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.

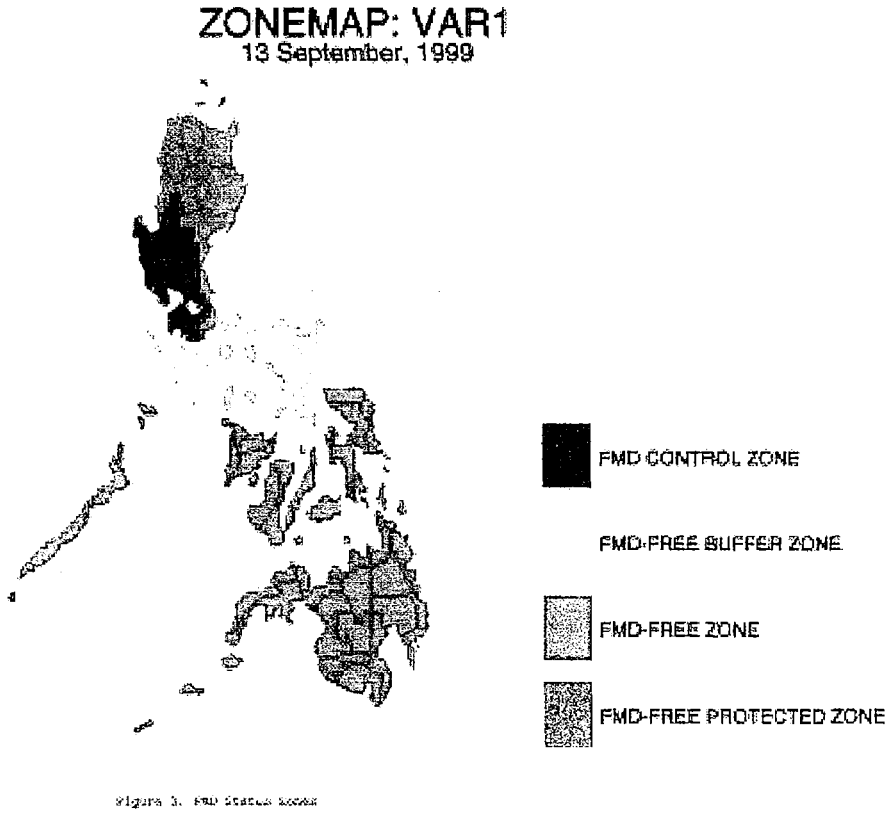

FIG. 1. Zonemap

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.

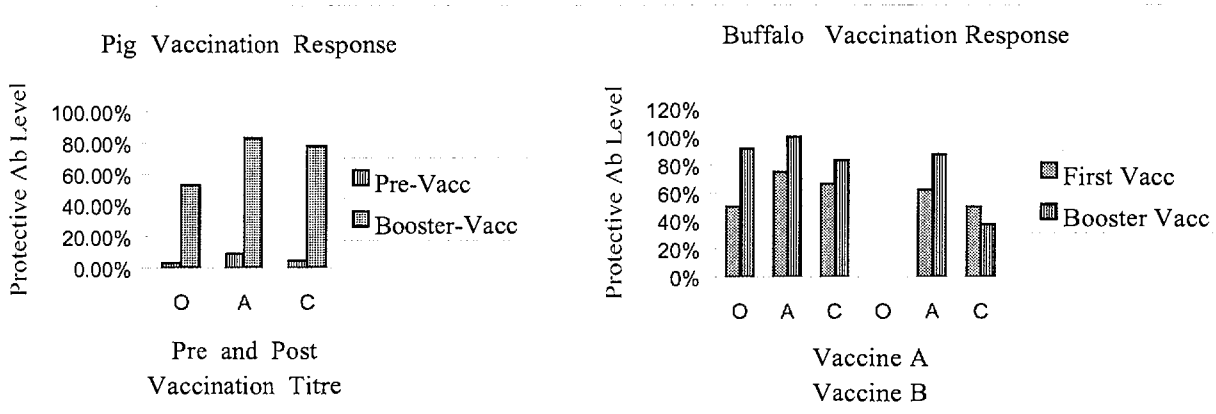

FIG. 2. Pig and buffalo antibody response after primary and secondary vaccination against FMD

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.

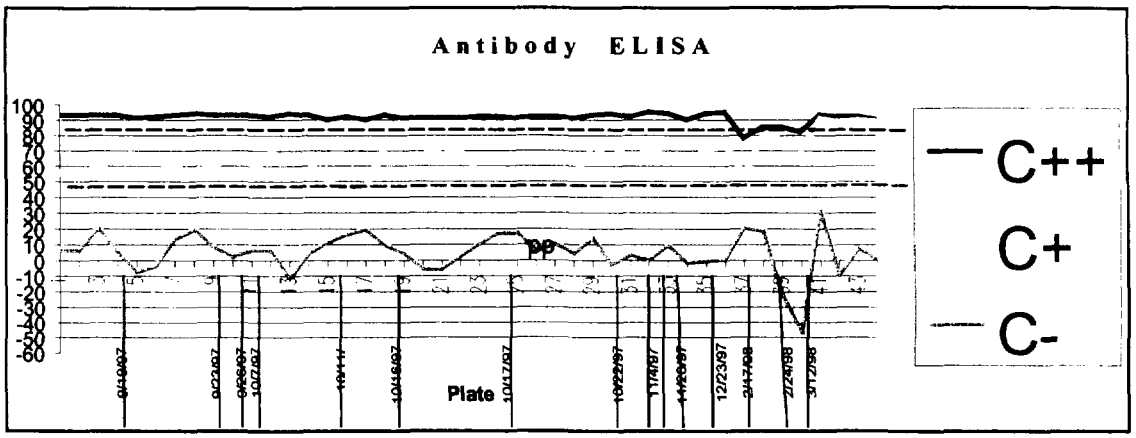

FIG. 3. Example of the FMD laboratory internal quality control of LPB-ELISA performance

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