench protocol September 1997 and October 1998 was followed. The diagnostic threshhold used for the screening assay was set at 50% Inhibition at serum dilution of 1:32. Serum samples positive by screening assay were subjected to titration assay to determine the protective level of antibody at two fold dilutions of 1:64 and 1:128. The protective level of antibody titre is considered to be > 90 and this indicate that at the time of bleeding, the animal was protective against infection from homologous antigen of FMD virus sero-type.

3. RESULTS

The protective level of antibodies detected in cattle and buffaloes in 17 districts are given in Table II. The percentage of mean protective antibody vary from 14% in Sabaragamuwa province to 67.5% in North Central Province (NCP). High prevalence of protective level of antibodies detected in NCP and North Western Province (NWP) could be attributed mainly to natural active immunity after the outbreak of disease in 1997. During 1995/96 the vaccination coverage in these provinces has been very low (NWP = 0.8% and 0.5%, NCP = 6.5% and 4.8%). The first case of FMD was detected on 3 January 1997 in the NWP. The index case was traced back to 29.12.96. In general, the vaccination coverage in the country during 1995 and 1996 was low, which was the main factor for low protective level of antibodies in most of the provinces. Poor vaccination coverage was mainly due to non-availability of adequate vaccine for an island-wide large scale vaccination programme and absence of the disease for nearly two years. After the disease broke out the vaccination coverage was improved (NWP = 25.5% and NCP = 61.6% in 1997).

The percentage of mean protective antibodies in Western Province (WP) and Central Province (CP) were found to be 46% and 44.6% respectively. However, the vaccination coverage in WP during 1995 and 1996 has been only 3.9% and 3%. CP has covered only 14% during 1996 vaccination programme. Thus,, one would not expect any protective antibodies in animals in these provinces indicating that natural active immunity after an outbreak is the reason for the antibodies detected in this study. Uva and Southern Provinces (SP) have 29.8% and 25.8% mean protective antibodies respectively. Vaccination coverage during 1995, 1996 and 1997 in Uva Province has been 12%, 20% and 11.6% respectively. Uva is the only Province where FMD has not been reported yet. Although the mean protective level of antibody in Uva is low, compared to the vaccination coverage, it is not clear how these protective levels of antibodies have developed. Vaccination provides relatively short-lived protection immunity. The duration of immunity is proportional to the potency of the vaccine following a primary vaccination [6]. In cattle, a single inoculation with a potent FMD vaccine provides protection for only 3–6 months [6,7]. A detailed epidemiological study needs to be carried out at Uva to get answers to these interesting questions.

Vaccination coverage during 1995, 1996 and 1997 in SP has also been low (3.4%, 4.45% and 3.5% respectively). However, during the outbreak of disease at Kalutara district in WP, vaccination in border areas was adopted to have a buffer zone to prevent the leak of FMD to SP. According to the manufacturers recommendation a booster dose was also given. Thus the mean protective antibody level in the whole district of Galle was found to be 42.4%.

TABLE II. PROTECTIVE LEVEL OF ANTIBODIES IN CATTLE/BUFFALOES

District		Sample size	FMD Type 'O'	Mean for Province
Anuradhapura	123	123	69.10%	
Polonnaruwa		123	65.80%	
NCP				67.50%
Puttalam		123	65.00%	
Kurunegala		102	63.72%	
NWP				64.36%
Matale		102	51.00%	
Kandy		102	61.76%	
NuwaraEliya		99	21.20%	
CP				44.65%
Kegalle		99	13.10%	
Ratnapura		102	17.60%	
Sabaragamuwa				14.90%
Badulla		99	30.30%	
Moneragala		99	29.30%	
Uva				29.80%
Gampaha		102	41.20%	
Colombo		99	49.50%	
Kalutara		99	47.40%	
WP				46.00%
Galle		99	42.40%	
Matara		99	16.70%	
Hambantota		99	18.20%	
SP				25.80%

4. CONCLUSIONS

To prevent FMD it has been suggested that at least 80% of a herd must be protected from infection [8,9]. It also documented that in order to prevent an epidemic 70% of the population need to be protected [10]. SP remained free until animals from NCP and Eastern Province (EP, not included in the study) were brought for a development project without informing the veterinary authorities. The infection at present has affected two districts, Matara and Hambantota. The district of Galle up to now remained free. Ring vaccination has been carried out to prevent the spread of disease.

FMD control and eradication strategy in Sri Lanka no doubt has to focus on preventing the free movement of animals without Health Certificate, regular mass vaccination in areas bordering the endemic Provinces NWP, NCP and EP to maintain a high herd immunity of more than 80% to prevent future outbreaks and also to protect the improved breeds in the field and on State farms [11].

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