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

L.D. SIMS, K.C. DYRTING, W.C. LO, K.W. WONG
Agriculture and Fisheries Department,
Castle Peak Veterinary Laboratory, Hong Kong, China

Abstract

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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.

ACKNOWLEDGEMENTS

We gratefully acknowledge the technical and financial support for this work provided by FAO/IAEA through the Co-ordinated Research Project on "Improved diagnosis and control of foot-and-mouth disease in Southeast Asia". The World Reference Laboratory, Pirbright, played a key role in supplying kits to Hong Kong and in processing diagnostic samples, both during this trial and in the past.

REFERENCE

[1] KITCHING R.P., SALT J.S., The interference by maternally-derived antibody with active immunization of farm animals against foot-and-mouth disease, Br. Vet. J. **151** (1995) 379–389.