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Management of classical swine fever and foot-and- mouth disease in Lao PDR

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128

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Management of classical swine fever and foot-and-mouth disease in Lao PDR

**Proceedings of an international workshop held in Vientiane,
Lao PDR, 20–21 November 2006**

Editors: J.V. Conlan, S.D. Blacksell, C.J. Morrissy and A. Colling



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Foreword

These proceedings are a compilation of research papers from collaborative work conducted by the CSIRO Australian Animal Health Laboratory (AAHL), Australia, and the Department of Livestock and Fisheries (DLF), Ministry of Agriculture and Fisheries, Lao PDR. The 3-year project commenced in July 2003 and at its conclusion was extended for a further 2.5 years. A workshop was held in Vientiane in November 2006 to share the research outcomes with partners throughout the South-East Asian region where foot-and-mouth disease (FMD) and classical swine fever (CSF) continue to be a problem for livestock producers. The workshop also provided a platform for scientists from neighbouring countries (Thailand, Cambodia, Vietnam, China and Myanmar) to provide up-to-date information on the disease situation in their respective countries. Likewise, organisations including the International Centre for Tropical Agriculture (CIAT) were able to present research conducted in Lao PDR, which reflects the multifaceted approach required to control these diseases.

Delegates attending the workshop were welcomed by Mr Michael Hassett, AusAID First Secretary, and Dr Somphanh Chanphengsay, Deputy Director of DLF, formally opened the workshop. Mr Hassett highlighted the strong commitment made by the Australian Centre for International Agricultural Research (ACIAR) and the Lao Government to better understand CSF and FMD. He also highlighted the importance of these diseases to Lao farmers and the strengths of the project to effect positive change at the village level through improved diagnostics and vaccine delivery. Dr Somphanh highlighted the strong and enduring link between DLF, ACIAR and CSIRO AAHL and the positive outcomes of almost 10 years of collaborative research for development. He emphasised the importance of strengthening food security, reducing poverty and strengthening the very important livestock sector by improving animal health.

These proceedings will provide readers with an insight into the complexities of disease control in Lao PDR and the wider region. The research presented in the following pages encompasses pig production, technically orientated laboratory research, social perspectives and epidemiological research, all of which are required as we endeavour to achieve positive change for livestock farmers in South-East Asia.



Peter Core
Chief Executive Officer
ACIAR

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Introduction

Laurence J. Gleeson^{1,2} and Axel Colling³

The strengthening of food security and the reduction of poverty in rural areas are major priorities of the government of Lao PDR, and improving livestock production systems has been assigned the highest priority in the government's rural development strategy. Livestock are an important source of cash income for Lao farmers, and disease is well recognised as a major constraint to livestock productivity with consequent significant impacts on the livelihoods of the rural poor. The control of two of these diseases, classical swine fever (CSF) and foot-and-mouth disease (FMD), is of high priority in the South-East Asian region due to their impact on productivity and trade and, in the case of CSF, its high mortality.

Significant advances in understanding the diagnostic and epidemiological issues of these diseases in Lao PDR were achieved during an earlier ACIAR-funded project (AS1/1994/038). During this project the capacity of the Lao Department of Livestock and Fisheries to carry out routine diagnosis and surveillance using participatory approaches was significantly enhanced. The project was concluded in June 2003 after more than 6 years of successful research and development. The natural progression of research was then to concentrate on disease management at the village level to achieve sustainable disease control for the village farmer. Hence, the project presented in these proceedings, AH/2003/001 'Management of classical swine fever and foot-and-mouth disease at the village level in Lao PDR' came to fruition.

The key objective of the new project, controlling CSF and FMD at the village level, was to be achieved through five activities:

- (i) development of a rapid, cheap, portable and sensitive diagnostic test for CSF to improve diagnostic output
- (ii) establishment of a system for the delivery of locally produced CSF vaccine to village pigs
- (iii) evaluation of the impact of CSF vaccination
- (iv) continued monitoring of the epidemiology of CSF and FMD
- (v) communication of research outcomes to all stakeholders at district, national, regional and international levels.

The successful development of a rapid and portable diagnostic test for CSF has been an important achievement of the project and the test is now used routinely in laboratories in four provinces—Luang Prabang, Oudomxay, Luangnamtha and Champasack. This has resulted in an improvement in the turnaround time from sample collection to providing the farmer and disease control authorities with a test result. Widespread farmer awareness of the test's applicability is critical to the long-term and successful uptake of the test in Lao PDR. Equally importantly, farmer participation will be affirmed if action is taken once an outbreak of CSF is identified. Nevertheless, the official confirmation of a notifiable disease outbreak, in this case CSF, will be undertaken by the National Animal Health Centre, requiring samples to be sent to Vientiane for confirmative testing.

The project was also successful in assessing issues related to CSF vaccination and identifying a significant need for improvements to production, storage and delivery. The vaccine was found to be less stable than anticipated. By international standards, a vaccine should be stable for at least as long as that stated by the manufacturer, but this was not the

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case with the locally produced CSF vaccine. However, the strict control of temperature and delivery shortly after manufacture resulted in successful outcomes when applied in village pig-raising systems.

As this proceedings goes to press, another ACIAR project, 'Improved control of pig diseases in Lao PDR', is in preparation. The main issues of this project are to estimate the risk of CSF outbreaks for the main village pig production systems. The project includes the development and comparison of suitable biosecurity interventions such as vaccination and quarantine, as well as the provision of assistance to establish a regulatory, advisory and marketing infrastructure that will improve the management and operations of the vaccine supply chain so that it becomes a cost-effective and sustainable enterprise capable of supplying quality vaccines to international standards. Some countries in the region, such as Vietnam, have successfully produced high-quality vaccines, and an approach to regionally produce and commercialise a powerful and quality controlled vaccine would be an attractive option. Regional and international organisations such as the Asian Development Bank, FAO and World Organisation for Animal Health / Office International des Epizooties

(OIE) strongly support initiatives to share knowledge and experience about modern methods for improved disease control and eradication.

During the course of this project, three students completed postgraduate and undergraduate degrees following studies related to project activities. Tess Vitesnik from the University of Melbourne came to Vientiane on the Australian Youth Ambassador for Development (AYAD) volunteer program for 11 months in 2006 to carry out research relating to her Bachelor of Animal Science degree. Kristina Osbjer from the Swedish University of Agricultural Sciences conducted research in collaboration with this project and the Centre for International Tropical Agriculture that contributed to her Master of Veterinary Science degree. James Conlan attained a Master of Science degree from the University of Melbourne following 2 years of research in Vientiane. While these were great achievements for the project, the critical shortage of Lao veterinarians and animal science specialists remains an important issue that should be addressed. It is unlikely that sustainable control of livestock diseases or any significant development of the livestock industries in Lao PDR will be achieved if this deficit is not corrected.

Section 1

Pig production and extension

International pig production and implications for Lao PDR

Ross Cutler^{1,2}

Worldwide change in the pig industry

Over the period 1990–2003, worldwide pig production increased by 37%, with the biggest increases being in Asia. From 1970 to 2003, pig production in Asia increased by 534% compared with 88% in Europe and 58% in North and Central America (Table 1; Windhorst 2004); over roughly the same period, production in Lao PDR increased by about 4%. In 2002 total pork production from the 1.4 million pigs in Lao PDR was about 31,600 tonnes (FAO 2005a). However, this is likely to be an underestimate as the pig population has subsequently been reported by the government to be greater than 1.6 million head in 2002—the correction did not reach FAO before the lower numbers were published.

The People's Republic of China is the dominant pig-producing nation in Asia. It currently produces about 47% of global pork and is expected to increase output to about 50% of global production by 2012 (Table 2). While China currently exports about 5% of production, it is forecast that by 2012 it will import about 589,000 tonnes of pork (Windhorst 2004). A key issue for China is whether it will be able to produce enough feed to meet its production needs. While China may not be a net exporter, it is likely that local trade will mean that some pigs find their way into Lao PDR over the next decade.

Thailand has been a consistent pigmeat producer. It currently produces about 655,000 tonnes annually but this figure has increased by only 3.5% over the last 10 years (FAO 2005b). It is likely that Thailand will take advantage of domestic market opportunities and is therefore unlikely to export more to Lao PDR.

Of direct and immediate interest to Lao PDR is Vietnam (Table 1). In 2003 it was the eighth highest pork-producing country in the world but by 1990 wasn't represented in the top 10 countries. Since 1990 Vietnam has increased its exports from about 2% to about 5% of production. Per capita consumption of pork has increased from about 10 kg per person to about 20 kg over the period 1990–2002. Vietnam produces about 1.795 million tonnes of pork from its 23 million pigs, and average carcass weight is about 81 kg (FAO 2005c). Vietnam can be expected to continue to export increasing numbers of pigs to Lao PDR.

The European countries have historically been the major exporting nations. Over the last 10 years Canada and the USA have increased their export market presence and Brazil has also become a major exporter (Tables 3, 4). By 2004 Canada and the USA were exporting in excess of 879,000 tonnes and 840,000 tonnes, respectively, and China was exporting 512,000 tonnes, but Denmark was still the world leader, exporting 1.7 million tonnes (Australian Pork Limited 2005). In the next 5 years, world exports will be dominated by the EU, Canada, Brazil and USA (Windhorst 2004).

Worldwide production and output changes

While the number of pig farms worldwide is falling, farm output is increasing. Figure 1 is derived from data

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from the Australian pig industry, but exactly the same trends are evident in every pig-producing country in the world. In the USA the concentration of ownership is particularly evident, with the 10 largest producers owning approximately 19% of the sows (Table 5).

Even though the number of farmers has decreased in Australia, the number of sows has remained constant over the last 30 years, with herd size increasing from about 12 sows in 1972 to 159 sows in 2004. Similar trends are occurring in every country in the

Table 1. Regional development of pigmeat production between 1970 and 2003, data in thousand tonnes (Source: Windhorst 2004 citing FAO database)

Region	1970	1980	1990	2003 ^a	Increase (%)
Africa	261	346	590	739	183
Asia	8,452	15,810	29,568	53,505	534
Europe	13,516	19,299	21,641	25,381	88
USSR	4,453	5,184	6,655	–	–
North and Central America	7,746	10,009	9,103	12,247	58
South America	1,306	1,741	1,900	3,373	158
Oceania	239	239	405	534	123
World	35,793	52,679	69,862	95,779	168

^a New regional classification by FAO

Table 2. The 10 leading countries in pigmeat production in 1990 and 2003 (Source: Windhorst 2004 citing FAO database)

Country	1990		Country	2003	
	Production ('000 t)	Share (%)		Production ('000 t)	Share (%)
China	24,016	34.4	China	45,567	47.6
USA	6,964	10.0	USA	8,931	9.3
USSR	6,654	9.5	Germany	4,123	4.3
Germany	4,457	6.4	Spain	3,200	3.3
Poland	1,855	2.6	France	2,350	2.5
Spain	1,789	2.6	Brazil	2,145	2.2
France	1,726	2.5	Poland	2,050	2.1
Netherlands	1,661	2.3	Vietnam	1,795	1.9
Japan	1,555	2.2	Denmark	1,761	1.8
Italy	1,333	1.9	Russia	1,657	1.7
10 countries	52,010	74.4	10 countries	73,597	76.8
World	69,862	100.0	World	95,779	100.0

Table 3. Regional development of pigmeat exports between 1970 and 2002, data in thousand tonnes (Source: Windhorst 2004 citing FAO database)

Region	1970	1980	1990	2002 ^a	Change (%)
Africa	9	3	3	7	-22
Asia	121	148	470	400	+231
Europe	1,495	2,207	3,298	5,206	+248
North and Central America	65	210	369	1,490	+2,192
South America	4	1	19	595	+13,875
Oceania	8	5	8	66	+725
World	1,702	2,574	4,167	7,764	+356

^a New regional classification by FAO

world. It is most obvious in the USA, where the sow herd population totals about 10 million.

Herd output is increasing as well, usually by increases in slaughter weight, although this fluctuates in response to market demand. Australia provides a good example, with slaughter weights increasing from around 55 kg carcase weight in the 1970s to a current average of about 72.5 kg (Figures 2 and 3).

In Europe carcase weights range from about 75 kg in Denmark to over 80 kg in the Netherlands and Germany; in North America they also exceed 80 kg. Carcase weights respond to market forces but higher weights confer higher herd efficiencies on overall farm production efficiency and reduce the

processing cost per kilogram. It costs just as much to kill a 50 kg pig as a 100 kg pig.

European countries, especially Denmark, have been able to demonstrate a significant genetic improvement in reproductive performance, which contributes significantly to national output (Table 6). The data also highlight the production skills of the top 25% of Danish producers.

Currency fluctuations and disease impact on markets

Markets are also changing. The EU, Canada, USA and Brazil are the dominant exporters of pork. New trading arrangements and animal and human health

Table 4. The 10 leading countries in pigmeat exports in 1990 and 2002 (Source: Windhorst 2004 citing FAO database)

Country	1990		Country	2002	
	Production ('000 t)	Share (%)		Production ('000 t)	Share (%)
Netherlands	771	31.0	Denmark	1,293	16.7
Denmark	471	19.0	Canada	772	9.9
Belgium/Lux.	278	11.2	Netherlands	750	9.6
Hungary	178	7.2	Belgium	722	9.3
Germany	164	6.6	USA	653	8.4
France	129	5.2	Germany	604	7.8
China	126	5.0	France	526	6.8
Canada	108	4.3	Brazil	511	6.6
USA	67	2.7	Spain	468	6.0
United Kingdom	49	2.0	China	336	4.3
10 countries	2,342	94.2	10 countries	6,635	85.4
World	2,485	100.0	World	7,764	100.0

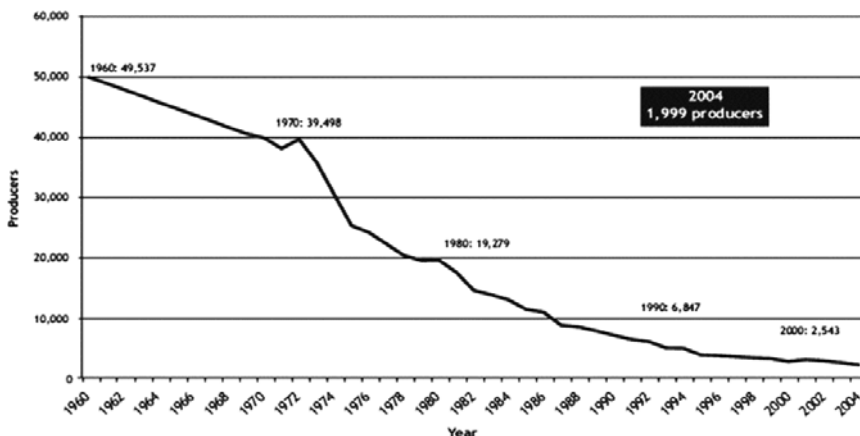


Figure 1. Number of pig producers in Australia (Source: Australian Pork Limited 2005)

concerns influence trade events. In response to the changing market landscape and World Trade Organization agreements, Australia first started importing pork in 1992. Movements in pork imports are also influenced by local demand and the relative strengths of international currencies. Fluctuations in Australian pork imports and the value of the Australian dollar (against the US dollar) are reported in Figures 4 and 5.

Two major diseases have also been responsible for stimulating export demand for Australia (Figure 6). An incursion of foot-and-mouth disease into Taiwan in 1996 meant that Taiwanese pig producers were no longer able to supply Japan, and Australia took up some of that market and grew it during a period of favourable currency exchange rates. In 1998 the outbreak of Nipah virus in Malaysia and the consequent death of an abattoir worker in Singapore provided a very significant market opportunity in fresh pork sales (Figure 6).

Cost of production has a major impact on international competitiveness. However, Denmark has demonstrated that it is not necessary to be the lowest cost producer to achieve market success (Figure 7). Denmark is assisted by favourable trading arrangements within the EU and its reputation as a supplier of high-quality pork.

Table 6. Reproductive performance in Denmark (Source: National Committee in Pig Production 2003)

	2000	2001	2002	2002 (best 25%)
Pigs/sow/year	22.5	22.7	23.1	26.1
Pigs born alive	11.9	12.1	12.3	12.9
Weaned/litter	10.4	10.5	10.7	11.4

Table 5. The 10 leading companies in pig production in the USA in 2003 (Source: Windhorst 2004, citing Freese 2003)

Company	Location of sows (states)	Number of sows
Smithfield Foods	NC, VA, UT, MO, OK, IA, TX	756,200
Premium Standard Farms	MO, NC, TX	225,000
Seaboard Farms	KS, CO, OK	213,600
Prestage Farms	NC, MS	129,000
Cargill	NC, AR, OK	118,000
Iowa Select Farms	IA	100,000
The Pipestone Farms	MN, SD, IA, NE, OH	100,000
Christensen Farms	MN, NE, IA	94,000
Goldsboro Hog Farm	NC	74,000
The Hanor Company	OK, NC, WI, IL, OH, IA	73,500
10 companies		1,883,300

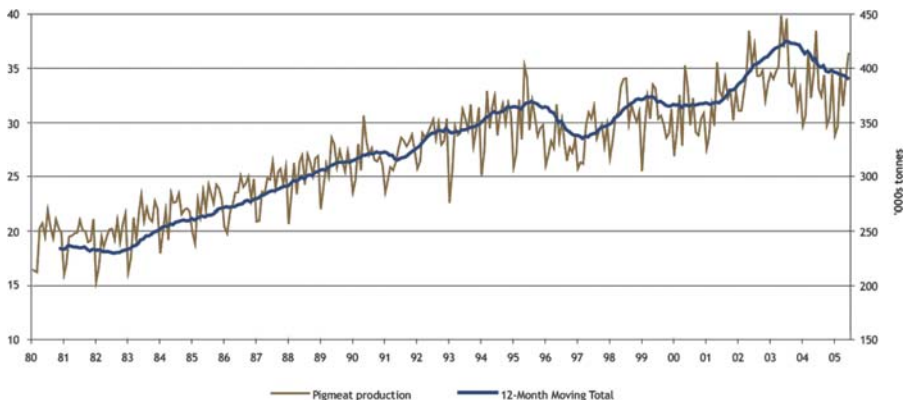


Figure 2. Pigmeat production in Australia (Source: Australian Pork Limited 2005)

Implications for Lao PDR

The data and experiences from around the world lead to the following conclusions:

- The output of pork is increasing worldwide.
- Herd sizes increase over time.
- Trade in pork is increasing worldwide.
- Production increases in Vietnam will lead to more exports to Lao PDR.
- Imports from Vietnam will likely place downward pressure on Lao pork prices; that is, villagers will get less for their pigs.
- To maintain profit, Lao pig producers will have to decrease their cost of production by increasing slaughter weight from the current base of 26 kg, increasing disease control and improving feeding practices.

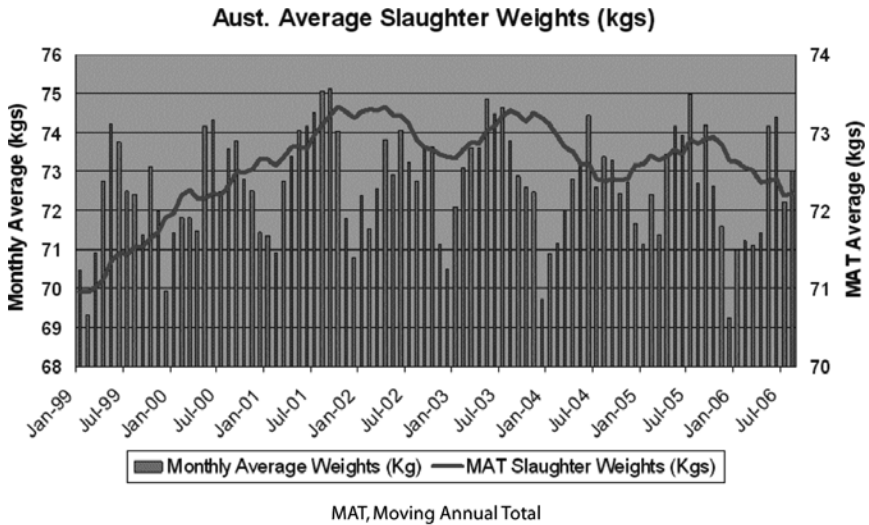


Figure 3. Increases in pig slaughter weights, Australia (Source: Australian Pork Limited 2005)

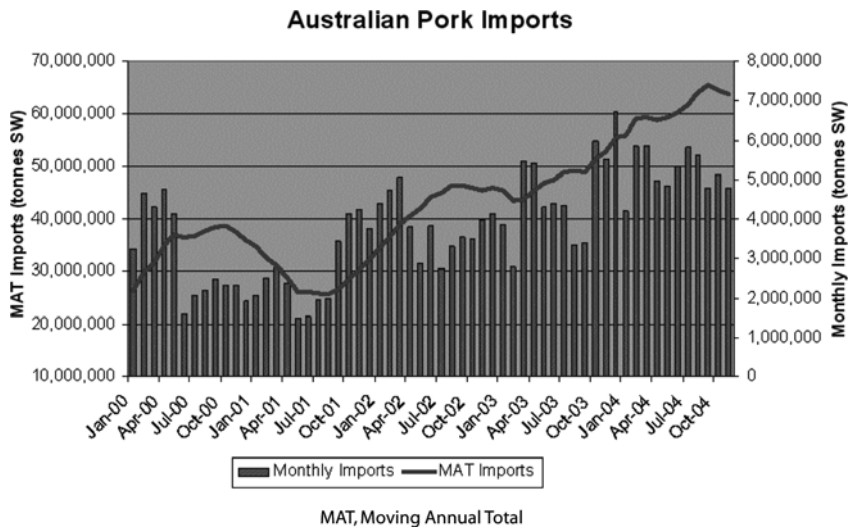


Figure 4. Australian pork imports (Source: Australian Pork Limited 2005)

- As Lao producers change their production methods in response to competition from other countries, they will encounter new health and management problems associated with increasing the intensity of production.
- If Vietnam cannot control foot-and-mouth disease and exports to Lao PDR are prohibited, Lao producers will have some time to adjust.
- In the longer term it is unlikely that Lao PDR will become a major force in pig production because

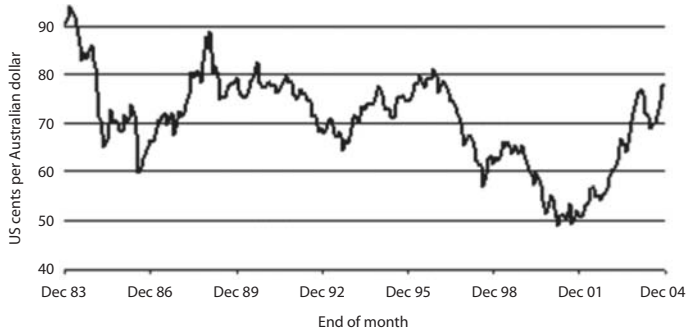


Figure 5. Changes in the value of the Australian dollar (Source: Baker and Barber 2005)

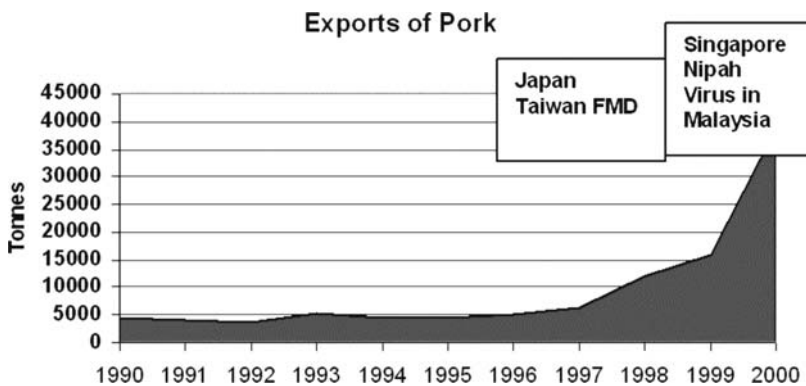


Figure 6. Exports of pork from Australia in relation to international events 1990–2000

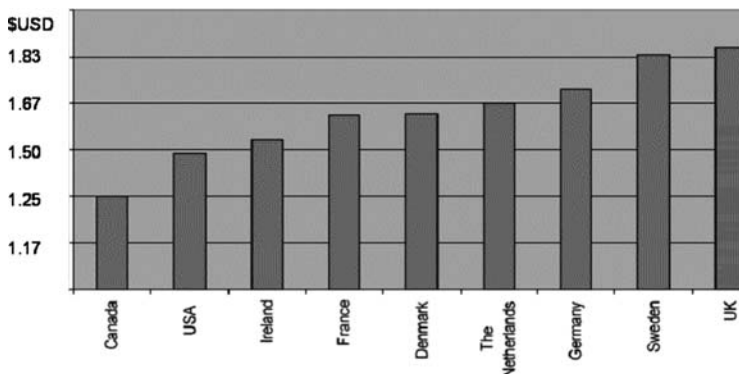


Figure 7. Cost of pork production per kg dressed weight (Source: adapted from Rasmussen 2004)

of genetics and the limited supply of feed grain. However, in the short term pig production will remain an important source of cash flow for villagers.

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Smallholder pig systems in Luang Prabang and Xieng Khouang provinces: Current feed situation and potential of forage legumes for improving pig productivity

Phonepaseuth Phengsavanh^{1,2}

Abstract

Pig production plays an important role in smallholder farming systems in upland villages of Lao PDR. It serves as an income source or as capital accumulation needed when crop (rice) production fails. Farmers raise pigs in many ways or systems depending on the availability of feed resources and the intensity of farming systems. There are two main problems in smallholder systems—disease and seasonal feed limitations.

Stylosanthes guianensis CIAT 184 (Stylo 184) has been introduced to overcome limitations to feed resources (in both quantity and quality). The impacts of using Stylo 184 are increased weight gain and savings in time and labour needed for finding and collecting feeds. Apart from these two main effects, other benefits include, for example, a systems change from free-range production to a more manageable penned system and a reduction in piglet mortality. The potential for introducing other high-quality legumes for feeding village pigs is high; however, they should suit the local farming systems.

Introduction

Pig production in Lao PDR is largely (>80%) in the hands of smallholder farmers. Pigs are mostly kept by farmers in the mountainous areas in the north, where average ownership is approximately 13 pigs per family and can be up to a maximum of 30 pigs per family. The commercial pig farms are mostly located near the big towns. According to Keoboulapheth (2003), pigs are kept in three major systems—free-range, confinement and pens.

The main traditional feed resources are agricultural by-products and vegetables or weeds that grow naturally in the forests, along streambanks and in

cropping areas. Naturally occurring feeds, maize and cassava, are always boiled and mixed with rice bran before being fed to the pigs. Feeding is usually twice a day—in the morning and in the late afternoon.

The two main problems in pig production are disease and seasonal feed shortage. Disease is the most serious problem and farmers report that, in the case of an outbreak of disease, especially swine fever, up to 70–80% of pigs in the village die (Phengsavanh and Stür 2006). Feed shortage, both in quantity and quality, severely limits productivity in smallholder pig production systems (Thorne 2005).

Since 1997 the Forages for Smallholders (FSP) and Forage and Livestock Systems (FLSP) projects have introduced *Stylosanthes guianensis* CIAT 184 (Stylo 184) to farmers for use as feed for cattle and buffalo. Innovation has now resulted in some farmers feeding Stylo 184 to pigs and discovering that they could use it to replace natural feeds.

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Current feed situation in smallholder pig systems

Farmers in upland villages of Lao PDR usually use locally available feeds such as rice bran, maize, cassava root and vegetables which occur naturally in the fallows, near crop areas and in the forest. The availability of these feeds depends on the seasons and locations. Farmers in the more upland areas provide more nutritional feeds to their pigs than farmers in lowland areas in terms of energy sources. Upland farmers usually use rice bran, cassava roots and maize as the main diet, mixed with other green feeds from the leaves of crops such as ‘Bone’ (*Colocasia*), ‘Phouk’ (*Alocasia*), paper mulberry, ‘Yahuabin’ (*Crassocephalum*) and others. Farmers in lowland areas seem to base feed more on rice bran, a little bit of broken rice and available green feeds. The sources of protein are mainly locally available green feeds, but they usually have a high fibre content which has low digestibility. Adding high-quality legumes can help to solve this problem.

Potential of forage legumes in improving pig productivity

Stylo 184 was originally introduced to farmers in upland villages as a forage legume to overcome feed shortages for ruminants, but through innovation it has also been used as a pig feed by many farmers. As a result, farmers are experiencing significant impacts on pig growth rates and are saving time and labour in feed collection and preparation. The details of impacts reported by farmers are as follows:

Impact of Stylo 184 on saving time and labour for collecting natural feed

One limitation for pig production in the upland areas is the time taken for the collection of natural feed—often more than 3 hours per day. The data in Table 1 show the benefit of using Stylo 184, as it reduces the time taken from 195 minutes to about 90 minutes if they still need to cook other feed such as cassava roots. For families that use fresh Stylo 184 and mix it with rice bran and maize meal, the preparation time could be reduced to 40 minutes.

Table 1. Labour and time savings (Source: Phengsavanh and Stür 2006)

Items	Before		Now			
	Time spent (min)	Who does the work?	Stylo shortage (min)	Plenty of Stylo (min)		Who does the work?
				+ cooking ^a	+ no-cooking ^b	
Collecting feed	125	W/M	55	0	0	W/M
Cooking	50	W	50	50	0	W
Feeding	20	W	20	20	20	W
Collecting Stylo	–	–	20	20	20	M
Total	195		145	90	40	

^a Cooking includes cassava roots and maize

^b Farmers who use only rice bran and fresh Stylo
min = minutes preparing feed; W = women; M = men

Table 2. Productivity of growing pigs supplemented with traditional green feeds or Stylo (Source: Phengsavanh and Stür 2006)

	Traditional green feeds (no Stylo)	Supplemented with fresh Stylo	SE ^b (±)
Duration of production cycle (months)	18.0	8.7	0.95
Initial weight (kg)	14.0	15.0	0.4
Final weight (kg)	65.3	65.1	3.2
Calculated ADG ^a (g/day)	107.0	207.0	12.2

^a ADG = average daily weight gain

^b SE = standard error

Stylo 184 has great benefits in that it can be adapted to different areas and grows well in a wide range of soils, it is easy to cut and it can be fed fresh to the pigs. Feeding fresh Stylo 184 can reduce the time of feed preparation by approximately 1 hour.

Impact of Stylo 184 on the growth rate of local pigs

The information presented in Table 2 is based on farmers' estimations of initial and final weights and their recollection of the time taken from the start of the fattening period to final sale. Average daily weight gains were calculated from these estimations.

Farmers usually fatten their pigs from about 4 months of age at a weight of about 14–15 kg and sell at an estimated 65 kg. To reach this weight in the traditional way, it takes about 18 months but, by using Stylo 184 as a supplementary feed, the fattening period was reduced to less than 9 months (Table 2). This means that the average daily weight gain of growing pigs was doubled from 107 g/day in traditional feeding systems to 207 g/day for pigs supplemented with Stylo 184. Phengsavanh and Stür (2006) explained that factors other than Stylo 184 supplementation could have played a role (e.g. better management, Stylo 184 being fed in addition to other feeds rather than as a substitute for other feeds), but the consistency and magnitude of the response shows that Stylo 184 has had a major impact on pig productivity.

Other benefits from Stylo 184

In addition to the main impacts mentioned above, there are several other benefits that have been mentioned by farmers, including: changes in pig-raising systems from free-range to semi- or full-confinement and penning systems, an increase in the sur-

vival rate of piglets as a result of better management (more time for taking care of pigs) and an increase in the number of pigs produced for the time input, which means that farmers can earn more income for their family.

Conclusion

The main direct benefits from the introduction of Stylo 184 for upland pig farmers are a 50% reduction in fattening time with an associated increase in daily weight gain (almost doubled), and labour and time savings for women, which is very important for upland farmers. Apart from these two main effects, there are also livelihood impacts, e.g. the changing gender roles in pig production. When Stylo 184 is introduced, men are more involved in pig production, especially in the daily harvesting of Stylo 184 for feed. The positive impacts of Stylo 184 open up other opportunities for improving feed and introducing additional improvements to production systems such as housing, management and animal health.

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Extension models for pig production in Lao PDR: The art of how to best use limited resources

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Introduction

Optimal systems for pig production involve adequate feed, assuring animal health and selective breeding. Because conditions in Lao PDR are diverse, the optimal system, that is the one which will best fit farmers' limited resources, will vary from site to site. Identifying the best system is therefore a challenge. Farmers typically do not adopt a new system in a single leap, but rather component by component. Thus, the second challenge is to identify the pathway that will lead farmers to progressively put each component together to build their optimal system.

This paper illustrates a natural pathway towards achieving an optimal system in rural areas of Lao PDR. It examines the different levels of intensification and the actions that can be applied, including participatory market chain studies, to move farmers along these pathways. Overall, it is an exercise in how to use limited extension resources most effectively.

Initiating system change

Pig production systems in rural areas of northern Lao PDR typically allow the animals to scavenge free-range, with the farmer providing mainly bran and forest greens as feed. As well as slow weight gain, many other problems result, including poor litter survival and easy transmission of disease. Most improved production systems are built around penning the pigs but, without adequate feed resources, farmers are unable to apply such systems.

The project 'Forage and Livestock Systems' (funded by AusAID and implemented by CIAT and the Lao National Agriculture and Forestry Research Institute during 2000–05) was able to initiate system change through three activities:

- problem diagnosis to reveal farmers' immediate constraints
- introduction of planted forages to resolve their immediate problems
- supportive follow-up and identification of innovative farmers who were able to make systems changes.

The application of problem diagnosis showed that, while the poor diet limited weight gain, the immediate problem for farmers was providing the time and labour to source feed for their pigs. Collecting forest greens typically took farmers 2–3 hours a day. Their initial interest in forages was thus mainly to save time and labour rather than any expectation for better weight gain. Working with a population of farmers across a number of villages provided the opportunity for innovative farmers to emerge. A few looked beyond the reduction in labour and, with an improved diet, began to pen their pigs. By carrying out follow-up, staff identified such farmers and supported them in their innovation.

In particular, *Stylosanthes guianensis* CIAT 184 (Stylo 184) was provided as a supplement to the normal bran-based diet, increasing the overall digestibility of the feed and enabling farmers to begin to pen their pigs. The result of this single intervention has had substantial impact on productivity, including a reduction in the time needed to fatten pigs from 18 to 9 months, an increase in survival rates of weaned piglets from four to six per litter and an increase in the number of litters per sow from 1.5 to 1.8 per year (see Phengsavanh 2008, these pro-

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ceedings). In turn, the following outcomes have begun to emerge:

- Pigs are now sold at regular intervals rather than just at times when cash is needed.
- Households previously not raising pigs have begun to purchase piglets for fattening.
- Traditional share-raising systems accessed by poor households now have the potential to make a significant impact on their livelihoods.

The change in productivity has thus altered the position of pig raising within village economies in quite profound ways.

Intensifying production systems

While the benefits of improved feed have been significant in themselves, they are just the beginning of the pathway towards optimal systems of pig raising, which include animal health, strategic feeding and selective breeding. To have introduced these interventions to farmers before they had begun to pen and feed their pigs would have gained little response and wasted scarce extension resources.

Although farmers who have adopted the use of Stylo 184 have taken the step to make a systems change, it is not necessarily assumed that this will flow on to the application of these more intensive interventions. Farmers require more technical knowledge, purchase of inputs and services, and perhaps also community agreements for the management of pigs within the village. Reaching these more intensive levels of pig production is a new challenge that we are just beginning to address. Farmers need specific incentives to invest in these interventions.

We have begun to learn from another project implemented by CIAT ('Small-scale Agro-enterprise Development for the Uplands' (SADU)) which pilots mechanisms to link farmers to markets. This is beginning to show that where farmers have developed a 'market orientation', they begin to seek specific technologies to meet market demands. This could play a role for pig production in a number of ways:

- investment in improving production systems, which results in a willingness to pay for veterinary services
- knowing seasonal demand and prices, which requires breeding cycles and feed mixes to achieve output that coincides with seasonal demand

- tailoring the size of the pigs to market demand (i.e. bigger is not always better)
- adapting to consumer preferences for types of flesh (which vary from rural to urban markets), which requires changes in the type of pig raised and/or the feed formula
- all-year fattening (i.e. in dry months forages are not productive), which requires alternative feed sources or technologies for storing feed (silage, leaf meal etc.).

Thus, instead of introducing a general package of improved technologies, it may be better to engage farmers in participatory market chain assessment to identify constraints and opportunities. The Agro-enterprise Development Process (AEDP) used by SADU uses simple tools that reveal market trends, seasonal variation in demand and prices, and preferred buying conditions. As these become clear, farmers should then demand the technologies to meet the specific market requirements. Uptake of more intensive technologies can then be very rapid. This role of market orientation will be piloted by the Asian Development Bank grant project 'Capacity Building for Smallholder Livestock Systems' (ADB Lao 4406) in Xieng Khouang and Luang Prabang provinces in 2007.

Concluding comments

There are two lessons that can be drawn from this work. First, there is a pathway for smallholder farmers to move from free-range low-input systems to optimal systems for pig production. The first step is for farmers to begin to manage their pigs. The introduction of Stylo 184 enabled farmers to pen their pigs and make a worthwhile enterprise. Achieving this step is a precondition for the introduction of typical interventions such as improved feed, animal health and selective breeding. It would be premature to introduce these interventions before farmers had the capacity to manage their animals.

The second lesson is that, in both these steps along the development pathway, the 'technologies' were not the message but a means to an end. The extension objectives were first to reduce time and labour for the farmers' immediate problem of providing feed to their pigs, even in a low-input system. Before they move on to apply more intensive technologies, farmers need specific production objectives, which can come from understanding market requirements. The next level of technologies then becomes the

means to achieve these market-linked objectives. Thus, it is important to appreciate the steps along a development pathway, both in terms of what is motivating farmers as well as the levels of technology required for each step.

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Improving pig production systems in the Integrated Upland Agricultural Research Project target area—Pak Ou district, Luang Prabang province, Lao PDR

Gavin Varney¹

Background

The 'Integrated Upland Agricultural Research Project' (IUARP) was an initiative of the National Agriculture and Forestry Research Institute (NAFRI), with funding and technical inputs from the Ministry of Agriculture and Forestry (MAF) and several international research agencies. The goal of the project has been to conduct multidisciplinary, integrated and participatory research that would address the major problems that farmers in the uplands of northern Lao PDR are currently facing. Activities commenced in 1999 in 10 villages in Pak Ou district, Luang Prabang province. A participatory problem diagnosis was conducted in the villages at the beginning of the project to identify the major constraints in local livelihood and agricultural systems. Livestock production was identified as being a key component to village and family wellbeing.

The International Centre for Tropical Agriculture (CIAT) has been involved in the IUARP since its inception. Major CIAT activities have included the provision of extension materials; training of district staff in technologies and extension methods; and the provision of quality animal feeds including forages, cassava and sweet potato. In late 2005 CIAT expanded their inputs to include improving livestock management systems, particularly in areas of pig nutrition, animal health and breeding management.

Activities commenced in seven villages in early September 2005 and continued until May 2006, with some ongoing support activities after this date.

Traditional IUARP pig production systems

Pig production is a traditional and important activity for IUARP families; between half and three-quarters of households are engaged in pig raising in some form. All villages have a combination of households that breed and fatten pigs. In most pig-raising households animal health inputs are minimal, and mortality and morbidity rates of both young pigs and breeding stock are high. Production levels are also low, due largely to poor nutrition, and knowledge of effective general health and management skills is limited.

The predominant breed has been the small black *Moo laht* (mulaht), and the low-input pig production systems have involved night penning and daily scavenging or free ranging. The majority of pigs roam freely and scavenge feed from within and around the village. Despite being free roaming, this system is still very labour intensive, with supplementary cooked feed being supplied morning and night. Feeds available are seasonally dependent and include rice bran, maize, taro, sweet potato, banana stem, paper mulberry leaf, cassava root and vegetation collected from the forests and fallow fields. No commercial feeds are provided. Sourcing and preparing feed can take as much as 2–3 hours per day and this is predominantly the work of the womenfolk.

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A small number of pigs can be confined post weaning in rudimentary housing where they remain until they are sold. The performance of these pigs is often lower than those that are free ranging. This reflects the poor feeding and watering of pigs in terms of both quality and quantity when put in confinement. Growth rates are slow and animals can take up to 15 months to reach a weight of 40–50 kg.

At the first signs of disease or ill thrift, it is not uncommon for farmers to sell or slaughter their ailing animals for home consumption.

Constraints that provide a challenge for IUARP pig producers

The main constraints facing IUARP pig producers are related to health and disease, feeding and nutrition, and breeding management. These constraints result from the lack of general livestock management skills and access to appropriate knowledge and information.

Disease is the most significant threat to IUARP pig farmers and, although unconfirmed, the most likely disease is classical swine fever (CSF). Vaccination against CSF has been variable and many farmers bemoan the fact that their animals continue to die even after vaccination. Even in the absence of epidemic outbreaks, mortality rates can be as high as 20%, and there has been some reluctance to invest more money and resources into pig production if the gains are cancelled out by outbreaks of epidemic disease.

Given the conditions under which the pigs are raised, the prevalence and impact of parasites must have a significant impact on the general wellbeing and growth rates of all animals.

Animals for fattening are often purchased from local markets, neighbouring villages and passing traders without quarantine or realisation of the consequences. Sick animals are not removed from their pen or herd and are very seldom given preferential treatment.

Poor diet quality is a greater problem than limited feed availability, and nutrient intakes are not well balanced for protein, energy, minerals and vitamins. This is most obvious during sow lactation, and in penned animals where diets are often energy rich but protein deficient. This problem arises not only because of the lack of a suitable protein source but also because of the lack of information and knowledge on how best to use existing available feeds.

Water, the cheapest and most available nutrient of all, is only on offer when mixed with the morning and evening feed rations.

There has been no managed breeding program for sows and only limited selection criteria for retaining animals for breeding. The availability of suitable boars has been an issue, with many farmers allowing their sows to range free in the hope of an opportune mating. Although farmers wish to do so, mainly because of market premiums, male piglets have not been castrated because of lack of knowledge and skills. Some of the pigs appear to be badly inbred and it is obvious that brothers are mating sisters, fathers mating daughters and sons mating mothers.

There is considerable variation in litter sizes and also in the number of piglets successfully raised by the sow. Up to 50% of piglets born do not reach weaning age, due mainly to smothering, starvation, disease and misadventure.

IUARP livestock producers recognised the above constraints and shortcomings in their pig production systems. Their involvement with IUARP researchers has focused on improving soils and increasing crop yield, but has not provided them with the necessary assistance, technologies and skills upgrading they need to improve their livestock management. Farmers no longer wanted to rely on the existing livestock support structure, including that of the village veterinary worker (VFW). Rather, they wished to form a village pig production focus group where they could have the opportunity to upgrade their management skills and eventually position themselves to provide and exchange information regarding livestock production and risk activities both within their villages and the IUARP target area.

Livestock management systems interventions

Cross visits

Following the formation of the seven pig production focus groups, a farmer-to-farmer cross visit to Oudomxay province was arranged. The purpose of this educational exercise was to expose the groups to 'progressive' farmers in Xai and Namor districts. These Oudomxay farmers not only enjoy the financial successes of improved pig management practices, but incorporate an integrated approach to their

livestock operations. Topics for discussion included animal health issues, nutrition, breeding, hygiene and housing, and general animal husbandry issues.

IUARP farmers identified the shortcomings in their own current pig management practices and all indicated a strong desire to improve. Discussions on the recognition and importance of the following were held:

- the quarantining of all new animals (pigs, goats, cattle, buffalo and poultry) coming into villages or on to farms to prevent the introduction of diseases
- the control of movement of pigs at farm and village levels to limit infection with CSF through penning and quarantining of suspected disease carriers
- the strategic rather than blanket vaccination against CSF of susceptible classes of animals such as sows and piglets
- the improvement of pig pen design and construction and the provision of clean water to minimise spread of CSF
- the promotion of village-based vaccinations against diseases
- appropriate treatment of both internal and external parasites
- early recognition of livestock diseases and the ability to respond with rapid quarantining and containment measures
- the planting of high-quality animal feed such as stylo, cassava, sweet potato and feed maize
- the advantages of recording animal performance, management and health issues
- suitable and appropriate breeding practices.

Pig production training course

In late January 2006 a 5-day animal health and production training course was held, ably led by Syseng Khounsy, National Animal Health Centre (NAHC), Vientiane. The training focused on the appreciation and reinforcement of the five principles of successful livestock production:

- unrestricted ready access to fresh water and a diet to maintain full health and vigour
- freedom from pain, injury and disease by prevention or rapid diagnosis and treatment
- appropriate breeding strategies
- the provision of a suitable environment including shelter, sufficient space and a comfortable resting area

- management and handling of animals in a stress-free manner.

Ongoing management

Farmers responded enthusiastically to their cross visit and training experience. Over the next 3 months many new pig pens were constructed providing a more hygienic, stress-free and relaxed environment for their animals. Drinking water supply systems were installed and feeding troughs redesigned and sited to minimise wastage. Seasonal feed calendars were prepared and plans made to correct the deficit areas. In two villages, in consultation with the village head, community quarantine pens were constructed to control animal movement into and within the village. A number of farmers who were interested in piglet production sold their mulaht pigs and purchased local Hmong sows more suited for breeding. Selection criteria included good length and conformation, quiet disposition, strong legs and feet, 10 well-shaped nipples, milking and mothering ability of dams, appearance and performance of sires, and litter size. A Chien Ji boar was purchased from Xieng Ngeun district. This animal will be the responsibility of one farmer who will provide a village boar service in return for one piglet per each successful mating.

Sweet potato production and multiplication workshop

This workshop focused on the potential of sweet potato to improve pig production levels through improved nutrition, varietal characteristics, multiplication, planting and management technologies.

The introduction of stylo and cassava

With the wet season approaching, activities focused on the nutritional needs of pigs during their growth and reproductive states, feeding strategies, and the planting and use of high-quality feeds including cassava and legumes. Workshops were held demonstrating planting methodologies for stylo and cassava. Topics covered included the treatment of stylo seed prior to planting by hot water immersion or scarification, seed bed preparation, sowing rates and depth of planting. Demonstrations were given on preparing cassava sticks for planting, and spacing and planting techniques.

Conclusions

For livestock production systems to be successful, a holistic approach covering the five principles of livestock management is necessary. This requires time. The 9-month input from the livestock management systems (LMS) consultant is too short a duration to develop 'best practice' in IUARP farmer livestock systems. However, many significant improvements have been made. There is now a better appreciation of disease, its consequence and the steps needed to provide a safe, healthy animal environment. Production levels have the potential to increase significantly with improved housing and

water supplies, and breeding and selection strategies. The use of introduced high-quality feed supplements remains an area of underdevelopment, but the proposed interventions of the new CIAT 'Legume for Pigs Project' (L4PP) in a number of villages in the IUARP target area will address this issue. This project will focus on nutritional aspects not addressed by the LMS program. The improved management regimes resulting from the CIAT LMS should mean that improved feeding will have a rapid and significant benefit, with farmers involved directly with the L4PP, and should spread easily to other farmers in IUARP villages where the LMS program was implemented.

Pig production and health in Bolikhamxay province, Lao PDR

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Abstract

Commencing in May 2002, a pig production and health survey was conducted in 16 villages of two districts in Bolikhamxay province to better understand the smallholder production system and assess the impact of infectious disease, with a particular focus on classical swine fever (CSF). The reproductive performance of sows in the smallholder sector was found to be low in comparison to tropical commercial pig production. The median litter size was 6.0 (range 4.7–6.8) and the median number of litters/year/sow was 0.8 (range 0.5–1.5). Piglets were traded out of the village production units early, with 76% of all sales comprising piglets in the 0–3-month age bracket. CSF had a major impact on village production units, affecting farmer confidence and sales patterns, and resulting in substantial piglet mortality. Overall, CSF incidence was 21 outbreaks per 100 village years, but it differed markedly in the two districts, with 38 and 4 outbreaks per 100 village years in Bolikhan and Pakading districts, respectively. A coordinated CSF vaccination program was, however, able to impact on CSF incidence in the target villages. Pig sales during disease outbreaks is a problem and facilitates disease spread within villages and to surrounding villages.

Introduction

The Lao People's Democratic Republic (Lao PDR) has a predominantly rural-based population and agricultural production is an important means of maintaining and improving livelihoods. The production of small livestock, including pigs and chickens, contrib-

utes significantly to household income. Greater than 50% of agricultural holdings raise pigs, compared to 14% raising cattle and/or buffalo (MAF 2000).

Approximately 88% of pigs are produced in the smallholder sector using labour-intensive but low-input traditional practices. Slow-growing indigenous breeds are raised and low-quality feed is used, leading to poor growth performance. Disease is also a major problem for pig farmers and classical swine fever (CSF), in particular, has been shown to be endemic and responsible for a large number of epidemic pig mortalities. Although CSF is a vaccine-preventable disease, only a small proportion (<10%) of pigs raised in the smallholder sector are vaccinated. This factor is compounded by vaccine delivery constraints, the most important being lack of an effective cold chain and inadequate vaccine stability (see Conlan et al. 2008 in these proceedings).

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A longitudinal survey was conducted in the central province of Bolikhamxay commencing in May 2002. The primary objectives of the survey were to better understand production and trading practices and the impact of CSF on village pig production. CSF vaccination was carried out in the second half of the survey and the impacts of this campaign were measured.

Study site, village selection and survey design

Bolikhamxay province was purposely selected due to its proximity by road to Vientiane Capital (Figure

1). Approximately 80–90% of all pigs in Bolikhamxay province are raised by smallholders and 47% of agricultural holdings raise pigs (MAF 2002). The total herd size in 2005 was 54,000 head (MAF 2006).

Eight villages from each of two districts, Pakading and Bolikhan, were selected for inclusion in the survey (Figure 2). The survey commenced in these villages in May 2002, was expanded in March 2004 with the inclusion of a further eight villages, and finished in August 2006. The village selection criteria included the following: (i) pig production was important for the agricultural economy of the village and for household livelihoods; (ii) the live-



Figure 1. Study site: Bolikhan and Pakading districts, Bolikhamxay province

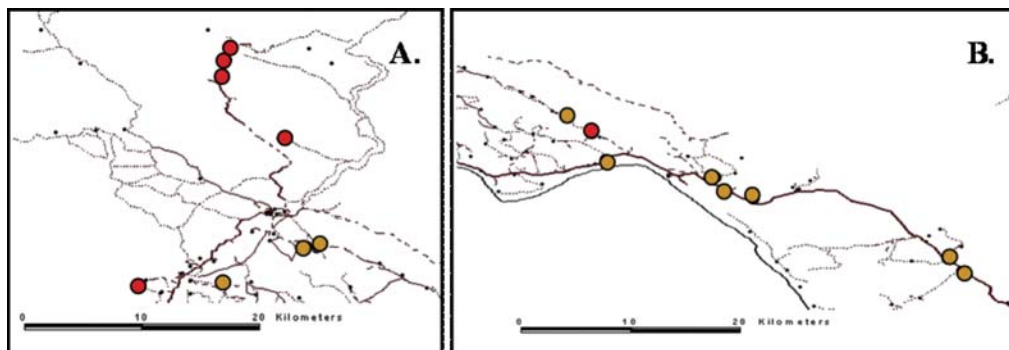


Figure 2. Study sites, A. Bolikhan district and B. Pakading district
 Note: Solid lines indicate good roads and dashed lines indicate poor quality roads. Survey villages are indicated by solid circles; orange = no CSF outbreaks, red = CSF outbreaks.

stock officers had a strong working rapport with the village administration; and (iii) the villages selected were accessible during the wet season (June to October).

The survey was initiated in each village at a meeting with village administration and pig farmers. All pig producers were recruited into the survey and the village veterinary worker or village chief compiled age-specific production and health data, which was collected by district and provincial livestock officers each month to determine reproductive performance, inputs and outputs from the production unit, and the impact of CSF on production.

Methods and results

Reproductive performance

Three animal-level indicators of reproductive performance were measured during this study, namely live-born litter size, estimates of litters/sow/year and estimated piglet mortality. The sow:boar ratio, a village-level indicator of reproductive performance, was also measured. During the survey period a total of 546 litters were observed in all 16 villages, with a median live-born litter size of 6.0 (range 4.7–6.8); in Bolikhan district the median litter size was 6.1 (range 5.4–6.8) and in Pakading district 5.8 (range 4.7–6.4). The estimated number of litters/sow/year was calculated using equation (1) (see below).

In all 16 villages the median number of litters/sow/year was 0.8 (range 0.5–1.5) and the median litters/sow/year were 1.2 and 0.7 in Bolikhan and Pakading districts, respectively. The median piglet mortality in all villages was 2% (range 0–15%). The median sow:boar ratio was 22.5:1 (range 6–81; $n = 14$) but two villages that had no boars were excluded from this calculation. The two villages that had no breeding boar did, however, have a total of 52 litters during the survey period.

Offtake and intake

During the survey period 84% of pigs left the village production units by way of sale, 4% by death and 12% for home consumption. Seventy-six per cent of all offtakes out of villages were in the age group 0–3 months. Similarly, 76% of all sales were

in the 0–3 months age group, 12% were 4–6 months old and 6% were sows. The remainder were either boars or were in the 10–12 months age bracket.

Intakes into the village production units were primarily by birth, with 76% born and 24% purchased from a nearby village, a trader or a market. Sixty-three per cent of all purchases were in the 0–3 months age group, 16% were 4–6 months old and 13% were sows. Fourteen of the 16 villages (88%) had a majority of pigs entering the production unit by way of births. Donsai village in Pakading district purchased 736 young piglets in the survey period compared to the birth of just 28 piglets from six sows.

CSF and trade patterns

In the survey period a total of 10 CSF outbreaks in six villages were identified, representing a total incidence of 21 outbreaks per 100 village years. Outbreaks were considered separate if they occurred in the same village 6 or more months apart. The results were very different for each district; in Bolikhan district the incidence of CSF was measured at 38 outbreaks per 100 village years, and in Pakading district the incidence was 4 outbreaks per 100 village years.

Sales patterns associated with CSF outbreaks and/or piglet deaths are illustrated graphically in Figure 3. Sale spikes were evident during or immediately after CSF outbreaks in four of the six villages—Ban Phonsavath, Ban Nalong, Ban Borthoun and Ban Phonethong. In all but Ban Phonethong, the observed sale spikes were associated with a large decrease in the number of farmers raising pigs (see next section). In Ban Houana and Ban Nampa, pigs were sold during or immediately after piglet deaths but the same levels of selling off were not evident.

Farmer confidence

During the course of this survey, six villages experienced CSF outbreaks. In four of these villages the number of pig farmers decreased substantially following these outbreaks. In Borthoun village the number of farmers raising pigs decreased by 40% following a CSF outbreak in June–July 2003, decreasing from a monthly average of 76 to 46. In Nalong village the number of farmers decreased by 30% following an outbreak of CSF in August–September 2004,

$$\text{litters/sow/year} = \frac{\text{total number of litters} \times 12}{\text{median monthly sow population} \times \text{number of months in survey}} \quad \text{equation (1)}$$

decreasing from an average of 104 to 72. The nearby village of Phonsavath also incurred an outbreak of CSF in August–September 2004 and the number of farmers decreased by 26%, from 109 to 81. In all these villages the number of farmers raising pigs did

not return to pre-outbreak levels by the time the survey finished. Nampa village also incurred an outbreak in May–June 2003 and the number of farmers decreased by 26%, from 43 to 31. However, dissimilarly to the other three villages, the number of farmers

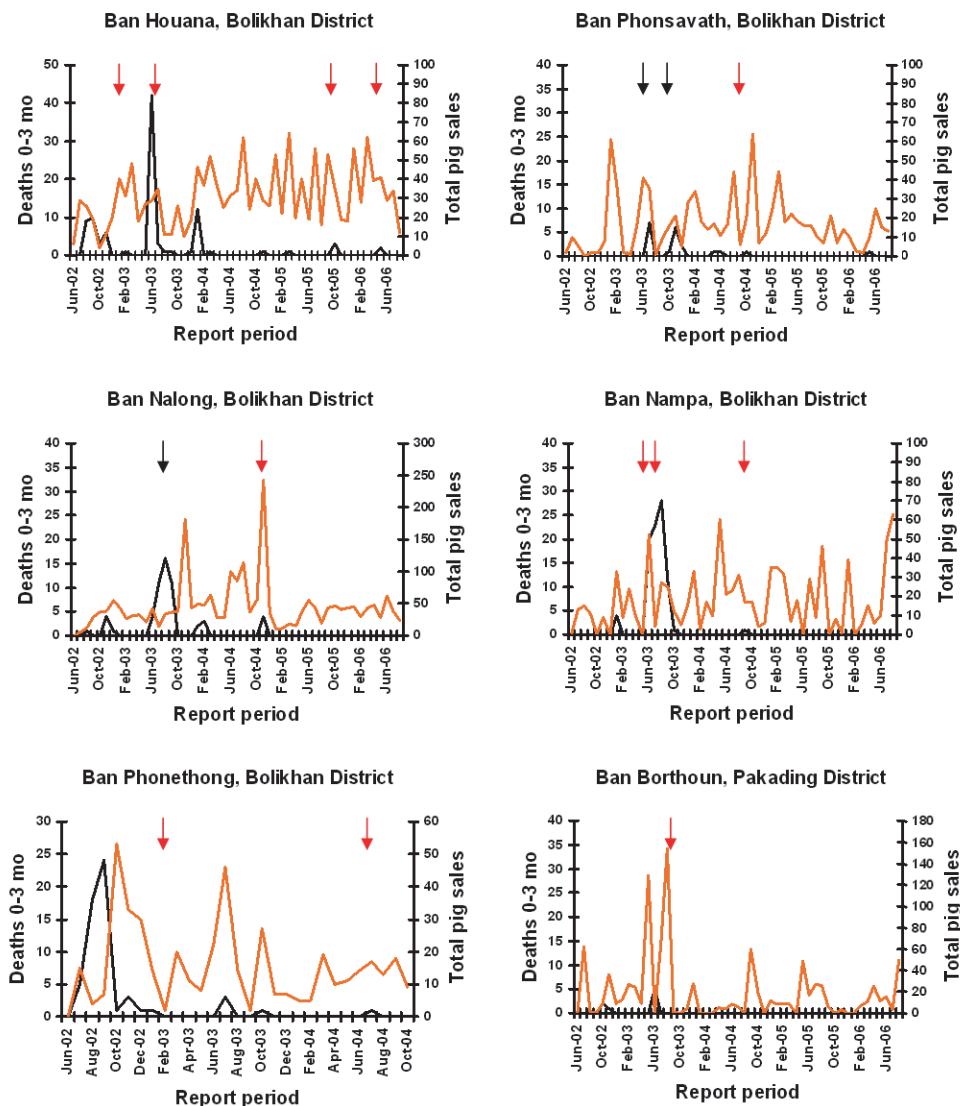


Figure 3. Piglet mortality and sale patterns associated with classical swine fever (CSF) virus outbreaks in villages in Bolikhamxay province, Lao PDR

Note: The black line represents monthly mortality of young piglets and the orange line represents total monthly pig sales. Red arrows represent when samples were first collected that tested positive for CSF virus antigen; black arrows represent suspected CSF outbreaks.

had increased to 41 in August 2006 when the survey was finished. This was despite another outbreak of CSF in August 2005.

Vaccination program

A CSF vaccination program was conducted in the 16 villages in December 2003 and January 2004. Vaccine was delivered under optimum transport conditions (see Khounsy et al. 2008 in these proceedings) to minimise any possible loss of quality. Training and awareness raising was conducted during the vaccination program to educate farmers about vaccination and places for purchase. Vaccination recommendations were made according to Khounsy et al. (2008 in these proceedings), and farmers and district agricultural extension officers were encouraged to continue vaccine delivery. The project thus provided a supporting rather than a driving role.

The vaccination program had a positive impact on the incidence of CSF in both districts. In Bolikhan district the pre- and post-vaccination incidences of CSF were 63 and 17 outbreaks per 100 village years, respectively, and in Pakading district 15 and 0 outbreaks per 100 village years, respectively.

Discussion and conclusions

The reproductive performance of the sows described in this study was poor in comparison to the commercial sector and other smallholder tropical pig sectors. The average live-born litter size in Bolikhamxay province does not compare favourably with the smallholder sector in Kenya or the Philippines, where sizes of nine (Wabacha et al. 2004) and eight (Lanada et al. 1999) were observed. The average live-born litter size in Thailand ranges between eight and 10 (Kunavongkrit and Heard 2000) and in European countries such as Denmark it is greater than 12 piglets (see Cutler 2008 in these proceedings). Good genetics and breeding is probably a substantial factor in the discrepancies seen between litter sizes in Bolikhamxay province and those in the smallholder sectors of Kenya and the Philippines. The pigs studied in both Kenya and the Philippines were predominantly native/exotic crossbreeds (Lanada et al. 1999; Wabacha et al. 2004), whereas in Bolikhamxay province the predominant breed was the indigenous black pig. The nutritional status of the sow and inbreeding are also important factors

affecting reproductive performance. A median sow:boar ratio of 22.5:1 indicates that significant inbreeding is occurring in villages, and this was further highlighted by two villages not having a recognised breeding boar at any stage during the survey. However, a low sow:boar ratio is not necessarily indicative of good reproductive management, and farmers need to understand and manage reproductive cycles and have access to boars when oestrus is detected.

A majority of pigs in the 16 survey villages left the village production unit by the age of 3 months, suggesting that the dominant form of pig production in the survey group was farrow-weaner, where each farmer owns a small number of sows and piglets are sold on to be consumed or fattened elsewhere. A number of farmers in each village also raise only growers, and some villages predominantly practise this form of production. The main reason for a majority of farmers selling pigs at this young age was limited feeding resources, and a contributing factor was fear of disease. The potential for economic gain would be greatly increased if smallholder producers could afford to hold pigs for a longer period and sell at a greater weight.

The incidence of CSF was substantially different between the two districts included in the survey, being almost 10 times greater in Bolikhan district than in Pakading district. Previous research in Lao PDR has also indicated that disease prevalence can vary quite substantially between districts (Blacksell 2001). The research presented in this paper indicates that Bolikhan district was hyper-endemic for CSF; further research is required to better understand the epidemiology of CSF at the village level. Neighbourhood infections play an important role in disease spread in Europe (Crauwels et al. 2003) and this is also likely to be the case in Lao PDR, although farming practices, pig densities and farm sizes are vastly different.

CSF outbreaks significantly impact on farmer confidence. Four of the villages experiencing outbreaks during the course of this study saw a substantial decrease in the number of farmers raising pigs following an outbreak, with 40% of farmers in one village discontinuing pig production. This research paper demonstrates that disease has a negative impact on production and trade in a village, with farmers selling off sick pigs when a disease event is recognised in order to maximise profits from a valuable yet vulnerable asset. This practice is not nec-

essarily limited to CSF; disease in general severely impedes production potential. However, the exodus of potentially infected stock from a village during an outbreak only serves to exacerbate the overall problem.

Effective CSF vaccination as a control strategy has the potential to provide benefit to smallholder pig farmers. While the cold chain continues to be a problem (see Conlan et al. 2008 in these proceedings), the research presented in this paper indicates that the incidence of CSF can be substantially decreased if a sustainable vaccination strategy is put in place and farmers are well informed.

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Pig production and disease management: A village perspective

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Abstract

Surveys that included 115 interviews were conducted in five villages of Bolikhamxay province, Lao PDR, to assess farmer attitudes regarding their pigs, pig disease, classical swine fever (CSF) and vaccination. Farmers had a strong appreciation of the value of their pigs due to the income they produce for their families. They demonstrated an appreciation of the attributes of a diseased animal and displayed some understanding of the contagious nature of disease. In the minds of pig farmers, CSF is the major disease affecting their pigs although knowledge about the cause was not evident. Fragmented knowledge about the spread and prevention of CSF was present. Even though farmers did not fully understand how a vaccine is able to protect an animal from disease, they were very positive about the outcomes of vaccination. The issue of water provision to pigs was also explored.

Introduction

The daily reality of classical swine fever (CSF) is dealt with by the Lao farmer. They are directly affected by disease outbreaks, as well as by any government or foreign aid attempts to improve the CSF situation. In order to enable a better understanding of CSF presence in Lao PDR, it is desirable to gain an understanding of the farmer's perception of the disease. The purpose of this research was to describe the views expressed by Lao village pig farmers in relation to their pigs, disease, vaccination and CSF. Women were the major focus of surveys conducted due to their status as primary pig producers.

It has been observed that pigs in the village setting in Lao PDR are not provided with adequate drinking water (Conlan 2006). This is a management issue

that affects not only the welfare of the pigs but also the health and production level of the animals.

Materials and methods

Four different surveys, including a total of 115 interviews, were conducted in five villages in Bolikhamxay province. Permission to undertake the surveys was obtained from the Department of Livestock and Fisheries (DLF), Bolikhamxay Provincial Agriculture and Forestry Office (PAFO), Bolikham District Agriculture and Forestry Extension Service (DAFES) and the chief of each village. Village women were requested to organise their working days around the surveys to maximise participation rates while minimising impact on normal daily activities.

Results

Pigs and their management

Two-thirds of the 40 participants in survey one stated that they kept pigs to create income for their

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household. The remainder stated that pigs were kept to be sold when money was needed by the family. Some people gave both answers. The advantages of pigs over other livestock species, according to the survey group, included the following: ‘pigs grow fast’, ‘they have a good level of productivity’, ‘they have high market demand’, and ‘pigs are a big animal and can be sold for a large sum of money’. Cattle were preferred for reasons such as ‘they bring in more money because they are bigger’ and ‘they can forage for their own feed’. Chickens were preferred because ‘it does not cost as much to feed them’ and ‘they can be grown out and sold more rapidly than pigs’.

Disease and health

When asked ‘What symptoms do you see when your pigs get sick?’, over half of the 40 participants in survey one responded that their pigs do not get sick. One-third of people described disease signs consistent with CSF. Five people mentioned diarrhoea, three talked about lameness and one mentioned bloating of the stomach. No CSF cases had occurred in the villages where survey one was conducted for at least 5 years. In contrast, when 35 farmers in villages where there had been recent CSF outbreaks were asked the same question, only six stated that their pigs had never been sick whereas 25 described the symptoms of CSF. The remainder described diarrhoea, lameness or unexplained deaths in piglets.

The most common answer in survey one when asked what causes disease was ‘don’t know’, with just over one-quarter of the 40 participants stating this. Another one-quarter attributed disease to poor hygiene, that is having a dirty pen. Other answers were varied and included ‘not vaccinating animals’ (four people), ‘buying infected meat from the market’ (four) and ‘letting pigs out of their pen’ (three). Five people showed understanding of the contagious nature of infectious disease.

Classical swine fever

Participants in survey one were asked what they thought causes CSF and how it is spread. The difference between these two questions was not distinguished. Just over half of the 40 participants stated that they didn’t know. One-quarter of participants mentioned poor hygiene in their answer, with a similar number suggesting that CSF could be caused/spread by people bringing infected pork into

the village. Participants also added factors such as pigs being free ranging (four answers) or pigs not being vaccinated (seven). Six farmers included answers that displayed their understanding of the contagious nature of CSF.

When a similar question, ‘Where do you think animals get CSF from? What makes them sick?’, was asked of the 35 farmers participating in survey four, a different set of answers was collected. Only six participants said they did not know. Fifteen stated that animals can get CSF from infected pork being brought into the village and eight mentioned that infected pigs being brought in to the village can make animals sick. Hygiene, that is a dirty pen or feeding dirty feed, was mentioned by five participants, as was pigs being allowed out of their pen.

Treatment and prevention

The treatment options available to village farmers when their pigs become sick are using the village veterinary worker (VW), buying drugs from a human pharmacy, using traditional treatments or administering no treatment to sick animals. Two-thirds of 35 participants stated that they treat disease using the treatments provided by the VW, just under one-third said they buy drugs from the pharmacy, and some people said they would do both. The remainder said they would use traditional treatments (five farmers) or provide no treatment (five).

Fifteen out of the 20 farmers in survey three agreed with the statement that before there was a VW, drugs and vaccine, their pigs died more often. Three participants didn’t use the VW and two said there was no difference. When asked if they have any traditional ways of treating animals that are sick, 10 people said ‘no’, seven said ‘yes’ and three said ‘yes but I do not use them’.

Vaccination

Sixty-five out of the 75 participants stated that they have vaccinated against CSF. Forty participants were asked how they tried to prevent disease in their pigs and 15 responded that they would vaccinate. In contrast, when multiple-choice prompts were provided, 33 out of 35 participants responded that they would vaccinate to try and prevent disease. When asked ‘How important do you think vaccination is?’ and given a scale ranging from very important to useless, all 35 farmers classed vaccination as either very important or important.

Water provision

When 40 participants were asked about water provision, all stated that they provided water once per day, usually at midday. Reasons for this were that the pig would not drink all the water if more was supplied, water has always been given like this, the pig tips the water out and dirties the pen, there is a lack of time to supply more water, and that native pig breeds do not require as much water as exotic pig breeds. The response to the introduction of water feeders to the villages was positive. All 20 recipients were still using their feeder 1 month later.

Discussion

Lao PDR is incredibly diverse in terms of people's access to infrastructure, the multitude of ethnic minorities and the country's geography; thus, caution should be exercised when comparing the results of these surveys in Bolikhamxay province with other parts of Lao PDR.

Lao farmers value their pigs for the income they provide for their families, as well as for their more traditional role as a method of accumulating capital. This is consistent with an increasing number of farmers becoming economically active in agriculture and moving away from subsistence farming. Pigs are seen as being important to the family but farmers recognise that each species has its advantages and disadvantages.

The farmers' reporting of sickness in their pigs is closely linked to CSF. In villages where there have been no recent CSF outbreaks, over half of the 40 farmers interviewed claimed that their animals do not get sick, whereas in villages where recent CSF outbreaks have occurred very few farmers made this claim. This discrepancy supports the claim that CSF is the infectious disease of greatest importance to pig production (Stür et al. 2002). Villagers in areas where there had been recent CSF outbreaks were better able to explain how CSF can be spread. This

difference is presumably due to the dissemination of information to villagers in CSF-affected areas by VVWs or government staff in an attempt to prevent further outbreaks.

All of the villagers' knowledge of the germ theory of disease, and disease treatment and prevention, is provided by the VVW, district and provincial officers, or foreign aid projects. This is also their source of vaccine. VVWs are provided with only 4 days of veterinary training. However, they are the closest thing to a veterinarian that the villages have and their actions can have a huge impact on the prevalence of disease seen in their village. Farmers saw vaccination as an effective disease prevention measure and had positive stories relating its success. In each of the five villages vaccination was carried out by the VVW on 1 day once every 6 or 12 months. If a sow is pregnant, the piglets are too young to be vaccinated or there are no family members at home on the day of vaccination, these pigs go unvaccinated. Farmers related stories whereby their vaccinated pigs survived an outbreak whereas any pigs that missed being vaccinated became infected.

The introduction of the water feeders to Ban Hadpho and Ban Naoh was seen as a labour-saving mechanism. Village women were pleased to only have to check if the container was full and then be able to leave the pigs for the remainder of the day. This small reduction in labour for the women meant they could spend a greater amount of time on other tasks such as feed collection.

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Section 2

Classical swine fever and foot-and-mouth disease country reports

Classical swine fever and foot-and-mouth disease in Lao PDR

Syseng Khounsy¹ and James Conlan^{2,3}

Introduction

Approximately 75% of the population of Lao PDR is engaged in agriculture and the vast majority (approximately 90%) of these producers are in the smallholder sector. Livestock are an important contributor to national, agricultural and village economies and are relied on for food security. The pig population has increased over the past 5 years at an annual average increase of 4.7% at the national herd level and up to 20% in some provinces. Cattle and buffalo populations have grown at more modest rates of 1–2% (Figure 1).

Disease, including foot-and-mouth disease (FMD) and classical swine fever (CSF), is a major constraint to efficient and sustainable livestock production. Up to 80–90% of pigs and 99% of cattle and buffalo are produced in the smallholder sector using low input practices; as such, there is limited private sector input. Disease reporting, diagnosis, control and prevention are addressed by the Lao Government through the National Department of Livestock and Fisheries (DLF) and local agriculture and forestry offices at provincial and district government levels. These activities are supported by international partners such as the Australian Centre for International Agricultural Research (ACIAR), Commonwealth Scientific and Investigation Research Organisation (CSIRO), Japanese International Cooperation Asso-

ciation (JICA), Food and Agriculture Organization (FAO), European Union (EU) and Office International des Epizooties (OIE).

Disease reporting and communication are passive and reports are made from villages through government administrations at district and provincial levels and then to the national level—the DLF and the National Animal Health Centre (NAHC). Communication of FMD-related information at regional and international levels is coordinated by the OIE South-East Asian FMD regional coordination unit (SEAFMD RCU), where reports are submitted monthly. Disease reporting for CSF is less well coordinated and information is provided to the OIE.

Disease diagnosis

Classical swine fever

Initial suspicion of CSF is based on clinical signs. Routinely, the laboratory test used to confirm an outbreak of CSF is the antigen capture (AC)-ELISA (Shannon et al. 1993) for the detection of viral antigen in tissue or the white cell fraction of whole blood. The immunoblotting (IMB)-ELISA (Conlan et al., in press) for antigen detection is used at the NAHC and will be used in provincial laboratories in the future. These tests are supported by ACIAR and reagents are supplied by the CSIRO Australian Animal Health Laboratory (AAHL), Geelong, Australia. Capacity at the NAHC for virus isolation and the fluorescent antibody test (FAT) has been developed in cooperation with JICA; however, these tests are not routinely used.

Antibodies to CSF are detected in serum using the complex trapping blocking (CTB)-ELISA (Blacksell 2001) with the support of ACIAR and CSIRO

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AAHL. The capacity to use the ‘gold-standard’ neutralising peroxidase linked assay (NPLA) has been developed but is not routinely used.

Foot-and-mouth disease

As with CSF, initial suspicion of FMD is based on clinical signs. Diagnosis of FMD is confirmed in the laboratory using the indirect sandwich ELISA and subtypes A, O and Asia 1 can be identified. Virus isolation in tissue culture can be done but is not routinely used.

Antibodies to FMD virus are detected in the serum of cattle, buffalo and pigs using the liquid phase blocking (LPB)-ELISA. During surveys to specifically look for naturally infected animals, the non-structural protein (NSP)-ELISA (CEDI Diagnostics, the Netherlands) is used.

Epidemiology of CSF and FMD

Classical swine fever

As the identification of CSF outbreaks is based on a system of passive surveillance, the incidence of CSF is probably under-reported. In 2003 five outbreaks in three provinces were laboratory confirmed: Bolikhamxay (3), Luang Namtha (1) and Xieng Khouang (1). In 2004, 11 outbreaks in six provinces were identified: Bolikhamxay (6), Khammuane (1), Vientiane Capital (1), Luang Prabang (1), Houaphan (1) and Bokeo (1). In 2005 five outbreaks in two provinces were confirmed in the laboratory: Bolikhamxay (2) and Vientiane Capital (3).

In 2006 up to October, 10 outbreaks in three provinces were identified: Bolikhamxay (2), Vientiane Capital (7) and Luang Namtha (1). Refer to Figure 2 for location of outbreaks and the season in which they occurred.

In 2006 a serological survey for CSF and FMD was conducted in five northern provinces, Oudomxay, Luang Prabang, Phongsaly, Xayabouly and Houaphan. The survey was conducted with the support of the FAO ‘Transboundary Animal Disease in the Greater Mekong Sub-region’ project, OIE SEAFMD RCU and the ‘Lao–Australian Animal Health Research’ project (ACIAR Project Number AH/2003/001). The CTB-ELISA was used to detect antibodies to CSF virus (Table 1).

Table 1. Sero-prevalence of classical swine fever in five northern provinces of Lao PDR, 2006

Province	Number of districts	Number of villages	Number of pig samples	Per cent sero-positive
Oudomxay	5	8	55	11
Luang Prabang	7	13	91	13
Phongsaly	7	13	88	15
Xayabouly	7	12	84	15
Houaphan	8	23	161	6

Foot-and-mouth disease

The predominant strain of FMD virus causing outbreaks in Lao PDR is type O. A small outbreak caused by type A occurred in Bokeo province in 2003, and no outbreaks of type Asia 1 were detected

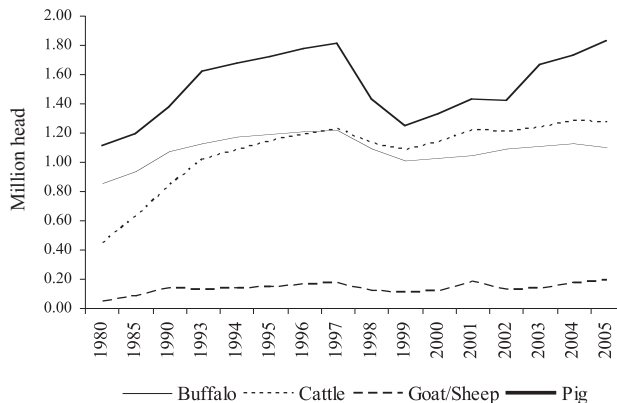


Figure 1. Livestock population trends from 1980 to 2005

in the Lao PDR in the period 2003–06. In 2006 no outbreaks of FMD had been reported up to October (Figure 3). As described above, a sero-prevalence survey for CSF and FMD was conducted in 2006 (Table 2). In 2005 a survey in four provinces, Savannakhet, Vientiane Capital, Huaphan and Xieng Khouang, was conducted to measure the serological prevalence of naturally infected animals (Table 3). In both sero-surveys the NSP-ELISA (CEDI Diagnostics, the Netherlands) was used to detect antibodies to FMD virus.

Table 2. Sero-prevalence of foot-and-mouth disease in five northern provinces of Lao PDR, 2006

Province	Number of districts	Number of villages	Number sampled	Per cent sero-positive
Oudomxay	5	8	112	0
Phongsaly	7	13	163	0
Huaphan Luang	8	23	323	1
Prabang	7	13	181	13
Xayabouly	7	12	168	14

Table 3. Sero-prevalence of foot-and-mouth disease in four provinces of Lao PDR, 2005

Province	Number sampled	Per cent sero-positive
Savannakhet	280	2
Xieng Khouang	765	0
Huaphan	458	0
Vientiane Capital	60	30

Disease control

Classical swine fever

There is no official policy for the control of CSF; however, vaccination is strongly encouraged and animal movement during an outbreak is discouraged. Prevention of CSF is also quite difficult to achieve in the smallholder farming sector. Vaccination is not routinely used (approximately 8% of the national herd is vaccinated) and regular trading of sick pigs facilitates disease spread.

Foot-and-mouth disease

Control of FMD is better coordinated than CSF control. It is highly reliant on a high level of awareness at village, district and provincial levels to rapidly report suspected cases of FMD and submission of samples for laboratory testing. National veterinary staff are responsible for implementing animal movement control once an outbreak occurs, and this may involve personnel from other ministries, including police notification to prevent animal movement. Livestock traders are also engaged, and trading of livestock and animal products during an outbreak is prohibited.

Lao PDR does not produce vaccine for FMD and does not have a routine vaccination program to prevent or control outbreaks of the disease. Bilateral agreements between the governments of Thailand and Lao PDR result in the supply of vaccine for emergency ring vaccination during an outbreak.

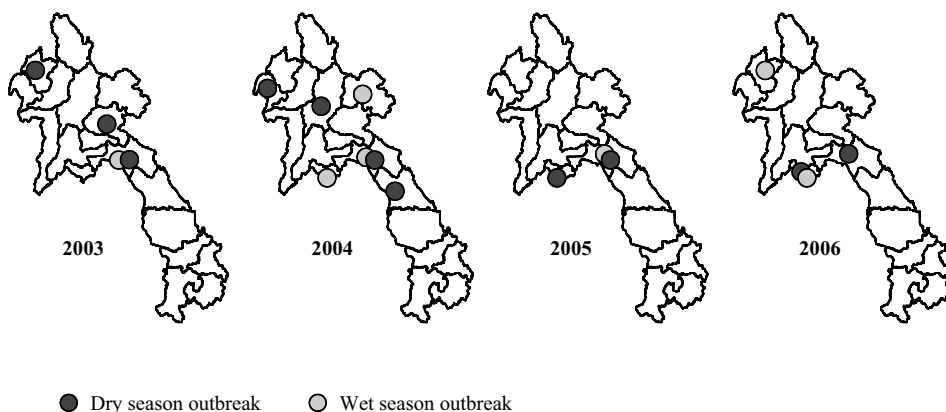


Figure 2. Outbreaks of classical swine fever, 2003–06

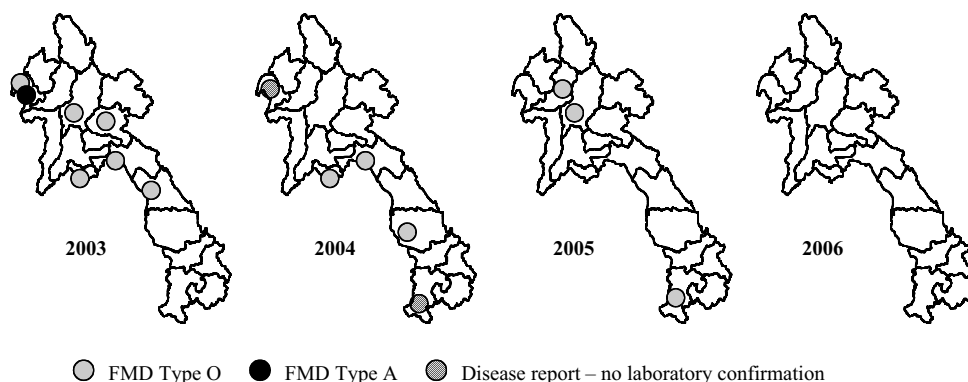


Figure 3. Outbreaks of foot-and-mouth disease (FMD), 2003–06

As mentioned above, control is reliant on a high level of disease awareness. During outbreaks, public awareness initiatives are undertaken to educate farmers and traders about the risks of disease spread.

Future activities

The control and prevention of these trans-boundary animal diseases require an ongoing commitment from all stakeholders involved in livestock production. In the future, activities will be undertaken in collaboration with international partners to strengthen the capacity of provincial and district livestock officers to recognise and control disease outbreaks. This will involve specialist training in disease recognition, disease reporting, sample collection and submission, and public awareness.

Other activities will include:

- introducing improved diagnostics and control methods for CSF, with a particular focus on implementation of the newly developed IMB-ELISA for rapid diagnosis in provincial laboratories
- scaling up vaccination programs for, and public awareness of, CSF

- engaging and working with livestock traders and providing education materials to minimise the risk of disease spread
- continuing to work with international partners to prevent the movement of illegal animals and animal products in the Greater Mekong subregion.

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Classical swine fever and foot-and-mouth disease in Yunnan province, People's Republic of China

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Introduction

China has an integrated veterinary administration system for animal health inspection and service that is divided into four levels—the national general veterinary station, provincial general veterinary stations, the region/prefecture level, and the county and township level. The county and township level is responsible for providing vaccination and collecting disease information, which is then sent to the region/prefecture level and the provincial general veterinary stations. Contagious diseases must be reported to the central government, which then issues the disease information through the government website <<http://www.agri.gov.cn>> in a monthly journal of agricultural information published openly by the government in the Chinese language. There is also international collaboration and communication between Yunnan province and other countries in South-East Asia. During 1998–2001 ACIAR, Lao PDR and Yunnan province participated in ACIAR project AS1/1994/038, which aimed to improve diagnostic methods and culminated in the research staff of Yunnan province participating in workshops and meetings held in this area. Training courses and meetings were also hosted in Yunnan province.

Many non-government organisations are also involved in disease diagnosis and control in Yunnan province. These are 'for profit' organisations, whose involvement in and capacity for disease diagnosis and control are quite varied.

Disease diagnosis

Classical swine fever (CSF)

Clinical diagnosis: this is still an important method of diagnosis for veterinarians at the county level and in non-government organisations.

Laboratory diagnosis: a variety of methods are used in different circumstances:

- a direct immunofluorescence test on cryostat sections of organs from affected pigs; however, due to non-specific reactions, it is not very popular
- virus isolation in cell culture, with virus detection by immunofluorescence
- RT-PCR, which is widely used at the provincial level due to its rapidity and convenience, with primers mainly targeting E0, E2 and NS5B genes
- indirect ELISA kits produced by IDEXX (USA) in some laboratories.

Serological tests: serum samples from recovering animals, from sows with suspected congenitally infected litters, or from pigs under surveillance are tested by indirect haemagglutination and indirect ELISA methods to detect serum antibodies. Only the attenuated vaccine of C strain is used nationwide; therefore, it is the only strain used for analysis of immune antibody titres.

Foot-and-mouth disease (FMD)

Clinical diagnosis: this is still an important method of diagnosis for veterinarians at the county level.

Laboratory diagnosis: a variety of methods are used in different circumstances:

- antigen capture (AC)-ELISA

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- virus isolation— inoculation of primary bovine thyroid cells, BHK-21 and IB-RS-2 cell lines; inoculation of mice
- RT-PCR, which is widely used at the provincial level; if the suspicious sample is positive, sequence analysis is needed to make a final diagnosis.

Serological tests include:

- liquid phase blocking (LPB)-ELISA
- virus neutralisation test.

Epidemiology of CSF and FMD in Yunnan province, PR China

Foot-and-mouth disease

There have been no reports of FMD in Yunnan province since November 2005, but CSF outbreaks occur every month.

From May 2005 to June 2006 (Tables 1 and 2), there were 12 FMD Asia 1 outbreaks in nine provinces in northern, north-western and eastern China. Among 4,608 susceptible animals, 634 were infected and showed clinical symptoms and one died. A total of 5,233 animals were slaughtered (Table 1).

Classical swine fever

According to statistical data, CSF occurred in 18 provinces / autonomic regions from January to November 2005. There were 1,221 disease cases and 85,010 pigs showed disease; 47,020 pigs died and 9,048 were slaughtered. There was no CSF reported in Liaoning, Jiling, Shandong, Hainan, Sichuan, Chongqing, Beijing, Shanghai, Tianjin, Shanxi, Inner Mongolia and Tibet. During 2005–06, 144 cases were reported in Yunnan province and 2,515 pigs died because of the disease (Table 3).

Table 1. Foot-and-mouth disease Asia 1 outbreaks in China in 2005

Date of announcement	Date of report	Place	Animal	Number of susceptible animals	Number of animals with disease	Number of deaths	Number of animals slaughtered
13.5.2005	24.4.2005	Shandong	Dairy cow	40	17	0	40
13.5.2005	24.4.2005	Jiangsu	Dairy cow	183	15	0	183
26.5.2005	18.5.2005	Xinjiang	Cattle	308	75	0	308
26.5.2005	5.5.2005	Beijing	Dairy cow	2,464	252	0	2,464
26.5.2005	–	Hebei	Beef cattle	512	0	0	512
20.6.2005	–	Xinjiang	Beef cattle	261	40	0	261
24.6.2005	–	Hebei	Dairy cow	263	4	0	263
20.7.2005	–	Qinghai	Beef cattle	168	95	0	168
20.7.2005	–	Gansu	Beef cattle	290	66	0	454
30.12.2005	–	Shandong	Bull	91	48	0	91
16.1.2006	–	Ningxia	Cattle	8	2	1	298
16.1.2006	–	Jiangsu	Dairy cow	20	20	0	100

Note: Diagnostic laboratory—National FMD Reference Laboratory, Lanzhou Veterinary Institute, Chinese Academy
Diagnostic methods used—AC-ELISA, LPB-ELISA, RT-PCR, virus isolation

Table 2. Foot-and-mouth disease Asia 1 outbreaks in China in 2006^a

Province	Serotype	Date of report	Number of animals with disease	Number of animals slaughtered
Jiangsu	Asia 1	16.1.2006	20 cows	100 cattle and cows
Ningxia	Asia 1	16.1.2006	1 householder, 2 cattle	89 cattle, 110 sheep/goats
Gansu	Asia 1	8.3.2006	1 householder, 11 cattle	18 cattle
Qinghai	Asia 1	12.3.2006	1 householder	19 cattle, 2 pigs
Qinghai	Asia 1	30.4.2006	4 cows	34 (cattle, cows, pigs and sheep/goats)
Gansu	Asia 1	30.5.2006	3 cattle	35 cattle
Hubei	Asia 1	30.5.2006	7 cattle	13 cattle, 32 pigs, 7 sheep
Gansu	Asia 1	23.6.2006	213 cattle	380 cattle

^a Data from the information network <<http://www.agri.gov.cn>>, Ministry of Agriculture, PR China

Table 3. Classical swine fever in Yunnan province in 2005–06^a

Province	Number of pigs with CSF	Number of deaths	Number of animals slaughtered	Total cases
Yunnan	3,693	2,515	83	144

^aData from the information network <<http://www.agri.gov.cn>>, Ministry of Agriculture, PR China

Risk factors for virus spread

There are three main risk factors for virus spread. High animal density, including diverse management styles, is the most significant factor for the disease. There are more pigs in Yunnan province than any country in the EU, but the level of management varies according to different farming practices. The second factor is that animal movement within the province and to and from neighbouring countries is difficult to control. The third factor is the lack of widespread use of improved diagnostic and serological methods for classical swine fever virus (CSFV) in Yunnan province.

Disease control

Vaccination

Vaccination against major animal diseases is a main strategy in Yunnan province. Vaccines against FMD and CSF are provided free of charge by the government, and vaccination campaigns are carried out twice a year in spring and autumn.

Disease monitoring

Regulated disease monitoring is carried out at the county level by disease monitoring stations. Specimens are collected from suspected diseased animals and sent to Yunnan Provincial General Veterinary Station or Yunnan Tropical and Subtropical Animal Disease Laboratory to do the preliminary diagnosis.

Serological survey

Sera are collected from vaccinated animals to test antibodies. LPB-ELISA and micro-neutralisation tests are used to assay antibody levels against FMD. Study results have indicated that when the antibody titre of a vaccine is higher than 2×10^5 to 2×10^6 , the animal is fully protected against virulent virus challenge.

Control measures

When there is a disease outbreak, farms or villages are quarantined for a time period during the outbreak, and vehicles which pass through the area are inspected and sterilised.

Future directions

Strengthening of veterinary facilities and services

A sum of 144.7 million yuan has been provided by both the central government and the Yunnan provincial government to upgrade veterinary facilities and services. The upgrade process is underway and includes the setting up of disease monitoring stations, the establishment of cold chains for vaccination campaigns, the upgrading of veterinary facilities at county and town levels, the establishment of a county- and town-level inspection and supervision system, and the building of a provincial residual chemical test laboratory.

Education and training

More training courses and meetings will be held in Yunnan province. Township-level veterinarians will thus be able to learn the basic methods of sample collection and make diagnoses in both the field and the laboratory.

Virus research

Studies about the molecular epidemiology of the CSFV should be strengthened. Some strains of field virus have been sequenced but more work still needs to be done. The current vaccination program needs to be reviewed, and should be based on information collected from virus activities and serological studies. It is necessary to assure vaccine quality and efficacy before carrying out large-scale vaccinations. New methods that are able to test potential virus-carrying animals are necessary.

International cooperation

ACIAR project AS1/1994/038 between China, Laos and the Australian Animal Health Laboratory improved the diagnostic ability for FMD and CSFV during 1998–2001. Cooperation between Yunnan

province and neighbouring countries is enabling mutual information exchange. This cooperation is important for disease control and it should be strengthened.

Classical swine fever and foot-and-mouth disease, Myanmar

Cho Cho Htun¹ and Mya Mya Oo²

Introduction

The Livestock Breeding and Veterinary Department (LBVD) is mainly responsible for the livestock sector and for animal health in Myanmar. It has assigned 14 state and divisional officers, 63 district officers, 281 township officers and 683 assistant veterinarians for village tracts. They all have responsibility for taking care of animal health and improvement of livestock productivity. A network of disease information and reporting systems has been established among the LBVD headquarters, field veterinarians, local authorities and livestock farmers. According to the Animal Health Development Law proclaimed in 1993, and related orders and directives issued in 1999, the owner of a diseased or dead animal, or a person who has in his possession such an animal, should report promptly to the LBVD employee-in-charge of either a village tract or township, or a person designated for this purpose by the Ministry of Livestock and Fisheries.

A veterinarian should report promptly to the nearest LBVD employee if he finds that an animal he is treating is suffering from a contagious disease. Based on this information, the Deputy Township Veterinary Officer takes responsibility for relaying the message through his superiors to LBVD headquarters or to the local authorities according to regulations (Figure 1). In the case of foot-and-mouth disease (FMD), the specimens are sent directly to the FMD laboratory for virus identification.

Disease diagnosis

Foot-and-mouth disease

The National FMD laboratory has capabilities for diagnosis and serological tests.

Antigen detection methods include:

- indirect sandwich ELISA prepared and standardised by WRL, Pirbright, UK
- ELISA for detection of FMDV antigen prepared and standardised by RRL, Pak Chong, Thailand
- BHK 21 monolayer cells to isolate the virus and confirm the diagnosis.

Antibody detection methods include:

- liquid phase blocking (LPB)-ELISA prepared and standardised by WRL, Pirbright, UK
- LPB-ELISA prepared and standardised by RRL, Pak Chong, Thailand
- FMDV NSP 3 ABC-ELISA manufactured by Bommeli Diagnostics
- FMDV NSP 3 B-ELISA manufactured by UBI, New York, USA
- FMDV NSP ELISA manufactured by CEDI Diagnostics B.V., Lelystad, the Netherlands
- virus neutralisation test.

Classical swine fever

Classical swine fever (CSF) is diagnosed by post-mortem examination and histopathological examination. The fluorescent antibody test (FAT) is used for the confirmation of CSF. The following methods are performed for virus isolation:

- egg inoculation
- animal inoculation
- tissue culture inoculation.

The Mandalay Regional Diagnostic Laboratory has one diagnostic facility for CSF by neutralising

¹ National FMD Laboratory, Livestock Breeding and Veterinary Department, Myanmar

² Research and Biology Section, Livestock Breeding and Veterinary Department, Myanmar

peroxidase-linked assay (NPLA) with the support of the JICA-ADC project in 2005. The Yangon Central Veterinary Diagnostic Laboratory has also planned to establish CSF diagnostic facilities using the NPLA method from the JICA-ADC project.

Statistical data from outbreaks of FMD and CSF are summarised in Tables 1 and 2.

Risk factors for virus spread in the livestock population of Myanmar

Disease outbreaks are caused by animal movement from endemic areas. Some outbreaks are associated with livestock owners who bring their animals to the market for sale, and with livestock traders moving from one market to another. In some areas there is limited grazing ground, so that farmers share

common pasture and the animals can easily come into contact with the disease.

Disease control

When outbreaks occur, the owner has to report to the Deputy Township Veterinary Officer (DyTVO) and the village headman. The DyTVO checks the outbreak and reports to the Township Veterinary Officer (TVO) and directly to LBVD Headquarters (HQ). The village headman reports to the Township Administration Authorities (Figure 1). The DyTVO collects diagnostic specimens and sends them to the diagnostic laboratory, destroys the carcasses, cleans the premises using disinfectants and notifies other offices of the outbreak (Figure 1). The DyTVO segregates the diseased and susceptible animals, bans animal movements and undertakes emergency vaccination.

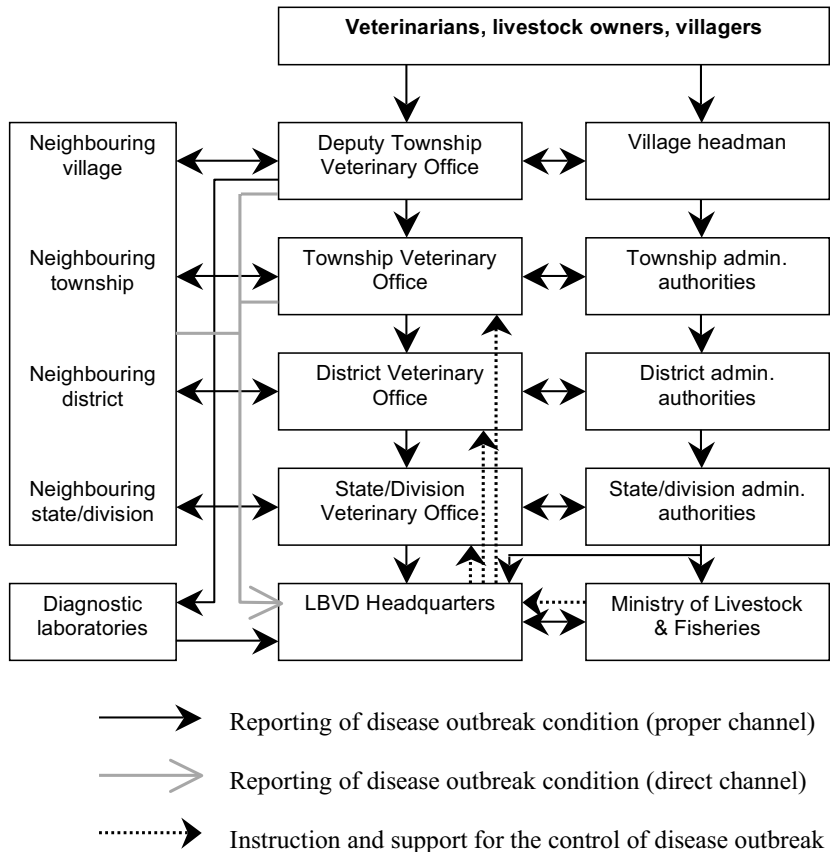


Figure 1. Disease reporting system in Myanmar

Table 1. Outbreaks of foot-and-mouth disease in Myanmar 2001–06

Year	Number of animals infected	Virus types	Locations
2001	2,303	O, Asia 1	Kachin, Sagaing, Magway, Mandalay, Rakhine, Yangon, Shan, Ayeyarwaddy
2002	11,712	O	Bago, Magway, Rakhine, Yangon, Ayeyarwaddy
2003	946	O	Sagaing, Bago, Yangon
2004	2,972	O	Sagaing, Bago
2005	1,103	O, Asia 1	Chin, Bago, Magway, Rakhine, Yangon, Kayah
2006 (to Sept)	1,671	O	Ayeyarwaddy, Bago, Yangon, Sagaing, Rakhine, Chin

Table 2. Outbreaks of classical swine fever in Myanmar 2001–05

Year	State/Division	Number of outbreaks	Pig population	Number of animals infected
2001	Yangon	8	19,325	11
2001	Ayeyarwaddy	2	44,669	81
2002	Yangon	n.a.	n.a.	n.a.
2003	Shan (South)	1	19,325	2
2004	Shan (South)	2	13,777	3
2005	Shan (South)	4	13,777	4
2005	Shan (East)	1	42,388	1

n.a. = not available

To minimise risk and prevent outbreaks, public awareness and communication programs are important. LBVD issues timely notification of FMD through public media such as daily newspapers, radio and TV programs, especially at the onset of the monsoon season, and conducts workshops and seminars on animal health and disease control for in-service personnel, farmers and livestock owners.

The national FMD laboratory produces 100,000–150,000 doses of monovalent type FMD vaccine every year with existing facilities. The vaccines are used for ring vaccination in case of outbreaks. CSF is controlled by local and imported CSF vaccines. Approximately 200,000 doses of CSF vaccine are produced by the Research and Biologic Section of LBVD.

Future directions

A draft national FMD control plan with a zoning approach has been prepared and initiated in Myanmar. The logical framework of the Office International des Epizooties (OIE) Regional Coordination Unit (RCU) will be a performance indicator

for FMD control in the South-East Asian region. Monthly, quarterly and emergency reports for FMD incidence in the country will be sent to OIE RCU. Country reports will be submitted to meetings of the OIE Sub-Commission for FMD in South-East Asia and to OIE RCU workshop meetings. FMD status in defined regions under the zoning approach will be monitored by sero-surveillance activities using improved serological tests including FMDV non-structural protein ELISA to differentiate vaccinated from infected animals.

The Research and Disease Control Division of LBVD continuously monitors the current status of contagious diseases in Myanmar and the preparedness for new or emerging diseases. FMD task forces at different levels and the national FMD laboratory will carry out control procedures and LBVD will hold evaluation meetings every 4 months at headquarters with the heads of state/division veterinary officers. Under the JICA project, an epidemiological survey on CSF was done in the Mandalay region in 2005 and a preliminary epidemiological survey on FMD was done in Sagaing division in 2006.

Classical swine fever and foot-and-mouth disease in Cambodia

Bun Chan¹

Introduction

In Cambodia the livestock sector comprises 12.8% of the gross domestic product (GDP) (World Bank 1995). It is considered the second priority after rice production and plays a crucial role in the national economy. Cattle and buffalo are widely used for draft power and means of transportation, being used on 85–95% of all cultivated lands. Pigs and poultry are normally raised for cash income and occasional home consumption. Most Cambodian farmers raise animals in the traditional manner without the use of modern technology. The animals are usually unpenned and fed by grazing the available natural feeds in the village, which leads to poor nutrition and susceptibility to disease. Classical swine fever (CSF) and foot-and-mouth disease (FMD) are endemic in Cambodia. FMD is considered an important disease because of high morbidity in affected cattle, buffalo and pigs, whereas CSF causes high mortality in affected pigs (DAHP 2001–04). Yearly animal population statistics are presented in Table 1 (DAHP 2005) and data from CSF and FMD outbreaks during 2001–06 are provided in Tables 2 and 3.

Disease diagnosis

The most common diagnostic method used for confirmation of CSF and FMD in Cambodia is assessment of clinical signs. However, enzyme-linked immunosorbent assays (ELISAs) are used for antibody detection of FMD and the neutralising peroxidase-linked assay (NPLA) is used for antibody

detection of CSF at the National Animal Health and Production Investigation Centre (NAHPIC). NAHPIC, the national laboratory, is capable of CSF and FMD diagnosis, but the facilities and human resources have limited capacity for the handling of specimens for field diagnosis.

Epidemiology of CSF and FMD

Cambodia uses passive epidemiological surveillance to investigate reported diseases. The animal disease reporting system was introduced in 2001 in the four target provinces of the APIP project by using monthly reports from veterinary field services. In 2002 the epidemiology unit extended this system to all provinces. The reports are sent from all provinces on the 25th day of the month to the epidemiology section of NAHPIC. Epidemiology staff collect the animal morbidity and mortality reports for the different diseases and enter the data into a computer. Serological surveillance has also been conducted to estimate the prevalence of CSF and FMD in Cambodia.

In 2006 we conducted KAP and serological surveillance to study the prevalence of FMD and CSF by randomly selecting 69 villages in eight provinces (Kompong Speu, Kampot, Kandal, Koh Kong, Phnom Penh, Prey Veng, Krong Preah Sihanouk and Takeo). These provinces represent FMD and CSF control as identified by the Lower Mekong Working Group. A total of 974 serum samples from cattle and buffalo, and 483 from pigs, were collected. The serum surveillance results were as follows:

- Fifty-four (11%) pig serum samples were positive for FMD antibodies.
- Of the cattle and buffalo serum collected, 76 (8%) were positive for type O, 49 (5%) for type A and 25 (3%) for type Asia 1.

¹ National Animal Health and Production Investigation Center, Department of Animal Health and Production, Phnom Penh, Cambodia

Table 1. Animal population statistics in Cambodia during 2001–05

Livestock	Year				
	2001	2002	2003	2004	2005
Cattle	2,868,727	2,924,457	2,989,416	3,039,945	3,184,146
Buffalo	625,907	625,912	660,493	650,572	676,646
Pigs	2,118,273	2,704,435	2,297,439	2,428,582	2,688,612

Table 2. Classical swine fever outbreaks during 2001–06

Year	Outbreaks	Number of sick animals	Number of dead animals
2001	8	1,862	233
2002	8	3,003	340
2003	9	2,972	333
2004	2	147	8
2005	0	0	0
2006	0	0	0

Table 3. Foot-and-mouth disease outbreaks during 2001–06

Year	Outbreaks	Number of sick animals	Number of dead animals
2001	0	0	0
2002	53	12,970	115
2003	21	3,983	38
2004	7	1,181	107
2005	53	55,785	706
2006	30	12,806	243

Disease control

There is no definite policy being implemented at present for CSF and FMD disease control. However, we have regulatory measures that prohibit animal movement into and out of infected farms, and control of importation and exportation of animals and animal products at borders. Every year before the rainy season, the Department of Animal Health and Production (DAHP) provides vaccine to all provinces, and in 2006 DAHP provided vaccine to prevent FMD and haemorrhagic septicaemia (HS) throughout the country. Ring vaccination is implemented in case of outbreak in any locality to control the spread of disease.

Future directions

The objectives of the government of Cambodia in the control and eradication of CSF and FMD are:

- strengthening veterinary services from the central level to the village level

- strengthening the NAHPIC's capacity and capability for the diagnosis of FMD and CSF virus
- improving national surveillance disease investigation of, and monitoring and reporting systems for, FMD and CSF
- studying the impact of FMD and CSF, and using the obtained results to indicate the direct and indirect impacts of FMD and CSF to farmers and the national economy
- implementing vaccination campaigns against FMD and CSF
- controlling movement of animals and animal products
- introducing training courses for district veterinarians and village animal health workers
- conducting serological surveillance in provinces that border neighbouring countries
- improving public awareness.

The classical swine fever and foot-and-mouth disease situation in Vietnam

Nguyen Thu Ha¹

Introduction

Vietnam, located in eastern Indochina, has an area of 332,000 km² and shares a 3,730-km border with China, Lao PDR and Cambodia. In 2005 Vietnam's population increased to 82 million, with 70% practising farming. Livestock production has played an important role in agriculture, with the livestock sector comprising 31% of total agricultural production in Vietnam in 2005. Livestock production based on economic output is increasing steadily in the following order: pigs, cattle, buffalo and goats (Table 1).

Table 1. Livestock population in Vietnam ('000 head) in recent years

Year	Buffalo	Cattle	Pigs	Goats
2001	2,950	4,200	21,000	480
2002	2,814	4,062	23,169	484
2003	2,834	4,394	24,884	612
2004	2,869	4,907	26,143	678
2005	2,922	5,540	27,435	685

The foot-and-mouth disease situation in recent years

From 2001 to 2005, foot-and-mouth disease (FMD) has occurred sporadically (Table 2). The number of provinces affected has increased slightly from five to nine, with the number of outbreaks being around 20 each year. In those 5 years FMD often occurred in cattle and buffalo. In 2001 and 2003 there were no FMD outbreaks in pigs. During 2001–03, all out-

breaks were type O only, but in 2004 and 2005 type A also occurred and there was a type Asia 1 outbreak in 2005. FMD outbreaks were numerous in early 2006. From January to June, 46 out of 64 provinces were affected by FMD, with 19,381 cattle and buffalo and 2,579 pigs infected. The disease is now under control and only six provinces are affected. There were three types of FMD detected: O, A and Asia 1 (O is the most common type). Type A was detected only in cattle and buffalo. According to the World Reference Laboratory (WRL) in Pirbright, UK, the type A strain which caused outbreaks in Ninh Thuan province showed a close genetic relationship to the type A strain from the Thai outbreak of 2003, and the type Asia 1 strain isolated in 2005 showed a genetic similarity to type Asia 1 from Myanmar.

The classical swine fever situation in recent years

The number of classical swine fever (CSF) outbreaks in Vietnam during 2001–05 is presented in Table 3. In Vietnam there are two forms of CSF: a chronic form, and a congenital form where only the foetus is affected and the sows are infected without showing any clinical signs. Occasionally there were outbreaks in fattening pigs. Many pigs, especially sows, become virus carriers even following extensive vaccination. There are many CSF vaccines available in the market but their effectiveness is variable. In all cases of CSF the clinical signs are not clear, so clinical diagnosis is very difficult and results must be confirmed by laboratory testing.

¹ Hanoi Regional Animal Health Centre Laboratory, Department of Animal Health, Hanoi, Vietnam

Table 2. The foot-and-mouth disease situation in Vietnam in recent years

Year	Number of affected provinces	Number of outbreaks	Number of infected cattle/ buffalo	Number of infected pigs	Serotype
2001	5	n.a.	644	0	O
2002	7	22	1,499	136	O
2003	5	13	397	0	O
2004	9	20	986	117	O, A
2005	8	20	1,031	565	O, A, Asia 1

n.a. = not available

Weaknesses in disease control

Foot-and-mouth disease

Weaknesses in FMD control include:

- late disease detection resulting in the spread of outbreaks
- existence of various types: O, A and Asia 1
- late reports, not enough information or no report, so that the real situation of the epidemic is not known well enough to issue appropriate control plans
- farmers having to pay for vaccines, high vaccine prices, insufficient government subsidies, and an unclear vaccine subsidy policy, all of which affect vaccination effectiveness
- cattle and buffalo being freely pastured in outbreak areas, which makes vaccination very difficult and may result in disease spread
- ineffective animal movement control because of the very long border and varied natural conditions; animal owners and traders not following veterinary ordinances and trying to sell infected animals illegally.

Table 3. Classical swine fever outbreaks in Vietnam in recent years

Year	Number of cases	Note
2001	15,483	Sporadic
2002	19,891	Sporadic
2003	13,947	Sporadic
2004	12,105	Sporadic
2005	9,773	Sporadic

Classical swine fever

Weaknesses in CSF control include:

- inability to detect and eliminate the CSF virus carrier
- late vaccination for weaned piglets
- animal movement without being quarantined.

FMD and CSF diagnosis

A laboratory for FMD diagnosis was established in 1996 and sponsored by an IAEA project; 1 year later another FMD laboratory was established by the FAO TCP project. Since then, these two laboratories (National Centre for Veterinary Diagnosis (NCVD) and Ho Chi Minh (HCM) Regional Animal Health Centre) have performed FMD diagnosis for the whole country. Currently, both laboratories use the antigen capture (AC)-ELISA to serotype FMD outbreaks, and other methods, such as RT-PCR and virus isolation, to detect the virus. The non-structural protein (NSP)-ELISA is also used in both laboratories to detect and differentiate infected and vaccinated animals. Every year, some FMD specimens are sent to reference laboratories (WRL, Pirbright, UK, and the Regional Reference Laboratory, Pakchong, Thailand) to research the genetic characteristics of the local strains.

Under the Department of Animal Health (DAH) of Vietnam there are a national diagnostic laboratory and six other diagnostic laboratories that belong to six regional animal health centres. In all laboratories CSF diagnosis is currently performed using AC-ELISA. Before ELISA was introduced, CSF had been diagnosed by the fluorescent antibody test. Since 2003, when highly pathogenic avian influenza (HPAI) occurred in Vietnam, and with great assistance from international donors, many real-time PCR machines have been provided to all the DAH laboratories, and these are also used for CSF diagnosis.

Foot-and-mouth disease surveillance in Vietnam

In Vietnam an FMD surveillance system has been established with assistance from FAO and OIE. In cooperation with OIE, Vietnam has drafted its own

National FMD Control Plan for the period 2005–10, which has been approved by the government. In Vietnam FMD is considered one of the most important diseases and its occurrence is reported on an emergency basis. Under the guidance of veterinary organisations, an animal disease information and disease reporting system has been established to report on outbreaks from the communal level up to the national level (Figure 1). Whenever an outbreak occurs, the Head of the Communal Veterinary Team reports to the relevant District Veterinary Station (DVS) immediately by telephone, which then reports to the Provincial Sub-DAH (SDAH) and, in turn, to the DAH by telephone or email with later confirmation by written letter. The SDAH reports and receives feedback reports on a monthly basis.

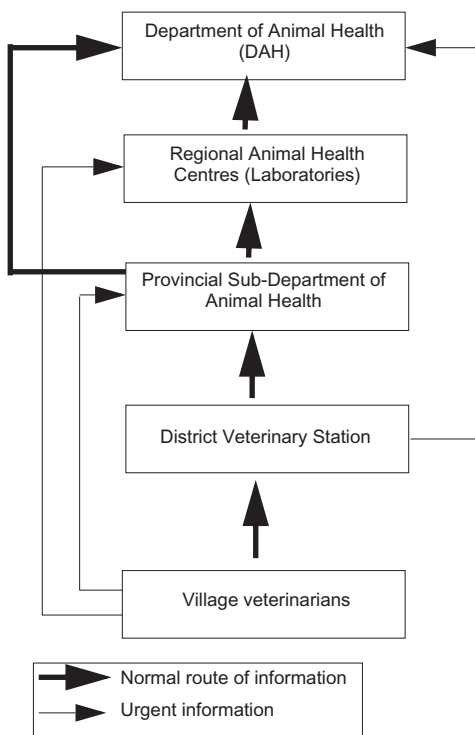


Figure 1. Disease reporting system in Vietnam

Respective Regional Animal Health Centres (Figure 2) send their staff members immediately to the field for further investigations and take samples for laboratory confirmation. Measures to control outbreaks will be decided upon according to the prevailing Veterinary Ordinance. The Animal Health Information System has been developed to some

extent with the FAO TADinfo database program and has been adapted for use under Vietnam circumstances. It was planned that the protocol for reporting CSF and FMD outbreaks be expanded to all 64 provinces throughout the country by the end of 2005. FMD vaccination campaigns were carried out regularly in provinces at higher risk, such as those that share international borders, have intensive live-stock production and have had previous outbreaks. Together with sponsors of some international projects such as CARD (Australia), SEA FMD and some surveillance programs on FMD and CSF have been carried out in some provinces along the borders with China and Lao PDR.

FMD and CSF prevention and control plan

Establishing the zones and the objectives of zoning

The plan includes establishment of the following zones:

- control zone: to control the disease and reduce disease incidence; reduce the number of outbreaks in border provinces; prevent transmission of the disease across borders; and minimise the control zone through a step-by-step process
- buffer zone: to control the disease in the zone; prevent transmission of the disease to the free zone; and successfully control disease, through a step-by-step process, to enable 80% of the provinces in the buffer zone to become disease free
- free zone (intended objective): to eradicate the disease in the delta areas of Red River and Mekong River, where animal populations are high, and to potentially become an exporting zone.

FMD plan

The plan includes the following objectives:

- carrying out the national program on FMD control and eradication 2006–10
- conducting epidemiological surveys to establish disease maps from year to year
- epidemiological surveillance to observe and record disease information, control animal movement and slaughterhouse activity, ensure isolated quarantine of imported animals and destroy affected animals

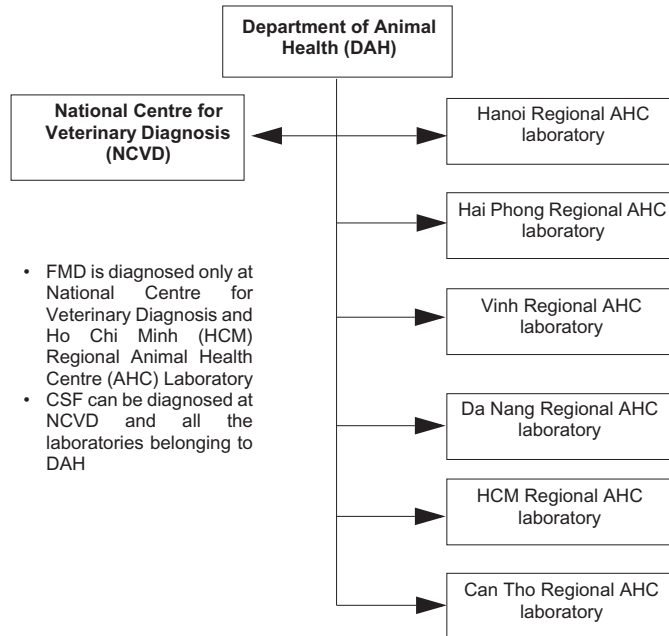


Figure 2. Diagnostic laboratory system

- sero-surveillance in order to detect outbreaks early, and type and subtype viruses to apply appropriate vaccination
- a vaccination plan to determine appropriate vaccines for each area, including monovalent (type O), bivalent (O and Asia 1 or O and A) and trivalent (O, A and Asia 1) vaccines
- public awareness to propagate knowledge of FMD prevention control measures directly to farmers
- training of staff of the Sub-department of Animal Health on procedures of disease control
- international cooperation with neighbouring countries through disease information exchange, carrying out of commitments on animal quarantine at borders, and participation in animal

disease prevention and control programs in order to prevent transmission of trans-boundary diseases.

CSF plan

The plan includes the following objectives:

- establishing CSF-free areas in two provinces in the Red River delta: Nam Dinh and Thai Binh
- a vaccination program twice each year and supplementary vaccination
- control of animal movement
- disinfection of the environment
- applying biosecurity to livestock
- strengthening the knowledge of farmers on CSF.

Classical swine fever and foot-and-mouth disease in Thailand

Nopphavanh Maiya¹ and Wichitra Wannawoharn²

Introduction

The pig-raising system in Thailand is divided into two categories: the agri-business level and the village-agricultural level. The agri-business level is handled by the private commercial sector. Because of good management practices such as standard farming, vaccination programs and biosecurity systems, foot-and-mouth disease (FMD) and classical swine fever (CSF) are not problems at this level. At the village-agricultural level, small-scale farmers learn how to adjust their production to fit within their crop production levels and socioeconomic constraints for the sake of survival of their families. Although there are occurrences of FMD and CSF at this level, the number of outbreaks is decreasing.

There are many organisations responsible for disease reporting, diagnosis and control of FMD and CSF in Thailand.

The Ministry of Agriculture and Cooperatives through the Department of Livestock Development (DLD) is the principal government agency responsible for controlling FMD and CSF. A reporting system and control strategies have been formulated by this organisation, which can also diagnose these diseases and provide FMD and CSF vaccines to support farmers, especially at the village level.

Faculties of veterinary medicine within universities have established laboratories for CSF or FMD diagnosis.

Private agencies maintain large intensive production and also provide health services for farm cus-

tomers, including the supply of drugs, vaccines and veterinary supervision.

Farmers must report disease outbreaks within 24 hours after finding sick pigs according to the Animal Epidemic Act B.E. 1956.

Village keymen provide voluntary help to government officers in clinical surveillance and vaccination.

In the case of FMD or CSF outbreaks, a report from farmers or village keymen or private veterinary farm advisers is sent through the district provincial and regional livestock office to the central office of DLD within 24 hours. Samples for identification of the causative agent are collected and submitted to the nearest laboratory. As soon as the disease is confirmed, the district officers, together with the provincial livestock officers of DLD are assigned to run the control program. In each outbreak the source and possible extent of infection is determined to give a better knowledge of the epidemiological picture of disease. This information is distributed to each organisation by the central office of DLD. Statistical information on the occurrence of the disease forms the basis of reports that are sent to the Office International des Epizooties (OIE) Regional Coordination Unit (RCU). Improved technology using facsimile and online computers has helped to minimise communication time among field personnel, the central office of DLD and the disease diagnostic laboratories.

Diagnosis

Classical swine fever

The diagnostic methods used for CSF are the fluorescent antibody technique (FAT) and cell culture. PCR is used for confirmation in some cases.

¹ Department of Livestock Development, Thailand

² The upper northern Veterinary Research and Development Center, Thailand

Foot-and-mouth disease

After field officers detect FMD-suspected cases by clinical surveillance, tissue culture and competitive ELISA methods are used for viral detection in tissue samples. For serum samples, non-structural protein ELISAs are used to detect FMD-infected animals. The liquid phase blocking (LPB)-ELISA is also used for detection to determine serum antibody titre and for evaluating herd immunity.

Epidemiology of CSF and FMD

Classical swine fever

In Thailand 40, 53, 50 and 25 CSF outbreaks were reported in 2003, 2004, 2005 and 2006, respectively. These occurred in small-scale farms in all parts of Thailand except the eastern region. From disease investigations, the main cause of CSF outbreaks is animal movement.

Foot-and-mouth disease

FMD outbreaks have decreased from 2003 to the present, with 205, 119, 117 and 46 outbreaks in 2003, 2004, 2005 and 2006 reported, respectively. These occurred in all parts of Thailand except the eastern region. The outbreaks mainly occurred in cattle. The strain of FMD virus diagnosed has appeared to change from O to A since 2003. No FMD virus type Asia 1 was found in these years. The last outbreak caused by FMD virus type Asia 1 was reported in Khon Khaen in 1998. However, the potential recurrence of type Asia 1 needs to be closely monitored. From disease investigations, animal movement was still a major factor associated with the occurrence of FMD. Animal movements were reported to be associated in 55, 51 and 43 outbreaks in 2003, 2004 and 2005, respectively. FMD sero-surveillance of 4,203 samples detected viral infection by the NSP method and immunisation from vaccine by the LPB-ELISA method. The results of this project, which is ongoing, indicate a prevalence of FMD outbreaks of 10.2% in 2005 and 7.2% in 2006.

Disease control

Vaccination

Vaccination is conducted at 6-monthly intervals with trivalent vaccines for types O, A and Asia 1, with the main objective being maintenance of herd immunity. The first vaccination round of the year is conducted in May and June and the second in November and December. The above-mentioned trivalent vaccines are produced by the Division of Veterinary Biologics under standardised quality control which complies with the OIE standard.

Animal movement control

According to the FMD information system, about 40–50% of FMD outbreaks in Thailand were associated with movement of animals, which included movement via animal markets, livestock vendors and directly by the owner. In general, the regulation of animal movement requires a formal approval from veterinary authorities when moving animals from one province to another or across the border. However, movement of animals within the province prior to the detection of FMD outbreaks is possible and difficult to control.

Public education

The main objective of this activity is to have farmers, livestock vendors and staff of related governmental organisations gain knowledge about the nature of FMD and the impact of the disease on them and the country. Mass media, such as radio and television broadcasting, newspapers, several forms of printed material and exhibitions, are used continuously to achieve this purpose.

Cooperation with neighboring countries

With assistance from international organisations such as FAO and OIE in coordinating plans and projects, cooperation between Thailand and its neighbouring countries can strengthen the national FMD control and eradication plan, not only in any individual country but also in the South-East Asian region.

Outbreak response

The following steps comprise the response to an outbreak:

- gathering information on animal populations, population distribution, the numbers of villages and farmers in the infected area, and specific routes of animal movement from the infected area
- formulating a program for vaccination, required staff, and materials needed including transport vehicles; and establishing the need for deployment of staff and facilities from another district or province
- strictly controlling animal movement in the infected area
- slaughtering all diseased and in-contact animals according to the regulated policies
- conducting ring vaccination around the infected area as soon as possible
- setting up a surveillance team to investigate the extent of FMD infection
- conducting an outbreak investigation in order to locate the origin of infection as soon as possible
- disseminating information on disease outbreak, vaccination and control measures to farmers and animal traders through various possible means and reporting to the authorities concerned.

Section 3

Diagnosis and vaccination

The molecular epidemiology of classical swine fever viruses from Lao PDR and Asia: A brief review

Stuart Blacksell^{1,2}

Introduction

Classical swine fever (CSF) is a highly contagious virus infection of swine caused by classical swine fever virus (CSFV), a member of the genus *Pestivirus*, family Flaviviridae. The CSFV genome is a positive-sense single-stranded RNA molecule of approximately 12.3 kb. The open reading frame (ORF) encodes a single polyprotein that is post-translationally processed into structural and non-structural proteins. Flanking the ORF are non-coding regions at the 5' (5'NCR) and 3' (3'NCR) ends of approximately 360–385 and 228 bases, respectively. Molecular epidemiology techniques (also known as phylogenetic techniques) have been successfully applied to the investigation of CSF viruses to explain geographical and/or temporal relationships.

Phylogenetic taxonomy

The first major study into the nature of CSF virus phylogeny examined the 5'NCR, E2 and NS5B genomic regions of a large number of CSF viruses that were divided, on the basis of genetic similarity, into two major genogroups and further subgroups (Lowings et al. 1996). These groupings have subsequently been adopted as the standard nomenclature for CSFV genogroup assignment. Minor refinements and expansion of the taxonomic groupings

have occurred with the introduction of a third genogroup and designation of additional subgroups (Paton et al. 2000).

Methodologies

Reference CSFV genetic data is stored in the form of nucleotide sequence in genetic databases such as GenBank. The University of Hannover's School of Veterinary Medicine in Germany has established a dedicated internet-based database that holds nucleotide sequences for 5'NCR, E2 and NS5B genomic regions (Greiser-Wilke et al. 2000), enabling researchers worldwide to access the sequences for local analysis.

Four regions of the CSFV genome—the 5'NCR (Hofmann et al. 1994), E2 gene (Lowings et al. 1996), NS5B gene (Paton et al. 2000) and 3'NCR (Björklund et al. 1998)—have been successfully employed for phylogenetic analysis of CSF virus isolates. The 5'NCR has proved to be a popular and reliable genomic region for the study of pestivirus phylogenetics. The first report of the successful analysis of the 5'NCR was by Vilcek et al. (1994), in which the 324/326 primer set (also employed in the 5'NCR analysis presented in this chapter) was employed to discriminate pestiviruses using phylogenetic and RFLP analysis. Further reports have employed the 324/326 primer set for amplification of the 5'NCR (Sakoda et al. 1999; Stadejek et al. 1996, 1997) for successful restriction enzyme (RE) or phylogenetic analysis of CSFV isolates. The E2 gene has also been used extensively for CSFV phylogenetic analysis. Investigation of a 190-nucleotide (nt) region at the 5' end of the E2 gene has formed

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the basis for the assignment of genogroups and is preferred over the 5'NCR because of the higher bootstrap confidence levels associated with E2 analysis (Paton et al. 2000).

Molecular epidemiology of Asian CSF viruses

South-East Asia

The distribution of CSFV genotypes in Asia is presented in Table 1. Studies of the distribution of CSFV genotypes in South-East Asia have been conducted in Lao PDR, Vietnam, Thailand and Malaysia.

In Lao PDR two studies have examined the phylogenetic relationships of the 5'NCR (Blacksell et al. 2005) and the E2 gene (Blacksell et al. 2004) of Lao PDR CSF virus samples. All Lao CSFV isolates examined belong to subgroups 2.1 and 2.2. There was a strong geographical relationship between CSFV isolates from the northern (subgroup 2.1) and southern (subgroup 2.2) regions of Lao PDR. As the viruses assessed in the studies were only collected over a period of 2.5 years, it was not possible to assess the level of temporal variation in Lao PDR CSFV isolates. The majority of Lao CSF viruses belonged to subgroup 2.2. Clear phylogeographic clusterings were evident for subgroup 2.2 viruses originating from provinces in the southern region, most notably in Champassak province. An important finding was that no subgroup 2.2 virus isolates were detected in the northern region of Lao PDR. Of the isolates belonging to subgroup 2.1, all originated from the northern and central regions of Lao PDR, with none being detected in the southern region. The northern region of Lao PDR is very mountainous with poor transport infrastructure. This isolation

forms a natural barrier from other regions of Lao PDR. It is therefore not unexpected that CSFV isolates from these areas may be distinct, as verified by the absence of subgroup 2.2 viruses in the northern region. The presence of subgroup 2.1 viruses in the central region is most probably due to the movement of infected pigs or pork products to Vientiane City for sale. A recent study of the viral diseases of pigs in Vietnam during 1999 to 2003 found subgroup 2.1 and 2.2 CSF viruses (Kamakawa et al. 2006) that are the same as those found in Lao PDR.

Other countries in South-East Asia demonstrate a large number of CSFV genotypes. In Thailand subgroup 1.1, 2.1, 2.2 and 3.4 CSFV strains have been identified from historical and contemporary virus isolates (Parchariyanon et al. 2000a, 2000b). Three viruses of Thai origin were shown to belong to genogroups 1 and 3 (Sakoda et al. 1999). Malaysian CSF viruses isolated in the 1980s were determined to belong to genogroups 1.2 and 2.1 by analysis of the 5'NCR, E2 and NS5B genomic regions (Lowings et al. 1996).

Northern/Eastern Asia

In northern and eastern parts of Asia, studies have been conducted in China, Japan, Taiwan and Korea.

The first report of the genetic characterisation of Chinese CSFV isolates was during a comparison of the NS5B genetic region of worldwide CSF viruses (Björklund et al. 1999). The Chinese 'Wuhan' field isolate and C-strain vaccine were both determined to be members of genogroup 1 (Björklund et al. 1999). More recent phylogenetic studies compared the E2 region and have identified CSFV isolates belonging to subgroups 1.1, 2.1, 2.2 and 2.3 (Tu et al. 2001). The majority of the CSFV isolates belonged to genogroup 2 (89.3%) with the viruses being almost

Table 1. Summary of CSF virus genogroups found in Asia

Region	Country	Sub-genotype	Reference
Central/Northern Asia	China	1.1, 2.1, 2.2, 2.3	Tu et al. 2001
Eastern Asia	Japan	1.1, 1.2, 2.2, 3.4	Sakoda et al. 1999
Eastern Asia	Korea	3.2	Paton et al. 2000
Eastern Asia	Taiwan	2.1, 2.2, 3.4	Pan et al. 2005; Deng et al. 2005
South-East Asia	Lao PDR	2.1, 2.2	Blacksell et al. 2004, 2005
South-East Asia	Thailand	1.1, 2.1, 2.2, 3.4 1.2, 3.3	Parchariyanon et al. 2000a, 2000b; Sakoda et al. 1999
South-East Asia	Malaysia	1.2, 2.1	Lowings et al. 1996; Vilcek et al. 1996
South-East Asia	Vietnam	2.1, 2.2	Kamakawa et al. 2006

equally divided between sub-genogroups 2.1 (48.1%) and 2.2 (41.2%) (Tu et al. 2001). Of most interest is the genetic composition of CSF viruses originating in Yunnan and Guangxi provinces of China that share common borders with Lao PDR and Vietnam, respectively. All virus isolates originating from Yunnan province belonged to sub-genogroup 2.1 (Tu et al. 2001). Somewhat surprisingly, CSF viruses belonging to a range of subgroups, 1.1, 2.1, 2.2 and 2.3, were detected in adjacent Guangxi province (Tu et al. 2001).

Analysis of the 5'NCR of a large number of Japanese CSFV isolates collected during outbreaks from 1951 to 1986 revealed that these viruses belonged to genogroups 1, 2 and 3 (Sakoda et al. 1999). In Taiwan subgroups 2.1, 2.2, 3.4 have been detected (Deng et al. 2005) and in Korea subgroup 3.2 (Paton et al. 2000).

Europe

The most recent CSF outbreaks in Western Europe, such as the Netherlands outbreak of 1997–98 and the United Kingdom and German outbreaks of August–September 2000, have been associated with sub-genogroup 2.1 CSF viruses (Widjoatmodjo et al. 1999).

Phylogeographic relationships between CSFV genotypes: Opportunities and limitations for disease control

There is considerable diversity in the distribution of CSFV genogroups throughout Asia and the rest of the world. Genogroup 2 viruses have the widest distribution in Asia and are the exclusive genogroup in Lao PDR and Vietnam. It is interesting to note the diversity of CSFV genogroups in Thailand and China. While it is not entirely clear why this is the case, trans-boundary movement of infected animals may be the cause. Given the diversity of genogroup distribution in Asia, the monitoring of CSFV genogroups following an outbreak is an excellent tool for tracking trans-boundary disease. A good example is the speculation on the possible origins of subgroup 2.1 viruses responsible for the CSF outbreaks in Western Europe during the 1990s that were thought possibly to be of Asian origin (Hofmann & Bossy 1998; Paton et al. 2000). Molecular epidemi-

ology will only be an effective tool if continued assessment of virus isolates from the region is performed. Mutations occur naturally within the RNA genome and there are potential incursions of new virus strains by the uncontrolled trans-boundary movement of animals within the region. Low-cost methods for genetic typing of CSF virus such as the use of restriction fragment length polymorphism (RFLP) (Parchariyanon et al. 2000b) may provide more appropriate and rapid methodologies for the assessment of field virus isolates in low-technology settings. Further investigations are, however, required to confirm the long-term usefulness of the proposed techniques.

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Diagnostic tests for the control of classical swine fever and foot-and-mouth disease in South-East Asia: An overview

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Abstract

Classical swine fever (CSF) and foot-and-mouth disease (FMD) are two major trans-boundary animal diseases (TADs) having economic impact on the South-East Asian region. This paper describes the various diagnostic tests available for CSF and FMD, the limitations of each and their potential application in a low-technology setting. The need to have complementary field and laboratory operations including suitable samples and transport methods are discussed, and examples are given. The importance of a quality assurance system to assess the accuracy and precision of diagnostic results is highlighted.

Introduction

Livestock are highly important in the agriculturally based economic and social structures of Asia. Endemic and periodically epidemic foot-and-mouth disease (FMD) has a serious impact on food security (including crop production through its effect on draught animals), rural income generation, and national economies by impairing livestock trade. Consequently, the poorest sectors of the community

are the most seriously affected. The progressive control of FMD is both a national and regional priority (Khounsy et al. 2008, in press). FMD is the most contagious disease of mammals and can cause severe economic loss in susceptible cloven-hoofed animals. While the disease usually does not cause high levels of mortality, it results in productivity losses and the lameness it induces severely limits the uses of cattle and buffalo for traction, which is of major importance to the livelihoods of poor farmers.

Classical swine fever (CSF) is known internationally as one of the most serious diseases of pigs. Infection may result in mortalities of up to 100% in the acute form or reproductive failure and increased susceptibility to other infections. CSF causes large financial losses to both commercial and smallholder pig farmers, contributing to rural poverty. Control of the disease is attempted by vaccination. The economic burden of CSF to the region is difficult to quantify without an accurate diagnostic capability, but there is consensus that it is the most serious disease faced by the pig industry.

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All farmers and governments in the region spend large amounts of money on FMD and CSF vaccines but outbreaks still occur, causing the farmers to vaccinate more frequently. Not all existing laboratories in the region have the necessary capability to confirm the efficacy of FMD and CSF vaccines and to provide an accurate diagnosis. This paper will discuss the different options available for the detection of FMD and CSF antigen and antibody that can be applied to control the diseases. The control of trans-boundary animal diseases (TADs) such as FMD and CSF can only be achieved by taking a regional approach with countries working together. The application of appropriate diagnostic tests under a quality assurance system to ensure accurate and precise results, combined with surveillance and a disease investigation program under a well-resourced animal health network, is vital for disease control.

Animal health network

The diagnosis and control of infectious livestock disease is an important role of the animal health network, comprising both field and laboratory personnel and requiring complementary field and laboratory operations. The animal health network is active during periods of disease surveillance and outbreaks.

During surveillance, laboratory staff conduct post-vaccination testing to both confirm vaccination success and to determine and monitor disease prevalence in target populations. The field veterinarians collect information on vaccination history, which includes vaccines used, disease outbreaks and health status of animals. It is important to involve laboratory diagnosticians, field veterinarians and epidemiologists in the planning of surveillance studies. This will ensure that critical parameters, such as suitable samples, test performance characteristics and accurate prevalence estimates, are available for the design of sampling frames for surveillance studies.

During a disease outbreak situation, field veterinarians are charged with the responsibility of collecting outbreak information, making a clinical diagnosis, collecting samples from suspected cases, and implementing control measures such as disinfection and quarantine and movement of animals. The laboratory staff support and complement the field investigation by conducting tests to confirm the clinical diagnosis and isolate the causative agent for

further characterisation, and performing molecular epidemiology studies to establish potential links with other outbreaks. In the case of CSF, a laboratory confirmation is required due to the difficulty of correctly identifying cases based solely on clinical signs. For FMD the laboratory confirmation is important to establish the serotype responsible for the outbreak.

Classical swine fever

Clinical diagnosis

It is difficult to accurately and confidently predict the CSF infection status of a herd based on clinical findings alone. The clinical signs and lesions associated with CSF can vary depending on the virulence of the virus and, importantly, individual pigs may show different signs when infected with the same virus strain. Clinical diagnosis is further complicated by inter-clinician variation. The experience of the clinician is very important but, in all cases, samples should be collected and tested in the laboratory to confirm or deny a suspicion based on clinical findings.

Laboratory diagnosis

The quality of laboratory testing is only as good as the samples collected and submitted. Suitable samples, which for CSF include spleen, tonsil, lymph node, whole blood and kidney tissue, will maximise the chances of making a correct diagnosis. Samples should be collected from no fewer than four animals showing clinical signs, with samples of approximately 2 g of tissue and 10 mL of blood from each animal transported on ice and reaching the laboratory as soon as possible after collection. A good history of the animals from which the samples were collected is required, as are details of the outbreak investigation or surveillance. This information links the diagnostic results with outbreak and control efforts.

Isolation and specific detection of virus in tissue culture

In-vitro isolation and subsequent detection of CSF virus is achieved on porcine kidney (PK15) cells or other suitable cells such as primary pig kidney (PK), swine kidney (SK6) or swine testis (ST), and is considered one of the most sensitive diagnostic tests. However, virus isolation (VI)

requires specialised facilities for cell culture and handling of virus, and is expensive to maintain. Reference laboratories require this test for characterisation of virus isolates. CSF virus grows in cell culture approximately 18–24 hours after inoculation but samples must be passaged on cells for three 4-day periods before being declared negative. The identification of virus isolates is carried out using specific antiserum- and immuno-techniques on fixed cell cultures, antigen capture (AC)-ELISA or polymerase chain reaction (PCR) tests, usually 48 hours post infection.

Antigen detection

There are a number of techniques for detection of antigen, allowing for rapid and cheap detection of CSF from field samples. In the case of ELISA, testing can be scaled up to process a large number of samples in a relatively short period of time. In-house antigen detection ELISAs (eg AAHL ELISA; Shannon et al. 1993) are commonly used and commercial antigen detection ELISA kits are available from many companies, most commonly CEDI Diagnostics (the Netherlands), IDEXX (USA) and Symbiotics (France). The antigen detection ELISA for CSF gives a result in 3–5 hours depending on the test used, with some tests having an overnight incubation step. New research in Lao PDR, as part of the ACIAR project, has led to the development of a rapid antigen detection ELISA test in a tube using immunomagnetic beads (IMB) as the solid phase (Conlan 2006; Conlan et al. 2008a). The IMB test can be used in the field and read by eye with a result in 60–90 minutes. Immunocytochemistry-based tests such as the fluorescent antibody test (FAT) and immunoperoxidase (IPX) staining are also used to detect CSF virus antigen in cell culture and tissue sections, and results can be achieved within 2 hours.

Molecular technologies

With improvements in PCR technology and advances in methodologies, the detection of viral RNA as a diagnostic tool has now largely surpassed the more traditional procedures such as virus isolation and FAT. There are a number of conventional and real-time PCR methods available for the detection of CSF genome. The real-time PCR (Ophuis et al. 2006) methods currently available are rapid and have high diagnostic sensitivity and specificity. Because the analytical sensitivity of PCR is also greater than other tests, viral genome can be

detected in smaller amounts and therefore sooner after infection, which has important implications for control efforts. Molecular technologies also allow the investigator to perform genetic characterisation of virus isolates and undertake molecular epidemiological studies to identify infection sources and virus evolution. Molecular technologies are, however, expensive and require high-quality samples with intact RNA. When samples are transported at ambient tropical temperatures, as is the case in Lao PDR, sample degradation has been shown to be detrimental to diagnostic performance (Blacksell et al. 2004).

Serological detection

Detection of antibodies to CSF virus has limited scope in diagnosis, particularly if the focus is on the early detection of virus in a herd or if vaccination is undertaken. Serum antibodies to CSF virus typically appear approximately 10–21 days after infection. Serological testing is, however, an important component of a disease control program to monitor the success of vaccination. Antibody detection is best achieved by the ‘gold standard’ neutralising peroxidase linked assay (NPLA). However, because this test requires tissue culture, it is time consuming, expensive and not suitable for the rapid screening of large numbers of samples. Other methods include in-house ELISAs (Colijn et al. 1999) such as the complex trapping blocking (CTB)-ELISA from the Australian Animal Health Laboratory (AAHL) and ELISA kits that can be purchased from commercial suppliers such as IDEXX and CEDI Diagnostics. Not all diagnostic tests are equally suitable to monitor sero-conversion after vaccination. An example is the AAHL CTB-ELISA that is of limited value to detect post-vaccination antibodies in pig sera because its MAb is specific for the NS3 protein of the crude antigen extract. These antigens are normally exposed after infection but only in limited quantities after vaccination. On the other hand, the NPLA and commercial ELISAs, such as the CEDI ELISA, are more sensitive for the detection of post-vaccinal antibodies because the CEDI ELISA uses a baculovirus expressed E2 protein subunit and an E2-specific MAb. Under experimental conditions with 20 vaccinated pigs, the CEDI ELISA showed a similar sensitivity to detect post-vaccinal antibodies as the NPLA, which is considered the gold standard (Conlan et al. in press; Conlan et al. 2008b).

Foot-and-mouth disease

Clinical diagnosis

Clinical signs of FMD vary between species. In cattle, onset of FMD is initially characterised by pyrexia, anorexia and shivering, followed by smacking of the lips, grinding of the teeth, drooling, lameness, and stamping or kicking of the feet. These symptoms are caused by vesicles on buccal and nasal mucous membranes and/or between the claws and coronary band that will rupture, leaving erosions. Recovery generally occurs within 8–15 days although complications can include superinfection of lesions with bacteria or screwworm infestation, hoof deformation, myocarditis, abortion, death of young animals and permanent loss of weight. Post-mortem lesions on rumen pillars and in the myocardium, particularly of young animals (tiger heart), may be evident. In sheep and goats the lesions are less pronounced and foot lesions may go unrecognised. Pigs may develop severe foot lesions, particularly when housed on concrete, and there may be high mortality in piglets. The differential diagnosis is species dependent and includes vesicular stomatitis, swine vesicular disease and vesicular exanthema of swine, which are all clinically indistinguishable from FMD.

Laboratory diagnosis

Virus isolation

As with CSF, virus isolation is expensive to maintain and requires specialised facilities for cell culture and virus handling. Virus isolation and characterisation is important to compare circulating viruses with vaccine strains (*r*-value) to maximise vaccine effectiveness. FMD virus (FMDV) will grow in a wide range of primary and continuous *in-vitro* cell cultures. The most sensitive cell culture for the isolation of FMDV is primary bovine thyroid (BTY) cells (House and House 1989). Continuous cell lines such as baby hamster kidney (BHK), lamb kidney (LK) and the pig kidney cell lines IB-RS-2 and MVPK-1 are also susceptible to FMDV infection. The sensitivity of virus isolation will depend on the quality and type of cells used as well as the quality of the sample.

Antigen capture ELISA

The antigen capture (AC)-ELISA or serotyping ELISA is the test of choice for countries endemic with FMD and is the recommended test for the detec-

tion of FMD antigen (Office International des Epizooties 2004). The FMD AC-ELISA provides detection of FMD antigen and identification of serotype in the case of an FMD-positive sample, and was developed in its current form by Roeder and Le Blanc Smith (1987) and Ferris and Dawson (1988). The FMD AC-ELISA replaced the complement fixation test for primary FMD diagnosis and serotype identification because of its increased specificity and sensitivity and because it is not affected by pro- or anti-complementary factors in the test sample. Standard reagents for the FMD AC-ELISA are produced at the World Reference Laboratory (WRL) for FMD, Pirbright, United Kingdom. At the Regional Reference Laboratory (RRL), Pak Chong, Thailand, reagents for the detection of serotypes A, Asia 1 and O are routinely produced for use in Asia. Sample quality is important as lesions older than 4–5 days have less antigen; however, samples unsuitable for virus isolation can be tested by ELISA. The ELISA allows high throughput testing of samples and is well suited to low-technology settings. Higher throughput can be achieved with robotics and other equipment and is mainly used in large laboratories which can afford to purchase and maintain this capability.

Molecular technologies

In the years since the advent of genetic diagnostic techniques nearly 2 decades ago, more than 50 different nucleic acid hybridisation and various PCR methodologies have been reported for the diagnosis of FMD. Recently, real-time PCR methods (TaqMan, molecular beacons, Primer-Probe Energy Transfer system) have been developed for FMD diagnosis and are now the mainstay for FMD genetic diagnosis (Reid et al. 2002; Oem et al. 2005). Evaluation of real-time PCR methods with conventional diagnostics (Shaw et al. 2004; Ferris et al. 2006) concluded that PCR was generally more sensitive and rapid, and is ideal for samples which contain low concentrations of virus. By introducing nucleic acid extraction and pipetting robotics, together with multichannel real-time PCR machines, diagnostic procedures have become rapid, robust and automated but may not be best suited to low-technology settings. Another promising development for developing country laboratories is the one-step, reverse transcription loop-mediated amplification (RT-LAMP) assay, which enables FMD virus to be detected in under 1 hour in a single tube without thermal cycling (Dukes et al. 2006).

Serological methods

The FMD liquid phase blocking (LP)-ELISA was developed for the detection of FMD antibodies because of the drawbacks of the conventional virus neutralisation tests (VNTs), which included slowness of the test (up to 3 days), the use of live virus and cell cultures, and the difficulty in reproducing results, all which could be countered by the use of ELISA. The FMD LP-ELISA can detect antibodies against all seven FMD serotypes using polyclonal rabbit and guinea pig IgG antibodies to detect residual FMD antigen following an in-vitro incubation of test serum and FMD antigen (the 'liquid phase'). Results from the FMD LP-ELISA indicated a high degree of correlation with VNT results for post-infection and vaccinated animals, and it was suggested to be a suitable alternative to the VNT (Hamblin et al. 1986a, 1986b, 1987). It was also suggested that the FMD LP-ELISA could be used to estimate in-vivo protection to FMD challenge (Hamblin et al. 1986a, 1986b, 1987).

The FMD LP-ELISA is one of the recommended ELISA methods for the detection of FMD antibodies (Office International des Epizooties 2004) and is the primary test for determining vaccine titres, being used throughout Asia (Blacksell et al., in press). Recently, the FMD competitive (C)-ELISA has been developed for all seven serotypes of FMD in response to the FMD LP-ELISA being less conducive to large-scale testing and automation. The FMD C-ELISA was developed using the same reagents as the FMD LP-ELISA but without the 'liquid-phase' step, allowing a result in the same day (4–5 hours). The FMD C-ELISA was found to be more robust, sensitive and specific than the FMD LP-ELISA, and was used in the recent UK FMD outbreak to allow rapid screening of serum samples for FMD antibodies.

FMD non-structural protein assays

Viral replication in FMD-infected animals induces an immune response against the non-structural (NS) protein of the FMD. The response against NS proteins is not serotype specific and indicates infection with any of the seven serotypes. Animals which are not infected with FMD but vaccinated normally don't develop a detectable antibody response against NS protein in the ELISA. Nevertheless, repeatedly applied, low-quality vaccines, (e.g. lack of viral inactivation and purification) may induce a false positive result in this test. In these

cases the history from the field, e.g. about potential outbreaks/infection, identification of vaccine and number of vaccinations received, is important for correct interpretation of the result.

The use of vaccine for control of FMD has led to the development of a number of assays for the detection of NS antibodies to discriminate between vaccinated animals and those that have been infected. AAHL, with the support of IAEA, has developed an in-house FMD NS 3ABC C-ELISA (Morrissey et al. 2007). It uses baculovirus expressed 3ABC antigen and a competing antibody, which is produced in chicken. This ELISA has been used and validated in the region (IAEA TECDOC 2007). There are a number of commercial ELISA kits (de Bronsvort et al. 2004) available from CEDI Diagnostics (baculovirus 3ABC expressed antigen), Bommeli (E-coli 3ABC expressed antigen) and UBI (synthetic 3B antigen), which are the most common NS-ELISAs in use in the region. The CEDI Diagnostics kit and the AAHL kit are both competitive ELISAs that can be used for all species, whereas the other kits are indirect ELISAs with separate kits for ruminants and pigs. The CEDI Diagnostics kit has been found to be the most sensitive and specific kit of those used in the region (Brocchi et al. 2006). Comparisons between the kits from CEDI and AAHL have shown that both ELISAs have similar performance characteristics when applied in the region.

Quality assurance and quality control (QA/QC)

'Quality is fitness for the intended purpose'. Quality assurance (QA) is a system designed to assure test facility management of compliance with a quality standard, e.g. AS ISO 17025-2005 'General requirements for the competence of testing and calibration laboratories'. Quality control (QC) is the technical realisation of the QA concept, e.g. calibration, assay validation, precision and accuracy of test results. QA and QC principles are crucial requirements to comply with quality standards such as ISO 17025-2005 or the OIE's 'Quality standard and guidelines for veterinary laboratories: Infectious diseases'.

The key components of QA are:

- paperwork/documentation of all tests into standard protocols
- validation data for diagnostic tests being used in the laboratory

- staff training and accreditation
- internal quality control (IQC)—positive and negative controls included in each test run
- analysis and charting to document results from IQC controls used in each test
- external quality assurance—successful participation in proficiency test rounds
- documentation on all sample collection, storage and transport from the field, and storage and handling in the laboratory
- calibration of equipment and calibration records
- laboratory accreditation to a standard, e.g. ISO 17025-2005.

Quality control of diagnostic tests is achieved through a combination of IQC and external quality assurance (EQA). Repeatability and reproducibility are measurements of precision and results are of particular value to monitor the validity of test results (De Clercq et al. 2008).

IQC is useful to measure the **repeatability** of test results in a laboratory. Ideally, internal controls should be included as replicates in each test run and should cover at least the critical range of test results to be expected, e.g. strong positive control (C++); weak positive control, which is slightly above the cut-off (C+); and negative control. Analysis of IQCs will give information about intra- and inter-assay variation, intra- and inter-operator variation, day-to-day variation etc. Critical parameters are basic statistics such as mean values, standard deviation, coefficient of variation, range, and upper and lower control limits. Results can be charted and recorded as Levey-Jennings charts. This approach helps to identify trends in assay performance and is useful to prompt preventive corrective actions or troubleshooting. IQC data can also be useful to assess measurement of uncertainty, e.g. continued measurement of replicates of an internal positive control close to the cut-off (see <http://www.scahls.org.au/policyguidelines/Worked_MU_examples.doc>).

EQA or proficiency testing (PT) measures the **reproducibility** of a test and its performance in different laboratories. It helps to standardise test results for the same test in different laboratories (inter-laboratory comparison, ring test or external quality assurance) or to harmonise test results from different tests in different laboratories (proficiency test round). Successful and regular participation approximately twice a year in EQA programs is an essential component of ISO 17025-2005 or OIE quality standard requirements, and therefore a pre-condition for accreditation.

Equipment calibration and maintenance is another important part of QA because it helps to ensure that tests are giving correct results. It is important that laboratories have a budget to allow them to maintain and calibrate their equipment. In summary, QA and QC are crucial elements in a laboratory's quality system and need to be well established to achieve accreditation to internationally accepted standards.

Discussion

Effective diagnosis and control of livestock diseases requires a strong animal health network where laboratory staff, field veterinarians and epidemiologists work together. Laboratories contributing to the diagnostic network must be able to carry out diagnosis with OIE recommended or alternative tests within a recognised QA system. OIE reference laboratories play an important role in monitoring the disease situation in a country and ensuring that continued, updated and accurate information is forwarded to OIE. This is especially important with TADs, zoonotic, and new and emerging diseases because of their global threat.

The laboratory network in a country is made up of laboratories at different levels of standard and capability, from the national laboratory down to the province and district levels. The diagnostic tests used in these laboratories will differ according to their respective capabilities (Tables 1 and 2).

The national laboratory may have the full range of diagnostic tests, which includes virus isolation and a molecular capability for PCR and sequencing, whereas a provincial or district laboratory will only have low-cost technology. Tests such as ELISAs are the most routinely used for CSF and FMD antibody and antigen detection. ELISAs are cheaper to run than virus isolation and PCR but reagents and equipment are still expensive for laboratories in poorer countries or at the district level. The development of cheaper or low-technology diagnostic tests such as the IMB-ELISA for CSF is important to allow rapid diagnosis close to the disease outbreak, e.g. in a district laboratory. The IMB-ELISA does not require any expensive equipment and can be easily quality assured.

For CSF serology the ELISA is the test of choice for sero-surveillance and post-vaccination testing. The VNT gives greater sensitivity and is used to support or confirm ELISA results. Normally it is available either at the national laboratory or a reference laboratory. The VNT test is still the test of

Table 1. Comparisons among classical swine fever diagnostic tests

Type of test	DSn	DSp	Speed	Cost	Quality of sample required	Degree of proficiency required	High sample throughput	Applicability to reference laboratory	Applicability in a low-technology setting
Virus isolation	•••••	•••••	•	•••••	•••••	•••	•	•••••	•
Molecular technologies	•••••	•••••	•••••	•••••	•••••	•••••	•••••	•••••	•••
AC-ELISA	•••	•••••	•••••	•	•••	••	•••••	•••	•••••
IMB-ELISA	•••	•••••	•••••	•	•••	•	•••	•••	•••••
Immuno-cytochemistry	•••••	•••••	•••••	•••••	•••••	•••••	•••	•••••	••
Serology	••	••	•••••	••	•••••	•••	•••••	••••• (NPLA and ELISA)	••••• (ELISA)

DSn = diagnostic sensitivity; DSp = diagnostic specificity

Table 2. Comparisons among foot-and-mouth disease diagnostic tests

Type of test	DSn	DSp	Speed	Cost	Quality of sample required	Degree of proficiency required	High sample throughput	Applicability to reference laboratory	Applicability in a low-technology setting
Virus isolation	••• (BTY)	••••• (BTY)	••	•••••	•••••	•••••	••	•••••	•
Molecular technologies	•••••	•••••	•••••	•••••	•••••	•••••	•••••	•••••	••
AC-ELISA	•••	•••••	•••••	•••	•••	•••	•••••	•••••	•••••
LPB-ELISA	•••••	•••••	•••••	•••	•••	•••	•••••	•••••	•••••

BTY = bovine thyroid cells (BTY is most sensitive cell line; other cell lines are less sensitive)

DSn = diagnostic sensitivity; DSp = diagnostic specificity

choice when studying maternal antibody levels in piglets to determine the best time for vaccination and vaccine protocols. For detection of CSF, an ELISA is recommended, especially where large numbers of samples are being tested. The CSF PCR for detection of genome is recommended where the laboratory has the capability in place and is testing small numbers of samples. Virus isolation is important for further characterisation and is best carried out in the national laboratory or a reference laboratory.

For FMD serology the LP-ELISA is the test of choice in a country where FMD is endemic, as it is still the only test validated for post-vaccination testing. The C-ELISA is used in FMD-free countries, and can be used to screen sera first as it has greater sensitivity and specificity and allows greater throughput. The NS-ELISA is used with the structural LP-ELISA or the C-ELISA to distinguish infected animals from vaccinated. The NS-ELISA does not identify carrier animals, but rather animals that have been infected with FMDV in the past. The NS-ELISA can be used to indicate disease prevalence, or when a country is declaring freedom from FMD, or in animal trading to indicate that animals have not been exposed to FMDV. The AC-ELISA is used for detection of FMD antigen and is the only test able to rapidly determine the serotype of an FMD outbreak. PCR is important as a confirmation for FMD genome and in further characterisation of FMDV by sequencing. Virus isolation is important in producing high-titred stocks of FMDV for characterisation or for growth of samples with low virus titre. Virus isolation is used in national or reference laboratories due to the high cost of maintaining tissue culture.

The quality of samples submitted to the laboratory is important in achieving precise and accurate results and involves:

- maintaining a cold chain
- collection of appropriate samples for diagnosis
- collection in the appropriate sample collection buffer (i.e. phosphate/glycerol for virus isolation and ELISA).

Training of laboratory staff in the different diagnostic tests for FMD and CSF is an important part of AAHL's overseas projects. Training includes aspects of test validation and application of internal and external quality control and assurance principles to monitor assay reliability. Quality results enable epidemiologists and policymakers to make informed decisions about animal health policies.

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Application of immunomagnetic bead technology for improved diagnosis of classical swine fever virus

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Abstract

Classical swine fever (CSF) is a highly contagious viral disease of swine that causes major losses in all pig production systems in many regions of the world. In Lao PDR, CSF is endemic and outbreaks have adverse effects on the predominantly smallholder farming sector. Laboratory testing is required to accurately identify CSF outbreaks because of the difficulty of making a diagnosis based solely on clinical signs. The National Animal Health Centre, located in the national capital, Vientiane, has the capacity to reliably detect CSF antigen in tissue samples using an antigen capture (AC)-ELISA, and antibodies to CSF virus from serum samples using the complex trapping blocking ELISA. This paper describes the use of immunomagnetic beads (IMB) as the solid phase for the portable detection of CSF antigen in spleen samples and for the reliable detection of antibodies to CSF in animals vaccinated with a lapinised C-strain vaccine. The portable IMB-ELISA for antigen detection was shown to be 100% sensitive and 91% specific in comparison to the AC-ELISA. The IMB-Antibody-ELISA was shown to be 97% sensitive and 95% specific in comparison to the gold standard—neutralising peroxidase linked assay. These new diagnostic tests have the potential to improve CSF management through portable and rapid identification of outbreaks and the reliable and inexpensive monitoring of vaccination programs.

Introduction

Laboratory testing of clinical samples is of paramount importance if classical swine fever (CSF) outbreaks are to be correctly identified (Elbers et al. 2004; Paton and Greiser-Wilke 2003; van Oirschot

1999). Likewise, a rapid turnaround from sample collection to reporting results is necessary to ensure control measures are enacted in a timely manner. In Lao PDR sample submission can be delayed after collection, and delays also often occur once samples have been received at the laboratory. To counter this

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problem, new diagnostic tests have been developed to simplify the process, decrease reporting times and provide reproducible results in a portable format without compromising results (Conlan 2006; Conlan et al., in press). The use of immunomagnetic beads (IMB) for the detection of CSF virus has been described previously (Conlan 2006; Conlan et al., in press), where the results are read by eye without compromising test integrity.

Vaccination is a very important control measure for CSF; however, to maximise the potential benefit of a vaccination program, farmers and veterinary authorities need to be able to monitor herd immunity post vaccination. The neutralising peroxidase linked assay (NPLA) is the gold standard for the detection of antibodies to CSF virus (OIE 2004); however, this test is slow, laborious and expensive, and requires tissue culture facilities. In addition, the complex trapping blocking (CTB)-ELISA currently being used at the National Animal Health Centre (NAHC) is not able to be applied in monitoring vaccination programs. Therefore, rapid inexpensive alternatives to these two tests are required.

The results presented in this paper describe the application of the IMB-ELISA (Conlan 2006; Conlan et al., in press) in a near-to-field format for antigen detection, and its adaptation to a format for antibody detection, the IMB-Antibody (Ab)-ELISA.

IMB-ELISA for antigen detection

Control antigens

Negative and positive control antigen extracts were prepared as pooled 5% w/v homogenates in buffered detergent (1% Nonidet P-40 in phosphate buffered saline (PBS) with 5% normal goat serum (NGS) and 0.07% Proclin 300) (Conlan et al., in press).

IMB-ELISA method

Immunomagnetic beads (Spherotech Inc., USA) were coated with anti-pestivirus goat polyclonal antibody according to methods previously described (Conlan 2006; Conlan et al., in press), with some modification. The IMBs were coated at room temperature overnight instead of for 2 hours. Following coating, the IMBs were blocked, washed and resuspended to 0.20% w/v in storage buffer as previously described (Conlan 2006; Conlan et al., in press).

IMB-ELISA test kits were prepared in dropper bottles (Nalgene, USA) and reagents were prepared at working concentrations. Monoclonal antibody (MAb) 24/10 (Kosmidou et al. 1995), specific for CSF virus E2 protein, was incubated with an equal volume of filter-sterilised NGS for 20 minutes prior to dilution in buffer containing 5% v/v glycerol, 0.5% w/v fish skin gelatine, 0.05% v/v Tween 20, 0.07% v/v Proclin 300 in PBS. Goat anti-mouse horseradish-peroxidase (HRP) conjugate (DakoCytomation, Denmark) was incubated with five volumes equivalent of NGS for 20 minutes prior to dilution in Guardian Peroxidase Stabiliser (Pierce, USA). The chromogen-substrate used in the test kit was TMB Liquid Substrate System (Sigma, USA) and was purchased as ready-to-use.

One drop (~35 μ L) of IMBs was added to 100 μ L of sample and controls in a 1.5 mL tube, mixed and incubated at room temperature (~25 °C) for 30 minutes. The tubes were placed on a magnet (Dexter Magnetic Technologies, USA) for 20 seconds and the supernatant discarded. Three drops (~100 μ L) of MAb were added, mixed and incubated at room temperature for 15 minutes. The tubes were again placed on the magnet, the MAb was discarded and three drops of conjugate were added, mixed and incubated at room temperature for 15 minutes. The tubes were placed on the magnet, the conjugate discarded and the IMBs were washed three times prior to transfer to a new tube. The final wash was removed and two drops (~65 μ L) of chromogen-substrate was added, mixed and incubated for 5 minutes at room temperature (Figure 1). Samples were considered positive if an obvious green/blue colour was visible (scored as 3+, 2+ or 1+ depending on intensity) and negative if the colour remained brown/red (scored as 0) (Figure 2).

Relative diagnostic performance

The relative diagnostic performance of the IMB-ELISA test kit was determined using an AC-ELISA (Fuqing et al. 2000; Shannon et al. 1993) as the reference comparator. During 2004–06, 110 spleen samples from CSF-suspected pigs were submitted to the NAHC, Vientiane. Specimens were transported in buffered glycerol (50% v/v glycerol in PBS), prepared as 5% w/v spleen homogenates in buffered detergent and stored at –85 °C prior to testing. The spleen samples were given a randomised number and tested in the IMB-ELISA test kit.

Kit stability

Four replicates of mid-positive and negative control antigens were tested in the IMB-ELISA at weeks 2, 4, 8 and 12 after preparing a kit. The kits were stored at 4 °C throughout. At the completion of the 5-minute chromogen-substrate incubation, 1N H₂SO₄ was added to stop the reaction, and the optical density was measured at a wavelength of 450 nm (OD₄₅₀).

Results

Of the 110 samples, 34 (31%) were positive by AC-ELISA and 41 (37%) were positive by IMB-ELISA. All samples positive by AC-ELISA were also positive by IMB-ELISA; that is, no false negatives were observed. The relative diagnostic per-

formance of the IMB-ELISA test kit was 100% (95%CI: 87–100) sensitive and 91% (95%CI: 81–96) specific in comparison to the AC-ELISA.

The kit was found to be stable for less than 3 months. After 12 weeks the detection efficiency as indicated by the optical density decreased substantially from an OD₄₅₀ of 0.80 to 0.36, a decrease of greater than 50%.

IMB-Antibody-ELISA for detection of antibodies to CSF virus

IMB-Antibody-ELISA method

The IMBs were coated, blocked and resuspended as described above to a final working concentration of 0.10% w/v. The MAb, conjugate and chromogen-

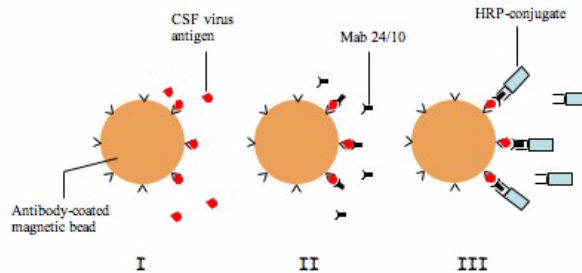


Figure 1. Principle of the IMB-ELISA for antigen detection. **I:** CSF virus antigen capture, **II:** specific detection by monoclonal antibody and **III:** chromogen-substrate colour development

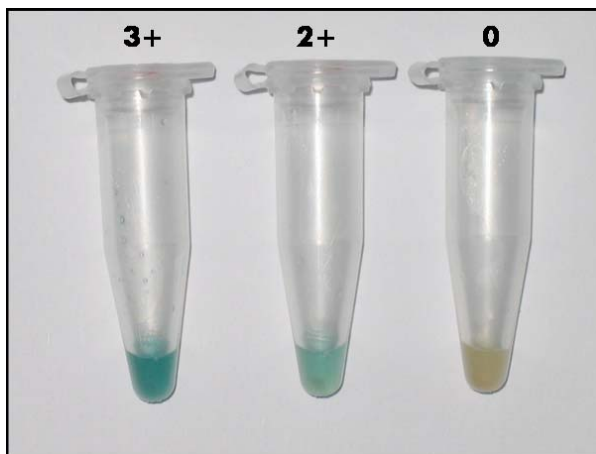


Figure 2. Colour range for test results; positive (bluest, score 3+) to negative (brownest, score 0)

substrate used were the same as those described above. The CSF virus antigen used in the blocking ELISA was the same as that described above diluted 1:2 to 2.5% w/v in buffered detergent.

Twenty-five μL of control or test serum was added to 25 μL of buffered detergent and incubated at room temperature for 5 minutes before the addition of 50 μL of CSF virus control antigen. The serum and antigen were mixed and incubated at 37 °C for 30 minutes followed by the addition of 50 μL of IMBs and shaking for 30 minutes at 37 °C. A no-serum control was included as OD_{max} . The tubes were placed on the magnet for 20 seconds, the supernatant was discarded and 100 μL of MAb added and shaken at 37 °C for 15 minutes. The MAb was removed and 100 μL of conjugate was added and shaken at 37 °C for 15 minutes. The IMBs were washed three times and transferred to a new tube, the final wash was removed and 50 μL of chromogen-substrate added and incubated at room temperature for 5 minutes. The reaction was stopped with 1N H_2SO_4 and the $\text{OD}_{450\text{nm}}$ measured; the percentage inhibition was calculated according to equation (1).

$$\text{PI} = 100 - \frac{\text{OD}_{\text{sample}}}{\text{OD}_{\text{max}}} \times 100 \quad \text{equation (1)}$$

Test samples

Twenty pigs were vaccinated with a lapinised C-strain CSF vaccine in two villages of Bolikhamxay province in central Lao PDR and bled at 0, 35 and 70 days post vaccination. In total, 57 serum samples were tested by NPLA (OIE 2004), Ceditest (CEDI Diagnostics, the Netherlands), CTB-ELISA (Blacksell 2001) and IMB-Antibody (Ab)-ELISA.

Data analysis

The diagnostic cut-off for the IMB-Ab-ELISA was visually assigned after graphically plotting the frequency against intervals of per cent inhibition for the 57 samples assessed, with the NPLA used as the reference test. The diagnostic performances of the IMB-Ab-ELISA and the CTB-ELISA were assessed by calculating relative diagnostic sensitivity and specificity using EpiCalc software (CDC, USA), with the NPLA and CEDI ELISA used as the reference comparators. The level of agreement of the tests was calculated using kappa statistic analysis (Smith 2006), where kappa scores of 0.41–0.60,

0.61–0.80, 0.81–0.99 and 1.00 correspond to levels of agreement of moderate, substantial, almost perfect and perfect, respectively.

Results

The diagnostic cut-off for the IMB-Ab-ELISA was set at greater than or equal to 50% inhibition (Figure 3). At this cut-off there were two false negatives and one false positive when compared to the NPLA. The relative diagnostic sensitivities and specificities of the IMB-Ab-ELISA and the CTB-ELISA are summarised in Table 1.

The levels of agreement between the CTB-ELISA and the CEDI ELISA, and between the CTB-ELISA and NPLA, were less than moderate. The IMB-Ab-ELISA showed almost perfect agreement with both the CEDI ELISA and NPLA.

General discussion

Antigen detection

Appropriate diagnostics are a critical component of CSF management and the speed and efficiency of application will, to a large degree, determine the outcome of a disease-control initiative. The research presented in this paper describes the application of IMB technology to CSF diagnosis in a portable and sensitive format suitable for use in the field. The detection of CSF viral antigen by IMB-ELISA was first described by Conlan et al. (in press), and was found to be a rapid, sensitive, specific and highly repeatable test format with demonstrated high levels of agreement between operators. Minimal training was required to implement the test in a laboratory and the test was not expensive. These combined factors make the test ideal for the conditions seen in a low-technology setting such as Lao PDR where sample submission from remote locations can be difficult. This research demonstrates that the test was successfully adapted to a portable format using dropper bottles for dispensing reagents, performing the test at room temperature and reading the result by eye. Diagnostic performance was good in comparison to an AC-ELISA, with 100% and 91% relative diagnostic sensitivity and specificity, respectively. The estimated shelf life was not as good as was expected using stabilised reagents. After 3 months the test performance dropped to unacceptable levels, and this will need to be corrected in the future.

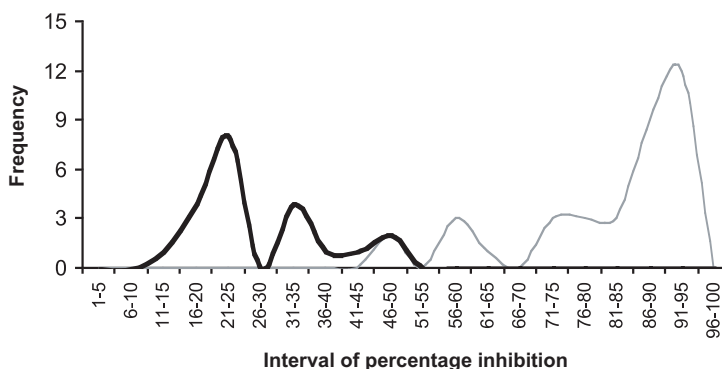


Figure 3. Analysis of the reactivity range of 57 serum samples tested in the IMB-Ab-ELISA. The black line represents samples negative for CSF antibodies (≤ 8 by NPLA) and the grey line represents samples positive for CSF antibodies (≥ 8 by NPLA).

Table 1. The relative diagnostic performance and level of agreement of the IMB-Antibody (Ab)-ELISA and complex trapping blocking (CTB)-ELISA in comparison with two standard antibody tests, the neutralising peroxidase linked assay (NPLA) and the Ceditest

	IMB-Ab-ELISA			CTB-ELISA		
	DSn	DSp	<i>K</i>	DSn	DSp	<i>K</i>
Ceditest	97	97	0.92	40	100	0.34
NPLA	97	95	0.92	39	100	0.32

DSn = diagnostic sensitivity; DSp = diagnostic specificity; *K* = kappa statistic

Monitoring vaccination

Vaccination is the only control and prevention measure undertaken in Lao PDR to minimise the occurrence of CSF in village production systems. A slaughter policy during an outbreak does not exist; however, in some villages, quarantine systems have been set up to decrease the risk of introducing disease into a village. Farmers and animal health officials are, therefore, highly reliant on the success of vaccine delivery and need suitable resources to accurately monitor vaccine uptake. The CTB-ELISA currently used in Lao PDR is not suitable for this purpose; this study demonstrated that its level of agreement with the NPLA test was very poor (0.32) and it showed low test sensitivity (39%) for the detection of vaccinated sero-positive animals. The adaptation of the IMB-ELISA into an antibody detection format has produced promising early results. The level of agreement with the NPLA was almost perfect (0.92) and the sensitivity and specificity were very high (97% and 95%, respectively). At this stage of test development, proof

of principle has been clearly demonstrated but too few samples have been tested to give a clear indication of test performance. Further work is required to adapt the test to a plate format to increase speed and the number of samples that can be tested.

Conclusions

Immunomagnetic bead technology is adaptable and versatile and can provide a platform for appropriate diagnostic test development in a limited-resource setting such as Lao PDR. The IMB-ELISA for CSF is inexpensive, portable, stable and a reliable test that requires minimal training to implement and will improve diagnostic services for pig farmers. The IMB-Ab-ELISA, while in the early stages of development, shows strong agreement with the 'gold standard' NPLA and could be a valuable tool for monitoring vaccine uptake.

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Classical swine fever virus vaccine stability in Lao PDR

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and Laurence Gleeson^{1,2}

Abstract

Classical swine fever (CSF) virus is a highly contagious but vaccine-preventable disease of swine. A locally produced lapinised C-strain vaccine is used to control CSF in Lao PDR; however, vaccine failure has been reported. The CSF vaccine is produced at the National Vaccine Production Centre (NVPC) as a freeze-dried rabbit spleen homogenate in a rubber stoppered glass vial and stored at -20°C with a recommended shelf life of 1 year. This paper describes two studies to (i) determine the stability of the locally produced vaccine when stored at 4°C and -20°C and (ii) determine if the vaccine elicits a protective immune response when delivered to village pigs under good transport conditions. The vaccine was found to be stable for only 4 months when stored at -20°C and for less than 3 months when stored at 4°C . Under field conditions, vaccine stored at -20°C for 2 months and transported at temperatures less than 1°C elicited an immune response in 89% of vaccinated pigs by day 35 and 100% of pigs by day 70 post vaccination.

Introduction

The control and management of classical swine fever (CSF) virus in an endemic country is reliant on rapid disease recognition and the use of an effective vaccine. Commercially available vaccines for CSF are widely available, the most common being live attenuated virus vaccines or subunit vaccines of the immunodominant E2 protein. The most effective live attenuated vaccines are based on wild-type virus strains and include the C-strain vaccine, the Japanese guinea-pig-exaltation-negative (GPE-) strain derived from the virulent ALD strain, the cell culture

adapted Thiverval strain derived from the virulent Alfort strain (de Smit 2000; van Oirschot 2003) and the PAV-250 strain (de Smit 2000). Traditionally, C-strain vaccine was produced from the organs of rabbits inoculated with a working seed (Terpstra et al. 1990). The C-strain virus has subsequently been adapted to cell culture systems for large-scale production of vaccine using the swine kidney cell line SK-6 (Terpstra et al. 1990) or minipig kidney (MPK) cells (Ferrari 1992; Rivero et al. 1988).

In Lao PDR the CSF vaccine is produced from the live attenuated C-strain virus from homogenised rabbit spleen and mesenteric lymph nodes freeze dried in the presence of stabilisers in a rubber stoppered vial (Khounsy et al. 2007). Only a small proportion of pigs are vaccinated in Lao PDR and vaccine failure has previously been documented and attributed to one or more of the following: (i) vaccine not viable at manufacture, (ii) inactivation due to incorrect storage and transport, (iii) incorrect administration of the vaccine or (iv) the presence of

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maternally derived antibodies. The research presented in this paper specifically addresses the second factor, vaccine stability during transport and storage. The 'cold chain' in Lao PDR is perhaps the most important factor limiting the successful delivery of temperature-sensitive live virus vaccine that requires storage at $-20\text{ }^{\circ}\text{C}$. Figure 1 represents a descriptive model of the cold-chain limitations under ideal conditions, where several temperature fluctuations occur and storage at provincial and district levels is inadequate according to the manufacturer's recommendations.

To determine if the temperature fluctuations could be eliminated from the transport equation by storing vaccine at $4\text{ }^{\circ}\text{C}$ instead of $-20\text{ }^{\circ}\text{C}$, the long-term stability of the vaccine was assessed at these two temperatures. Secondly, the ability of the vaccine to elicit a protective immune response in village pigs was assessed.

Vaccine stability

Materials and method

A single batch (05/2004) of the lapinised CSF C-strain vaccine was procured from the National Vaccine Production Centre (NVPC) and transported on ice to the National Animal Health Centre (NAHC), Vientiane, Lao PDR. The vaccine batch was assessed by vaccinating four pigs that had been brought into the laboratory pens, allowed to accli-

mate and treated with ivermectin and antibiotics to eliminate any infection. Two pigs were included as non-vaccinated controls.

One month after procuring the vaccine, an adequate volume of vaccine was stored at $4\text{ }^{\circ}\text{C}$ and the remainder kept at $-20\text{ }^{\circ}\text{C}$. Temperature was monitored for both lots of vaccine using a temperature logger (Thermocron, OnSolution, Australia) at 20-minute intervals. After 3 months' storage at $4\text{ }^{\circ}\text{C}$, one group of four pigs was vaccinated with vaccine stored at $4\text{ }^{\circ}\text{C}$ and a second group with vaccine stored at $-20\text{ }^{\circ}\text{C}$ for 4 months, and one unvaccinated pig was included as a control. As above, pigs were treated with ivermectin and antibiotics to eliminate infection prior to vaccination. After 4 months' storage at $4\text{ }^{\circ}\text{C}$, the above protocol was repeated. Pigs were bled on days 0, 10, 14, 21 and 28 post vaccination (pv) to monitor neutralising antibody titre by the neutralising peroxidase linked assay (NPLA) (OIE 2004).

Results

Vaccine stored frozen during the experiment was held at an average temperature of $-18.28\text{ }^{\circ}\text{C}$ with a standard deviation of 1.28. Vaccine stored in the refrigerator was held at an average temperature of $4.34\text{ }^{\circ}\text{C}$ with a standard deviation of 0.73.

All pigs vaccinated during the pre-trial assessment of batch number 05/2004 were CSF antibody negative on day 0 and all four vaccinated pigs were

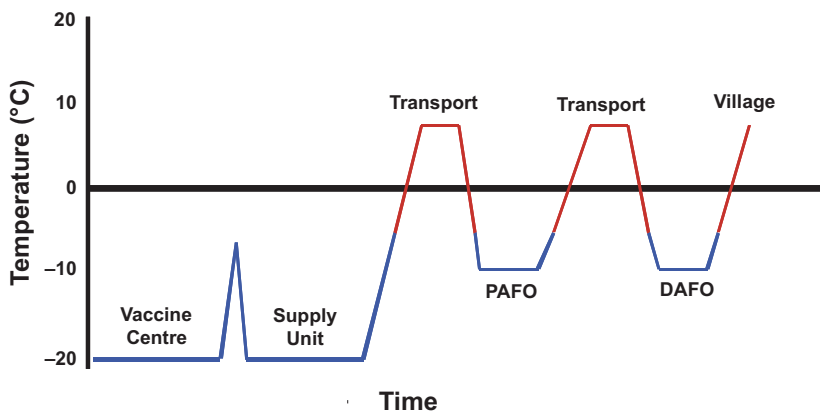


Figure 1. Conceptual model of CSF vaccine delivery in Lao PDR. The blue line represents adequate temperature and the red line represents substandard storage temperatures. PAFO = Provincial Agriculture and Forestry Office; DAFO = District Agriculture and Forestry Office. Storage times at PAFO and DAFO can be variable, depending on demand for vaccine.

positive for the presence of CSF virus neutralising antibodies on day 35 pv, with a median titre of 1:44 (range 1:32–1:44). The median antibody titre on day 28 pv was 1:32 (range 1:22–1:32). Antibodies were first detected in one pig on day 10 pv, three pigs on day 14 pv and all pigs on day 21 pv.

For the pigs vaccinated with vaccine stored at 4 °C for 3 months, the median neutralising antibody titre was 1:4 (n=4; range 1:4–1:32); no significant difference could be demonstrated in comparison to the pre-trial assessment (Fisher Exact Test, p=0.07) due to the small sample number. However, storage at 4 °C for 3 months was four times more likely to result in vaccine failure than new vaccine (risk ratio=4, 95%CI: 0.73–21.84). For the pigs vaccinated with vaccine stored at 4 °C for 4 months, the median neutralising antibody titre was 1:4 (n=4; range 0–1:8), a significant difference when compared to new vaccine (Fisher Exact Test, p=0.01).

For the pigs vaccinated with vaccine stored for 4 months at –20 °C, the median neutralising antibody titre was 1:36 (n=4; range 0–1:352); no significant difference could be demonstrated in comparison to the pre-trial assessment (Fisher Exact Test, p=0.5) and the risk of failure after storage for 4 months at –20 °C was low (risk ratio=1.33; 95%CI: 0.76–2.35). For the pigs vaccinated with vaccine stored at –20 °C for 5 months, the median neutralising antibody titre was 1:4 (n=3; range 0–1:8), a significant difference when compared to new vaccine (Fisher Exact Test, p=0.01). One of the four pigs in this final group died during the experiment and no CSF virus antigen was detected in its organs.

Discussion

The cold chain for the delivery of frozen vaccine in Lao PDR is poor; as a result, the delivery of frozen vaccine to village pigs requires several freeze–thaw cycles and substandard storage temperatures. It is well recognised that CSF virus is adversely affected by temperature fluctuations such as repeated freezing and thawing. The principal aim of this research was, therefore, to determine if the locally produced CSF vaccine could be stored at 4 °C for prolonged periods of time and remain immunogenic. An added component of this research was one of quality assurance—determining an estimate of vaccine shelf life when stored at –20 °C.

The results of this study show that, for this batch at least, the vaccine cannot be stored at 4 °C for extended periods of time and remain viable. After

3 months' storage at 4 °C at the NAHC with no temperature fluctuations, the vaccine was not able to elicit a good immune response in test animals. Somewhat surprisingly, this study found that the vaccine is not stable for at least as long as the manufacturer recommended. The vaccine was still viable after 4 months' storage at –20 °C but was unable to elicit a good immune response in test animals when stored for 5 months. This experiment was conducted under ideal conditions of storage and transport; it is anticipated that under field conditions the vaccine stability would be even less. Many provincial and district vaccine storage freezers are unable to maintain temperatures in the range of –15 °C to –20 °C. To navigate through the constraints of delivering a quality CSF vaccine to a village pig, a great deal of planning will be required on the part of the Lao animal health service. More research and investment is required to address the quality of CSF vaccine produced at the NVPC and its subsequent delivery to village farms.

Vaccine delivery at the village level

Material and methods

Two villages in Bolikhan district, Bolikhamxay province, were selected for this study. Thirty CSF vaccine vials (300 doses) were procured from the NVPC (batch number: 03/2006) and stored frozen at the NAHC for approximately 2 months. The vaccine was transported in an ice box to the selected villages and the temperature was monitored throughout with a temperature logger (Thermocron, OnSolution, Australia) at 20-minute intervals.

All pigs in the villages were vaccinated (excluding pregnant sows and piglets <1 week of age). Blood samples for serology were collected from 10 pigs in each village prior to vaccination and again 35 and 70 days post vaccination. Sera were tested in the complex trapping blocking (CTB)-ELISA at the NAHC, and a portion of the samples were also sent to the CSIRO Australian Animal Health Laboratory (AAHL), Geelong, Australia, for testing by the NPLA and Ceditest (CEDI Diagnostics, the Netherlands) for CSF antibody.

Results

The average storage temperature at the NAHC over the 2 months prior to vaccination was –21.2 °C (standard deviation: 1.8). During transport to the village, the temperature within the icebox was main-

tained at or below 0 °C, with logger readings gradually increasing from -14.5 °C to 0 °C.

By NPLA, one pig was weakly positive for antibodies to CSF virus on day 0 pv (titre=1:8) and the remaining 18 pigs were negative for neutralising antibodies. One serum sample could not be tested by NPLA due to cell toxicity. By day 35 pv, only 19 pigs remained in the cohort and 17/19 (89%) were positive for the presence of neutralising antibodies; however, only 6/19 (32%) pigs had antibody titres \geq 1:32. On day 70 pv, 18 pigs remained in the cohort and all were positive for the presence of neutralising antibodies, with 17/18 (94%) having an antibody titre \geq 1:32. The Ceditest had very strong agreement with the NPLA results ($\kappa=0.88$) and diagnostic specificity and sensitivity were 90% and 97%, respectively. The CTB-ELISA, on the other hand, had very poor agreement with the NPLA results ($\kappa=0.32$) and diagnostic specificity and sensitivity were 100% and 39%, respectively.

Discussion

This study has clearly demonstrated that, under ideal storage and transport conditions, a relatively new batch of vaccine can elicit a protective immunity in village pigs. Seventy days after vaccination, 94% of pigs had an antibody titre \geq 1:32, which, for epidemiological purposes, affords complete protection and prevents virus shedding (Terpstra and Wensvoort 1988). Post-vaccinal antibody titres can continue to increase for up to 12 weeks (Dahle and Liess 1995; Terpstra et al. 1990; Terzic et al. 2003), and this was observed during this study. The NVPC recommends that the vaccine be stored at -20 °C for up to 1 year; however, as demonstrated above, the vaccine loses viability after 4 months' storage under recommended conditions. The capacity of rural agricultural offices to hold vaccine at -20 °C is low regardless of the timeframe it can be stored for; therefore, the critical issues of vaccine stability and delivery remain. Future strategies for the improvement of vaccine delivery at the village level need to be put in place if farmers are expected to embrace this technology.

This study has also highlighted the critical issue of having the capacity to monitor vaccination success. The only antibody detection test routinely available for CSF in Lao PDR is the CTB-ELISA. However, this test was unable to reliably detect vaccinated positive animals, with a sensitivity of just 39% and a

very low level of agreement with the NPLA ($\kappa=0.32$). Additional work is required to increase the capacity of the laboratory to enable the detection of vaccine-related serological responses. The Ceditest and NPLA are expensive tests in comparison to the CTB-ELISA, and could not be introduced into mainstream laboratory testing without the continued support of foreign donors. Research is required to develop a simple and inexpensive alternative to the CTB-ELISA that is capable of sensitive and specific detection of vaccinated positive animals.

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Recommended vaccine programs for village-based pig production systems in Lao PDR

Syng Khounsy¹, Tess Vitesnik^{1,2} and James Conlan¹

Introduction

Classical swine fever (CSF) virus is endemic in Lao PDR, with major outbreaks each year resulting in significant production losses in all farming systems, including smallholder, semi-intensive and intensive farms. CSF is a vaccine-preventable disease and there are many vaccines, both live attenuated and subunit, commercially available. In Lao PDR a variety of livestock vaccines are produced at the National Vaccine Production Centre (NVPC), which is located at Nongteng village 15 km from Vientiane. The NVPC was established in 1980 and produces vaccines for CSF, haemorrhagic septicaemia, Newcastle disease, fowl cholera, infectious bronchitis, fowl pox and duck plague. CSF vaccine is produced from the live attenuated C-strain virus from homogenised rabbit spleen and mesenteric lymph nodes freeze dried in the presence of stabilisers in a rubber stoppered vial and stored at -20°C .

Storage and transport of CSF vaccine

The NVPC recommends that, under correct storage conditions, the shelf life of CSF vaccine is 12 months; however, many provincial and district vaccine storage freezers do not have the capacity of -20°C storage (-10°C is the normal limit in small domestic refrigerator-freezers). The CSF virus is an

enveloped virus and, because rapid and frequent temperature fluctuation results in viral death, there can be a significant decrease in the live virus titre of each dose of vaccine under such conditions. Therefore, temperature fluctuations should be avoided to maximise the amount of live virus in each dose administered to the pig.

Vaccine should be transported on ice in a well-insulated 'cool-box' to prevent the transport temperature exceeding 4°C ; if long transport times are anticipated, sufficient ice or ice-packs need to be included. In Lao PDR vaccine delivery follows a chain from the NVPC through different government offices before delivery to the farm (Figure 1).

Delivery of vaccine at the village level

The CSF vaccine is supplied freeze dried in a 10-dose vial and needs to be reconstituted in 10 mL of sterile distilled water which is provided by the manufacturer. Once reconstituted, the vaccine should be used as quickly as possible and not re-used the next day. The following procedures should be followed during a vaccination program in a village:

- Sterile technique should be used when reconstituting vaccine.
- Unused reconstituted vaccine should be discarded.
- Once reconstituted, vaccine should be used in only one village.
- Pigs should be adequately restrained and 1 mL of vaccine administered intramuscularly.
- A pig snare can be used to restrain larger pigs.
- Sterilised/sterile syringes should be used when administering vaccine.
- Syringes and needles should be sterilised between uses in different villages.

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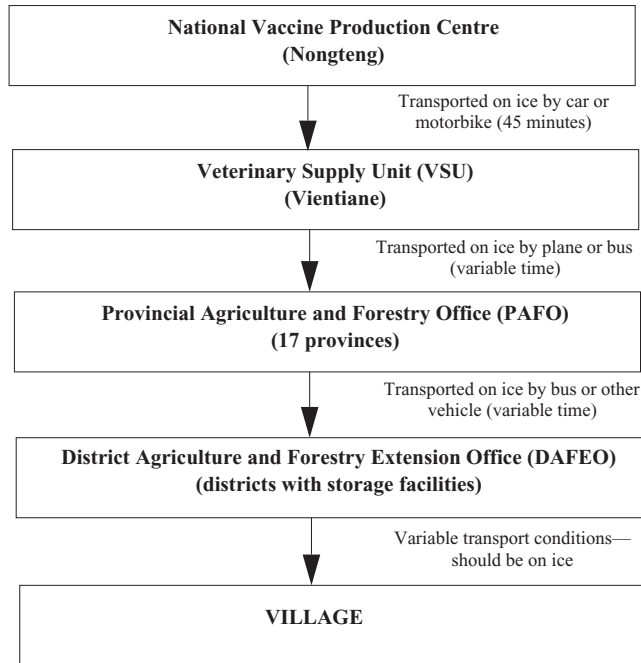


Figure 1. CSF vaccine delivery chain in Lao PDR

Recommended vaccine strategy by animal age class

Sows: Sows should be vaccinated every 6 months or at least once per year. Pregnant sows should not be vaccinated.

Boars: Boars should be vaccinated every 6 months or at least once per year.

Piglets (of vaccinated sow): Every effort should be made to allow newborn piglets access to colostrum within the first 12 hours after birth. Piglets should be vaccinated no earlier than 5–6 weeks of age to avoid virus neutralisation by maternally derived antibodies. A booster dose is recommended 4 weeks after the initial dose.

Piglets (of unvaccinated sow): Piglets should be vaccinated at 1 week of age and a booster dose given 4 weeks later.

Weaners and growers: If vaccinated as a piglet, a booster dose should be given after 12 months.

Purchased pigs: New pigs purchased from a market, middleman trader or another farmer should be vaccinated and kept in quarantine for a minimum of 2–4 weeks and a booster dose given 4 weeks after

the initial dose. Subsequent vaccinations should be given according to information for boars and sows above.

Factors contributing to vaccination success

Animal-related factors include the following:

- no subclinical infection present
- livestock well fed and watered
- low parasite burden
- livestock old enough for maternal antibodies to have declined
- livestock not challenged with CSF virus before vaccine can generate an immune response.

Vaccine-related factors include the following:

- vaccine not expired
- vaccine proved to be effective by the manufacturer
- vaccine stored and transported at correct temperature and fluctuations avoided or minimised
- vaccine not exposed to heat for long periods of time.

Vaccination procedures include the following:

- correct route of administration
- animal restrained to ensure delivery of the correct dose
- use of diluent supplied by the manufacturer
- leftover doses from multidose containers disposed of safely
- sterile equipment used in multidose containers.

Future research directions for classical swine fever and foot-and-mouth disease in Lao PDR: A facilitated session to capture the skills and experience of workshop delegates

Ross Cutler^{1,2} and James Conlan³

Introduction

At the close of the workshop, a facilitated session was held to explore future directions for the control and management of classical swine fever (CSF) and foot-and-mouth disease (FMD) in Lao PDR. The workshop was attended by experts in their fields from the People's Republic of China, Thailand, Myanmar, Cambodia, Vietnam, Lao PDR and Australia, and other representatives from a range of non-government organisations and international development projects. The session was designed in such a way as to best capture the skills and experience of attending delegates to progress ideas in an environment where all participants were able to make a contribution to the discussion.

The workshop participants were divided into five groups in a manner to ensure an even representation across countries. Two leaders were assigned to each group, one to record answers on a whiteboard and the other to facilitate discussion within the group. The leadership personnel comprised Lao and Australian project staff and the session was facilitated by the first author. The session method is described in Figure 1.

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Outcomes

The first question to be addressed by the groups was:

With regard to FMD and CSF projects in Lao PDR, what have been the successful elements?

The key responses are summarised as follows:

Information exchange (regional and local) during outbreaks

- established networks of farmers—district, provincial, national, international
- network of people to report and respond

Knowledge

- improved management skills, training, disease control skills and public awareness
- international cooperation
- improved capacity at all levels
- farmers sensitised about diseases and control

Vaccination

- benefits of vaccination, how to use, information about transport and storage
- improved knowledge of how to produce and deliver a good-quality vaccine
- increased vaccination coverage
- standardised laboratory and vaccine production and training

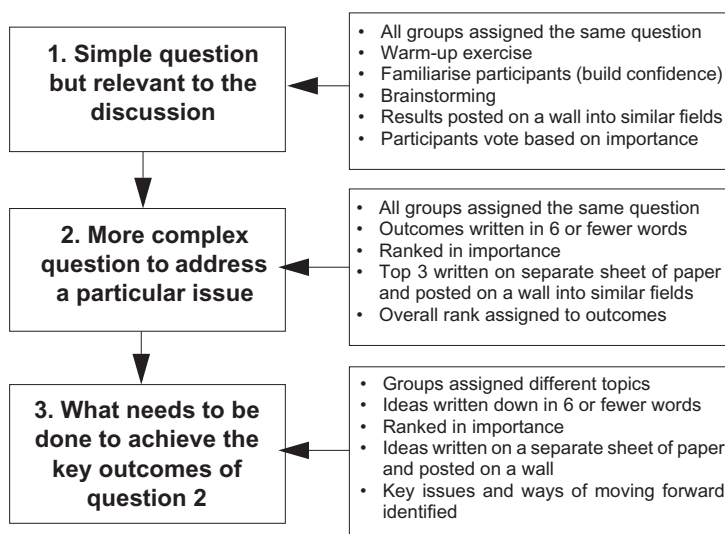


Figure 1. Flow diagram describing facilitated session method

Improved diagnostic capacity: new diagnostic tests

- IMB-ELISA for rapid CSF diagnosis
- quality control improvements
- strengthened sample collection and transport (diagnostic network)

Animal movement control

- local and trans-boundary control.

Each delegate voted for the three most important outcomes of CSF and FMD projects and the results are as follows:

Outcome	Score
Knowledge	15
Vaccination	13
Information exchange	10
Improved diagnostic capacity	7
Animal movement control	2

The session proved successful in introducing the participants to brainstorming and understanding the session format. Delegates were now able to proceed to exploring future possibilities.

The second question was:

What more would you like to see achieved?

The responses and relevant scores were:

Establishment of regional CSF reference laboratory (score 9)

- provision of standard reagents to participating countries
- strain characterisation
- training

Greater disease awareness for farmers and advisers (score 20)

- capacity building for farmers and district agricultural extension staff
- increased capacity of district agricultural extension staff
- organised education material for farmers and provision of better access to education
- increased disease recognition, diagnosis and control capacity
- networking for reporting and response

Training of more veterinary staff (score 20)

- more veterinarians needed in Lao PDR
- training of more veterinary staff

Vaccination (score 36)

- research to develop a heat-stable CSF vaccine
- simple test to check vaccine immunogenicity
- simple test to check post-vaccine immunity
- improved vaccine program (production, delivery and timing)
- encouragement for vaccination (with high-quality vaccine).

Emerging from this question, the most important issues were vaccination, disease awareness for farmers and advisers, and an obvious shortage of veterinary personnel in Lao PDR.

The next question was aimed at addressing these three key points by asking:

What do we do next to:

- a) improve CSF vaccine quality? Group 1
- b) increase the capacity for vaccination? Group 2
- c) increase incentives to vaccinate and promote awareness? Group 3
- d) train more veterinary staff? Group 4
- e) develop greater disease awareness for farmers and advisers? Group 5

The outcomes from this session are summarised below:

a) Improve CSF vaccine quality

- Improvements are needed in quality control at the vaccine production laboratory.
- Testing is needed post vaccination to ensure livestock are protected.
- Improvements need to be made to control the cold chain.

b) Increase the capacity for vaccination

- Ensure enough vaccine is produced to meet demand.
- Ensure enough trained staff are available to supply and administer vaccine.

c) Increase incentives to vaccinate and promote awareness

- Make the vaccine free.
- Raise awareness of the benefits of vaccination among farmers and allied veterinary staff.
- Develop effective advertising to coincide with public awareness.

d) Train more veterinary staff

- Long-term vision is required to correct the human resource deficiency that now exists.
- Short-term specialist training is needed for existing veterinarians and technicians at the district level.
- Encourage and lobby regional governments to provide university places for Lao veterinary students.
- Create university places in regional universities for Lao students.
- Make the options donor friendly—veterinary degrees require a 5–6-year investment, which in many cases is beyond the time frame of a project.
- Develop, in conjunction with universities and international governments, scholarships for Lao students at regional and Australian and New Zealand universities.
- Establish joint funding agreements between Lao and regional governments.
- Encourage donor support.
- Establish postgraduate training for Lao students with a Bachelor of Science degree to upgrade to a Master of Veterinary Science or Master of Science. This is more likely to be a donor friendly option as it will require only a 1–2-year investment; however, there will still be a shortage of trained veterinarians.

e) Develop greater disease awareness for farmers and advisers

- Target farmer groups, exchange information and determine the factors that influence farmers' decision making.
- Assist district and provincial agricultural officers to understand improved techniques of communication.
- Produce effective materials adapted for all people in a range of languages, including all media types (e.g. oral, written, pictorial).
- Improve and extend the university curriculum to include communication skills to upgrade the communication capacity of district and provincial staff.
- Create a suite of education materials and media/ education kits directed specifically at farmers, traders and district agricultural extension officers.
- Use 'mentors' at provincial and district levels to facilitate the dissemination of skills and information.

Conclusions

The facilitated session was able to identify three important areas to focus on in the future for CSF and FMD research. Vaccination was seen as a very important issue for the sustainable prevention of disease, and further research and development will be required to ensure the delivery and use of a quality

vaccine. The human resource deficiency in the veterinary field was highlighted but it was noted that this will be difficult to correct; making options 'donor friendly' will be important. The final key issue highlighted during this session was that of public awareness. For disease control measures to be successful, greater disease awareness at farmer and district levels will be required.

Maximising training outcomes in diagnostic laboratories: A two-way process

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Abstract

In this paper we describe the Australian Animal Health Laboratory (AAHL) experiences and approach with training and technology transfer of diagnostic tests for major livestock disease in South-East Asia. Examples are given of successful achievements in Vietnam, Thailand, Indonesia and Lao PDR. In brief, AAHL follows a 3-step approach. The laboratory diagnostician is trained at AAHL in the test methodology (e.g. TaqMan PCR) for a particular disease—for example foot-and-mouth disease (FMD) or highly pathogenic avian influenza (HPAI)—under ideal conditions and in a quality controlled and quality assured environment. The trainee then takes test reagents and protocol to his/her own laboratory to establish and standardise the test locally. Follow-up is provided by a consolidation and troubleshooting visit by AAHL staff to the overseas laboratory and includes a hands-on workshop on diagnostic techniques to remedy in-situ constraints.

Quality control procedures are built into the technology transfer. AAHL organises external quality assurance rounds to monitor the success of the technology transfer and to obtain useful information about potential sources of error. Other important aspects of training are related to biosecurity and biosafety. The close collaboration with South-East Asian counterpart laboratories increases AAHL's awareness of potential new and emerging diseases in the region. Although being directly involved in the development and validation of diagnostic tests for exotic diseases such as FMD, classical swine fever (CSF), HPAI, severe acute respiratory syndrome (SARS) and henipavirus, AAHL scientists are confronted with the limitations of a country (Australia) where these diseases do not exist. The collaborative nature of the projects in South-East Asia allows AAHL to use and validate these tests in an environment where the diseases are present. In summary, these activities enhance Australia's emergency disease preparedness and pre-boundary protection, which are the pillars to maintaining and improving its competitive international trade status. In turn, collaborating South-East Asian (SEA) countries strengthen their own diagnostic and disease surveillance capacity that subsequently leads to improved disease control—a win-win situation for all involved.

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Introduction

The Australian Animal Health Laboratory (AAHL), in its role as an OIE collaborating and reference laboratory, has a strong commitment to the development, validation and transfer of diagnostic technologies in the South-East Asian region. This process also includes training in the use of the technologies, interpretation of results, and aspects of internal and external quality assurance to ensure ongoing precision and accuracy of diagnostic results. An efficient network between clinical information from the field, laboratory diagnostic results and epidemiological data is the basis for informed decisions and adequate livestock health policies.



Figure 1. Major collaborating countries in South-East Asia

Funding agencies

The ability of AAHL to perform training depends on funds from a number of Australian sources including AusAID and AusAid programs (e.g. via the Collaboration for Agriculture and Rural Development (CARD) program), the Australian Centre for International Agricultural Research (ACIAR) and the Department of Agriculture, Fisheries and Forestry (DAFF). International funding agencies that have contributed to programs include OIE, FAO, IAEA and USDA. The majority of countries that AAHL collaborates with are located within the South-East Asian region (Figure 1).

Projects and training courses

AAHL provides support to the region through two types of training programs: workshops/programs lasting 1–2 weeks to 6 months; and projects of 1–3 years' duration. The training is based around the major trans-boundary diseases (TADs) of concern to Australia, the region and the world including foot-and-mouth disease (FMD), classical swine fever (CSF), duck virus enteritis (DVE), avian influenza (AI), and new and emerging diseases including Nipah, Hendra and severe acute respiratory syndrome (SARS).

AAHL has also organised veterinary training courses for Australia and the region. The focus of these courses is on recognising the clinical symptoms of these diseases and the differential diagnoses available, together with the selection of suitable specimens, protocols for their collection and transport, and information needed from the disease investigation. Below is a list of some of the projects in which AAHL has participated as manager or collaborator.

- FMD in Thailand — ACIAR (AS1/1988/35 1985–91; AS1/1992/04 1994–96), AusAID (2005–07)
- CSF and FMD in Vietnam — ACIAR (AS2/1993/875 1995): 'CSF in Vietnam'; AusAID (CSF 2001–03, FMD 2005–08).
- Duck virus enteritis (DVE) in Vietnam — ACIAR (AF2/1991/022 1996–99) in collaboration with University of Queensland: 'Duck plague: improved diagnostic methods and vaccination'.
- FMD and CSF in Lao PDR — ACIAR (AS1/1994/038 1997–2003): 'Improved diagnostic and control methodologies for livestock diseases in Lao PDR and Yunnan Province, PRC'; and ACIAR (AH/2003/001 2003–08): 'Management of CSF and FMD at the village level in Lao PDR'.
- Quality assurance project in Thailand and Indonesia (EQA program in six laboratories) — DAFF (2000–01) in collaboration with Attwood laboratory (ANQAP).

Workshops and training programs are aligned with AAHL's key deliverables in diagnostics and the support of Australia's preparedness for disease emergency. These include diagnostic techniques (i.e. ELISA, haemagglutination assay (HA) and haemagglutination inhibition assay (HI), cell culture, virus isolation, PCR and sequencing); veterinary training (i.e. disease diagnosis, disease investigation and

sample collection); major disease threats; and new and emerging diseases such as FMD, CSF, Nipah virus (Malaysia, Thailand, Indonesia, Taiwan and Japan) and AI (the Philippines, Vietnam, Indonesia, Nepal and Myanmar). There has been an increase in provision of molecular diagnostics training to the region, which gives diagnostic laboratories the ability to perform rapid testing of samples from the field. The PCR test provides increased sensitivity, ability for higher throughput and a rapid result compared to virus isolation. With the aim of determining the need for, and gaps in, improved diagnosis in regional laboratories, AAHL has undertaken an increased number of laboratory assessments.

Major collaborations and outcomes

Vietnam

ACIAR duck plague project

The ACIAR duck plague project in Vietnam, in collaboration with Queensland University and the Cambodian National Veterinary Company (NAVETCO), was a very successful ACIAR project with clear impacts. This project developed new diagnostic tests for diagnosis of DVE that were previously lacking both in Vietnam and Australia. The project's achievements included: the development of diagnostic tests for detection of antigen and antibody including indirect antibody ELISA, antigen capture ELISA, cell culture and virus isolation, and PCR; the development of a new cell culture vaccine that is cheaper and of higher quality; and a leadership role for other projects in Vietnam for CSF and FMD.

AusAID CARD FMD project

The current CARD FMD project in Vietnam has been successful for both Vietnam and Australia. It has given AAHL scientists a chance to enhance AAHL's FMD diagnostic capability as well as better understand FMD at the field level. The aims of the project are to establish an effective laboratory network for the diagnosis and control of FMD by providing resources and training staff in required methods and quality assurance; and to provide accurate data that will help to explain the failure of vaccination to control the FMD virus and develop new effective vaccine application strategies. The CARD project's achievements include:

- The Ho Chi Minh City and Hanoi (National Centre for Veterinary Diagnosis) Department of Animal Health laboratories now have established diagnostic tests for FMD diagnosis including ELISA, cell culture, virus isolation, PCR, nucleotide sequencing and the production of FMD antigen for use in ELISA (reagent production).
- The regional laboratories of Da Nang and Can Tho now have capability for detection of FMD antibody and antigen using ELISA.
- The project has improved the quality control and quality assurance procedures for diagnostic tests.
- Field specimens from FMD-infected animals and other reference populations were received to assist with further validation and sequencing of field isolates.
- The project is being used as a model for Vietnam's National Control Plan for FMD.
- There have been requests for assistance to improve diagnosis and outbreak investigation for AI and goat pox in Vietnam and further collaborations with DAH.

Lao PDR

AAHL has managed ACIAR-funded projects conducting studies on CSF and FMD in Lao PDR since 1997, with a focus on improving the country's animal health network from field to the laboratory. The project has been vital for improving the national diagnostic capacity through development of the laboratories and training of national staff to enable and enhance understanding of the disease situation in Lao PDR. Project outcomes include the implementation of conventional laboratory diagnostics for the diagnosis of CSF and FMD using ELISAs for antibody and antigen detection, as well as studies to determine the most appropriate samples for CSF diagnosis (Khounsy et al. 2007). Furthermore, a novel immunomagnetic bead (IMB)-ELISA was developed for the rapid diagnosis of CSF in low-technology field settings (Conlan et al., in press). Extensive training of local staff in disease investigation and surveillance, and vaccination and quarantine procedures, was conducted. The outcome was the development of a national sample submission network that resulted in a better understanding of the distribution of CSF virus strains from outbreak samples using molecular epidemiology (Blacksell et al. 2004; Blacksell et al. 2005). Detailed studies of FMD epidemiology were per-

formed using serological surveys and antigenic analysis from FMD outbreaks (Blacksell et al., submitted; Khounsy et al., submitted). Studies also demonstrated that local native pigs were less susceptible to CSFV infection when compared to improved breeds (Blacksell et al. 2006). In addition, the project provided opportunities for improved livestock production and understanding of livestock marketing.

Thailand

AAHL has had a long relationship with Thailand, having worked on FMD since 1985. Projects were based at the FMD Regional Reference Laboratory located at Pak Chong in north-eastern Thailand and the Northern Veterinary Research and Diagnostic Center in Lampang in northern Thailand. During the life of these projects, numerous Thai scientists visited AAHL for short- and long-term studies, and Australian scientists were resident in Thailand providing training to local scientists and gaining experience in FMD diagnosis and control measures. The early projects were funded by ACIAR and concentrated on the development, transfer and validation of FMD diagnostic technologies (Blacksell et al. 1994a), examination of local FMD epidemiology (Gleeson et al. 1995; Chamnanpood et al. 1995) and characterisation of Thai FMD strains (Blacksell et al. 1992; Lunt et al. 1994; Doughty et al. 1995a, 1995b). In addition, ACIAR projects developed internal quality assurance programs for the diagnostic assays in use at the time (Blacksell et al. 1994b; Blacksell et al. 1996). The current AusAID FMD CARD project is focusing on quality assurance and the completion of the BSL3 laboratory at Pak Chong. Current project outcomes include:

- BSL3 laboratory completed and in use
- accreditation to ISO 17025 Standard to obtain OIE status as a regional reference laboratory (RRL)
- harmonisation of diagnostic protocols for FMD diagnosis in the region
- training of regional countries to send samples to RRL
- training of regional scientists in FMD diagnostics.

Malaysia

AAHL assisted Malaysia in the control and eradication of Nipah virus from Malaysian pig herds. Nipah virus is a zoonotic disease affecting both animals and humans, and AAHL was able to apply

its experience with Hendra virus, another emerging disease that affected horses and humans in Australia. Providing support in both the laboratory and the field, AAHL staff worked in-country with Malaysian scientists and veterinarians. Major outcomes from the collaboration were:

- the development of diagnostic tests for detection of Nipah virus antigen and antibody including indirect ELISA (Nipah virus ELISA) for antibody detection; virus neutralisation test (VNT) for detection of antibody; and virus isolation, PCR and EM tests for detection of antigen or genome
- AAHL Nipah virus ELISA used for sero-surveillance and eradication of Nipah virus
- support in the laboratory and in the field
- AAHL becoming the OIE collaborating laboratory for new and emerging diseases.

The newly developed indirect Nipah ELISA was vital for the control and eradication of Nipah virus from Malaysia. The test was used to decide on the status of pig farms for the presence of Nipah virus and the culling of pigs in infected premises. The indirect Nipah ELISA had a relative diagnostic sensitivity of 98.99% (94.50–99.97% at 95%CI) and a relative diagnostic specificity of 99.91% (99.78–99.97% at 95%CI) with a kappa value of 0.9697 for ELISA and VNT (Table 1).

Table 1. Relative diagnostic specificity and sensitivity of indirect Nipah ELISA

ELISA	VNT	
	Positive	Negative
Positive	98	5
Negative	1	5333

Technology transfer and training programs: AAHL protocol

AAHL's training program consists of three phases (Figure 2).

During phase 1, trainees receive training at a reference laboratory, such as AAHL, where testing can be performed under ideal and standardised conditions in a quality controlled manner. In phase 2, trainees receive reagents and standard protocols to establish and perform the test at their own laboratories. During phase 3, trainees will be visited by an expert in their own laboratory and given more focused advice and attention based on reported problems including troubleshooting with the test.

During this phase the laboratory will improve its quality system by using and analysing internal quality controls and, ideally, through participation in an external quality assurance program. To make the technology transfer successful and sustainable, the following support systems need to be in place:

- appropriate staff, both trainers and trainees, from collaborating institutions
- appropriate diagnostic methodology to suit the purpose of the new test and the fitness of the test to fulfil this purpose (e.g. whether it is to be used as a screening or as a confirmatory test)
- suitable laboratory infrastructure and budget to implement and maintain the new methodology (e.g. appropriate equipment, suitable working space, supply of critical reagents).

The chances for the training program and technology transfer to be successful increase if a two-way process is followed in which both sides benefit and where participating scientists have ownership and understanding of the outcomes. Monitoring of assay performance is easier and more reliable in a system with established internal and external quality control and quality assurance (OIE Quality Standard 2008). Good and continued communication about the outcome of analysis and interpretation of quality control samples between the two laboratories is an important basis for success. Training in data analysis and interpretation of results is an important step at the end of the diagnostic testing.

There must be a clearly established procedure when a sample needs to be retested, or when a result is inconclusive and a confirmatory test is required. For example, during a surveillance program, a screening test such as ELISA with a high sensitivity, high throughput, short turnaround time and low cost is required. This test may produce some false positive results which will need to be retested with a more specific confirmatory test. Another important consideration is the positive and negative predictive values of a test. For example, during a disease control program, the prevalence of the disease may drop dramatically from 5% to 1% or less. This drop will have a serious impact on the predictive values of a diagnostic test. The lower the prevalence (pre-test probability), the lower the positive predictive value and the higher the percentage of false positive results.

Diagnosis and outbreak investigation

The training program includes disease diagnosis and outbreak investigation for both laboratory and field staff. Recognition of clinical signs and basic understanding of pathology, specimen collection and transport are essential to improve animal health. Also crucial is the use of standardised protocols for diagnostic tests including quality control and quality assurance, and record keeping and data collection for a disease database.

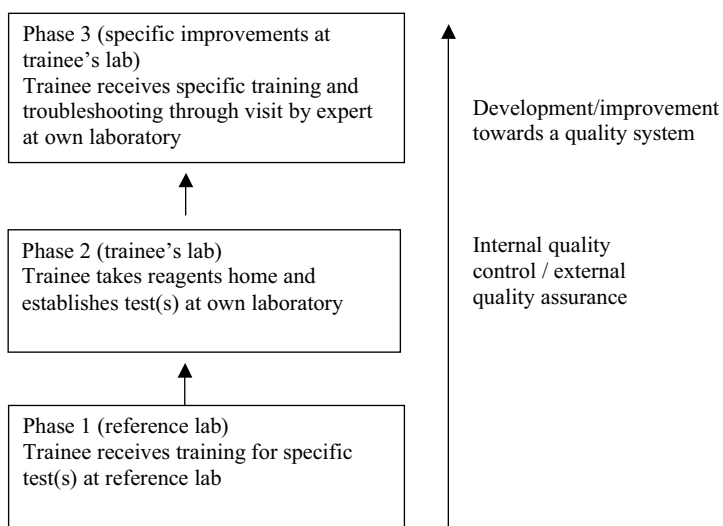


Figure 2. The three phases of AAHL's training program

In our experience a major constraint in the successful realisation of a laboratory test is the suitability of the specimens. Ideally, the technology transfer will therefore involve field veterinarians and village para-veterinary workers who interact with farmers and provide the primary link in the diagnostic chain by selecting and submitting specimens for laboratory examination. Training in submission of appropriate specimens becomes part of the training package, with a focus on building a specimen submission network and improved interaction between field veterinarians and farmers. Involvement of the farmer at the early stage of this process and communication of the benefits of timely and accurate diagnostic results will help to contain the spread of disease and turn the farmer into a major stakeholder in the project.

The collection of specimens should follow a standard protocol. Once the specimens have been collected it is important to transport them under the correct temperature conditions. Serum and tissue samples should be transported at 4 °C and, if possible, tissue samples at -20 °C. The quality of the specimens when received at the laboratory will determine the quality of the diagnostic results. The history of the disease and other important field information about the species (age, sex, vaccination etc.) are important and need to be recorded on the specimen submission sheet. The result of a diagnostic test is only one component of the information required to arrive at a final diagnosis. Clinical and epidemiological findings from the field are also crucial.

Biosafety and biosecurity

Training in biosafety and biosecurity is an important part of a disease investigation, both in the field and in the laboratory. Biosafety is required when collecting specimens or carrying out a post-mortem in the field. Veterinarians need to wear correct personal protective equipment including gloves, gown, mask or respirator, and eye protection. Similarly, laboratory diagnosticians need to follow biosafety rules to be adequately protected against microbiological contamination by using gloves, gown and eye protection and working in class II microbiological safety cabinets (BSCII). Biosafety protocols and standard operating procedures need to be in place to prevent human infection and ensure the safe handling of disease agents. They are an important part of all outbreak investigations, especially with

an increasing number of new and emerging zoonotic diseases.

Biosecurity is related to the physical containment of microbiological contamination and starts in the field when carrying out a post-mortem. Transport of specimens from the field to the laboratory must be done in the correct containers to prevent leakage. Work areas in the laboratory must be designed to prevent release of an agent to the environment. This is achieved through the use of flow hoods and BSCII and HEPA-filtered exhaust air.

Quality assurance and quality control

All training is carried out under a quality system. AAHL is an ISO 17025 accredited laboratory and all its procedures are documented in a quality manual. The overall goal of the training is to enhance the trainee's understanding and application of quality control and quality assurance protocols and the need to have quality controlled and valid test procedures. The use and analysis of internal quality controls such as strong positive, weak positive and negative controls over a critical range of potential test results is an essential technical requirement of ISO 17025-2005 'General requirements for the competence of testing and calibration laboratories'. Results are useful to ensure that a test run is valid and provides important information about test precision. Trends in test performance can be detected early before results get out of control and therefore are useful parameters for preventive troubleshooting (Crowther et al. 2006).

External quality assurance (EQA) or proficiency testing (PT) is carried out by an external laboratory. The PT provider sends out a proficiency panel and samples need to be tested by each participating laboratory. Results are then returned to the PT provider who does the analysis and sends out a brief report to the participants. PT programs are useful to identify sources of bias such as random versus systematic errors and loss of test sensitivity or specificity, and are important tools in the ongoing assay validation process (De Clercq et al. 2008). As part of the technology transfer monitoring process, AAHL has organised a number of PT rounds. Successful participation in PT programs is a crucial requirement for an ISO 17025 accredited laboratory. Australia's National Quality Assurance Program (ANQAP) organiser offers PT rounds for a wide range of tests

on a global level (<<http://www.anqap.com>>). Supranational organisations such as FAO or IAEA have organised a number of external quality assurance programs (e.g. for FMD ELISAs) and participants have used the experience to establish quality systems in their own laboratories (Colling et al. 2008).

Conclusion

The major objective of Australia's disease control projects over the past 25 years has been to provide training to local South-East Asian staff to increase capacity in the diagnostic and clinical aspects of disease control. To maximise training and project outcomes, it is important that training be a two-way process using a collaborative approach (Figure 3).

Such projects also have benefits for AAHL, including opportunities for the validation of current and new diagnostic tests; provision of education and training in support of pre-border quarantine; and training of Australians in disease diagnosis, disease control and surveillance. A collaborative approach improves commitment from the involved parties, communication and understanding, and ownership of project outcomes. OIE has recognised the benefits of this approach and has initiated an era of 'twinning projects' involving OIE collaborative or reference laboratories with other laboratories. Training is more efficient if it is done under a system using internal and external quality control and assurance procedures because results provide useful troubleshooting

information. EQA improves the communication between laboratories about assay and staff performance. Data analysis, interpretation of results and graphical display of mass data are needed to convert pieces of information into knowledge. Field staff must be included in the training to collect suitable and fresh specimens and so improve the quality of information about the disease outbreak. Training in biosafety and biosecurity is very important to minimise human exposure and spread of the disease agent in the environment and to prevent laboratory infections.

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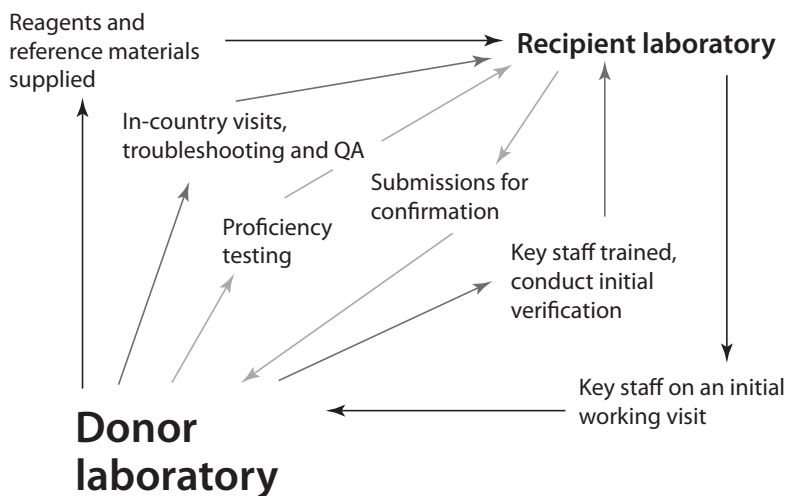


Figure 3. Inter-dependent pathways of successful technology transfer

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