Australian Government

Australian Centre for International Agricultural Research

Final report

project

Management of classical swine fever and foot and mouth disease at the village level in Lao PDR

project number	AH/2003/001
date published	March 2010
prepared by	Dr. Axel Colling, Veterinary Diagnostic Scientist, CSIRO AAHL
co-authors/ contri	Dr. Syseng Khounsy Dr. Phouth Inthavong Dr. Laurence Gleeson Mr. Jamie Conlan Dr. Ross Cutler Dr. Stuart Blacksell Mr. Chris Morrissy Ms. Kim Newberry
approved by	Dr Doug Gray
final report numbe	FR2010-08
ISBN	978 1 921615 83 2

published by

ACIAR
GPO Box 1571
Canberra ACT 2601
Australia

Contents

1 Acknowledgments

The authors would like to thank the Lao village farmers, especially the pig producers and village veterinary workers who were the main focus of the project, for their interest, support and hospitality. Their contributions and cooperation made the project possible and successful and helped us to better understand the epidemiology of CSF and FMD at the village level. We would like to acknowledge the friendship, enthusiasm, knowledge and co-operation of the staff of the National Animal Health Centre in Vientiane and Lao project leaders, Dr. Syseng Khounsy, Dr. Phouth Inthavong, Mr. Jamie Conlan and laboratory project staff Ms. Manivanh Phrouaravanh, Ms. Vilaywan, Mr. Laphinh. The significant support of the Deputy Director General of DLF Dr. Somphanh Chanphengxay and Dr. Mahanakhone Souriya is also greatly appreciated.

We would like to sincerely thank the Australian experts Dr. Laurence Gleeson, project author and project leader from 2003 to 2005, Professor Colin Wilks, Mr. Chris Morrissy, Mrs. Mehan Greer, Mrs. Kim Newberry, Dr. Ross Cutler and Dr. Stuart Blacksell for their significant contributions.

Thanks also to the ACIAR program managers Dr. John Copland, Dr. Peter Rolfe, Dr. John Skerritt and Dr. Doug Gray and program assistants Ms. Sally Moon and Ms. Julie Hart for their continued support and contributions.

2 Executive summary

The strengthening of food security and the reduction of poverty in rural areas are major priorities of the government of Laos 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 being a major constraint to productivity coupled with significant impacts on the livelihoods of rural poor. The control of classical swine fever (CSF) and foot-and-mouth disease (FMD) is of high priority in Laos and the Southeast Asian region due to their impacts on productivity, trade and, in the case of CSF, high mortalities.

The project aimed to use improvements in husbandry and disease control as an entry point to conduct research on the implementation and impact of CSF vaccination in the village pig production systems. It was build on the achievements of AS1/1994/038 by assisting in the collection and analysis of epidemiological data on FMD and CSF that has contributed to a better understanding of disease control.

The key objective of controlling CSF and FMD at the village level was to be achieved through five objectives, (i) development of a rapid, cheap, portable and sensitive diagnostic test for CSF to improve diagnostic output, (ii) establish a system for the delivery of locally produced CSF vaccine to village pigs, (iii) evaluate the impact of CSF vaccination, (iv) continue to monitor the epidemiology of CSF and FMD and (v) communicate research outcomes to all stakeholders at the district, national, regional and international levels.

The successful development and validation of a rapid and portable diagnostic test for CSF has been an important achievement and its application at the NAHC in Vientiane and two regional laboratories has resulted in an improvement in the turn-around time from sample collection to providing the farmer and disease control authorities with a test result.

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 and by international standards, a vaccine should be stable for at least as long as that stated by the manufacturer; this was not the case with the locally produced CSF vaccine. However, in saying this, the strict control of temperature and delivery shortly after manufacture resulted in successful outcomes when applied in village pig raising systems. Based on these findings two new ACIAR projects concerned with the improved vaccine quality control and the development of a sustainable business model were implemented.

A baseline and longitudinal survey in Southern Laos provided crucial baseline data about pig health, production and the impact of CSF at the village level. This included interviews and market observations to better understand the drivers for demand and offer. An interesting observation was the sell-when-sick practice, which applies to sick or sick-looking and usually very young pigs by mobile pig traders or salesmen. This in combination with lack of vaccination and use of quarantine pens were identified as the major risk contributors to the spread of CSF.

During the course of the project the diagnostic capability for CSF and FMD was improved through better diagnostic reagents and training of laboratory staff in quality control and quality assurance practices. In consequence the National Animal Health Centre (NAHC) was able to respond to CSF and FMD outbreaks by providing accurate diagnosis and animal health reports to national and international bodies including numerous publications in peer reviewed journals.

Project findings and results were disseminated to a wide range of stakeholders from village farmers and veterinary workers and NGOs to ministry officials and supranational

organizations such as FAO, IAEA and OIE. Workshops, training courses and educational material, seminars, conference proceedings, papers and network management were the main knowledge dissemination carriers. The ACIAR publication "Management of Classical Swine Fever and Foot-and-Mouth disease in Lao PDR" is a central reference for the achievements of this project (http://www.aciar.gov.au/publication/PR128).

It was a particular challenge to deal with a chronic lack of delivery mechanisms in Laos. In response the project took a multi-disciplinary and participatory approach, which had been developed and already successfully applied by the Forages and Livestock Systems Project, FLSP implemented by the Centre for International Agriculture and Technology, CIAT and National Agriculture and Forestry Research Institute, NAFRI with AusAID funding. At the same time it was recognized that without effective control of the high pig mortality caused by CSF it would be difficult to convince village farmers to invest in improvements to nutrition and animal management strategies that can significantly enhance village pig production. The FLSP link improved the successful introduction of the CSF vaccination program to villages and other simple preventive quarantine measures such as penning of recently purchased or sick pigs. Results from this successful collaboration have given birth to a new ACIAR project entitled: "Increased productivity and reduced risk in pig production-market chains for improved livelihoods in Lao PDR", which considers 4 major areas to overcome constrains to market access, such as 1) enhanced pig production, 2) manage of impact of pig disease, 3) reduce the risk of zoonotic disease and 4) deal with environmental issues associated with intensifying smallholder pig production

During the course of this project three students completed post-graduate and undergraduate degrees by undertaking project-related studies.

3 Background

Poorer households in remote areas in Laos are rely heavily on pigs and poultry for generation of income, often contributing greater than 50% to household income. One pig can contribute 5-10% of the yearly income of a rural family and make a critical difference for social and economic growth, e.g. the average income of a rural family is approximately 200 US$/year and the price of a pig ranges between 10-20 $US.

Pig raising is popular throughout Laos although the northern Lao people tend to keep a higher number of pigs because of traditional customs. In northern Laos it has been reported there is an average of 3.7 pigs per family, in Central Laos 1.4 pigs and in Southern Laos 2.3 pigs per family. According to Vongthilath et al. (2000) there are three pig raising systems in Lao PDR: smallholder (96%), small family business (3%) and semi intensive (1%). For smallholders the three main pig production systems in Laos are: a) seasonal or year round free-range scavenging, b) confinement in an enclosure with the provision of feed and basic housing and c) confinement in a pen with a raised slatted floor and the provision of feed (Phengsavanh et al. 2006). Production systems can be further categorized into fattening or farrow-to-finish and breeding or farrow-to-weaner systems. For example, in the northern mountainous areas sows are raised for piglet production in a free range scavenging environment, whereas in the lowlands and valleys fattening of weaners is common. Semi-intensive and intensive production systems are found close to cities to supply urban markets and trade exotic, e.g. Landrace, Duroc, crossbreed and indigenous breeds, e.g. Moo Laat.

In a review paper (Classical Swine Fever and Emerging Diseases in Southeast Asia, ACIAR proceedings 94, S.D. Blacksell, 2000) for pig systems in Laos,T. Gibson & I. Wilkie wrote

“Attempts to control pig diseases in SEA, e.g. Vietnam and Lao PDR have been going on for over a decade but even after many years high death rates for village pigs are observed."

The authors further conclude that data on which development has been based are largely anecdotal and there is a great need for more reliable data such as can be obtained from the Animal Health Project. Villages experienced regular (often yearly or twice a year, epidemics in which the reported death rate of pigs varied from about 20 to almost 100%. There a belief that animal vaccination is ineffective and often diagnostic laboratory results are not received at the village. The only effective means the villages know to prevent pig (and poultry) death was isolation. The disadvantage of having pigs isolated, e.g. in pens is that they need to be fed as opposed to free ranging pigs.

Kamakawa et al. (2006) recently identified CSF within the Mekong delta region as "a cause of piglet mortality that disastrously affected backyard farming. Vaccine standardization and proper instructions are needed to simplify diagnosis and complement established simultaneous vaccination of sows with piglets”.

Vaccination of pigs in an endemic environment is probably the most important control tool available to farmers and animal health staff in Laos, and the ability to monitor vaccination success is a crucial component of the control program. This is particularly important in the Lao context where constraints to vaccination have been identified; including limitations to the cold chain and possible problems with vaccine stability.

Significant advances in the understanding of diagnostic and epidemiological issues of these diseases in Lao PDR were achieved during an earlier ACIAR-funded project (AS1/1994/038) entitled "Improved diagnostic and control methodologies for two major livestock diseases in Lao PDR and Yunnan Province, China. 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.

This project was concluded in June 2003 after more than six years of successful research and development. The natural progression of research was to then concentrate on disease management at the village level to achieve sustainable disease control for the village farmer. Hence, this project "Management of CSF and FMD at the village level in Lao PDR" came to fruition.

4 Objectives

The overall aim of the project was to improve the management of CSF and FMD at the village level in Laos. To achieve this, the following 5 objectives were identified and revised during two project reviews and variations:

1. Development, evaluation and implementation of a simple, rapid diagnostic test for CSF including

   a) Undertaking experiments to validate the IMB-ELISA and develop a system to introduce the test into the mainstream of CSF diagnosis nationally and regionally.

   b) Supply CSF IMB ELISA reagents and internal quality controls as a near-to-field diagnostic tool to other interested countries in the region, e.g. Vietnam, China, Indonesia and in Europe.

   c) Investigate options for other diagnostic platforms for IMB CSF ELISA such as point-of-care rapid test.

2. Establishment & validation of a system to apply locally produced CSF vaccine in Lao villages.

3. Evaluation of the impact of the CSF vaccine program in the village pig production system.

   a) Develop and implement a program for the adoption of CSF vaccination as a control strategy in village production systems,

4. Monitoring the epidemiology of FMD and CSF in Lao PDR.

   a) To maintain a diagnostic capability for FMD by providing FMD reagents

   b) Supply diagnostic and control reagents for CSF and FMD, update standard operating procedures (SOPs) and

   c) Deliver training in their application and in trouble shooting in order to maintain a diagnostic capability for these diseases in Lao PDR.

   d) Alternatives for continued long-term supply of diagnostic reagents are to be investigated.

   e) Initiate molecular epidemiology analysis of selected sets of archived CSF and FMD samples at CSF reference lab in Hannover, FMD-WRL, Pirbright, UK and AAHL.

5. Communication of project findings to extension staff and animal health and production scientists in national, regional and international networks.

   a) Develop and distribute an education package for the control of CSF at the village level

   b) Produce manuscripts for publication about important project results, e.g. 1) reduced sensitivity of CSF CTB ELISA to detect maternal antibodies in CSF vaccinated piglets in comparison with NPLA and CEDI tests, 2) Results from molecular epidemiology studies for CSF and 3) Results from molecular epidemiology studies for FMD

5 Methodology

5.1 General

The project aimed to use improvements in husbandry and disease control as an entry point to conduct research on the implementation and impact of CSF vaccination in the village pig production systems. It was build on the achievements of AS1/1994/038 by assisting in the collection and analysis of epidemiological data on FMD and CSF that has contributed to national and regional understanding of disease control.

A fundamental characteristic of this project was its multi-disciplinary approach which helped to overcome the lack of delivery mechanisms in Lao PDR by applying the participatory approach developed by the CIAT/AusAID Forages and Livestock Systems Project (FLSP). At the same time it was recognized that without effective control of the high pig mortality caused by CSF it would be difficult to convince village farmers to invest in improvements to nutrition and animal management strategies that can give a significant enhancement in village pig production. The linkage with the FLSP improved the introduction of the CSF vaccination program to villages and other simple preventive quarantine measures such as penning of recently purchased or sick pigs.

During the course of this project two students from the University of Melbourne were trained at AAHL and then worked in Lao PDR on the Australian Youth Ambassador for Development (AYAD) volunteer program. Mr. Conlan was trained at AAHL and used AAHL's facilities and reagents to develop the IMB ELISA. Ms. Vitesnik also received a short training at AAHL to become familiar with diagnostic methodologies for CSF and FMD. Ms. Kristina Osbjer from the Swedish University of Agricultural Sciences conducted research in collaboration with the project and the Centre for International Tropical Agriculture that contributed to her Master of Veterinary Science degree.

To a smaller extend Lao staff was also trained at AAHL, e.g. Dr Khounsy received training in EPI info at the start of the project. Most of the training of Lao staff was provided in Lao PDR, e.g. through workshops and field visits and training courses given by AAHL staff, e.g. Jamie Conlan, Laurence Gleeson, Axel Colling, Chris Morrissy, Greer Mehan, Kim Newberry and selected experts, e.g. Ross Cutler, Colin Wilks and Trevor Ellis.

There was also training provided by Lao project staff to Lao staff, e.g. 12 students from the Agriculture Faculty and Technical College of the National University of Lao were trained in basic diagnosis of FMD and CSF between 2003-2005. The former Lao project leader Dr. Khounsy provided training in public animal health (sampling, diagnosis, analysis of results) at national and international conferences and meetings held by OIE, SEAFMD, FAO/ADB. Jamie Conlan provided training in the production of reagents, use and troubleshooting of the IMB ELISA to Lao staff at NAHC and provincial laboratories. He also produced a manual in the form of a simple Standard Operating Procedure.

The project also paid salaries for two laboratory staff, a project car, equipment (printer/fax/scanner, 2 desktop computers, 4 centrifuges and pipettes), diagnostic reagents and consumables for FMD (serotype specific Ag and Ab ELISA and group-specific non-structural antibody ELISA) and CSF (including dropper bottles, beads and magnets to assemble IMB CSF ELISA kits) and running costs for communication, e.g. telephone, fax, internet access and fuel and car maintenance costs.
The project also supported 2 project staff (Mr. Lapinh and Ms. Vilayvanh) to attend an English course.

Specific methodologies to achieve objectives

In the following pages the methodology to achieve each major objective is described:

1. Development, optimization, evaluation and implementation of a simple, rapid diagnostic test for CSF.

5.2 Laboratory

This aspect of the project work was conducted principally by Mr James Conlan who was enrolled as Masters Degree student at Melbourne University through the School of Veterinary Science. Mr Conlan started his program in 2003 at the CSIRO Australian Animal Health Laboratory in Geelong. Some of the test reagents were developed or obtained at AAHL. In addition the Lao DLF project leader (Dr Syseng Khounsy) serially immunised rabbits with the local CSF vaccine strain to produce a hyperimmune serum suitable for application to the test format. The proposed format for the test (a bead binding assay using coloured beads) was not successfully replicated when a new batch of monoclonal antibody was produced and subsequently was adapted to an immuno-magnetic bead ELISA for detection of CSF antigen. Preliminary results indicated that this could be a suitable near to field test system with the advantages to be faster and possibly more sensitive than the current antigen detection ELISA, which can only be performed in a well equipped laboratory. The binding conditions of the reagents and the conditions for the performance of the IMB test were optimized and standardised. Experimental work was undertaken to test the possibility to link the mab directly to the conjugate as this would shorten the turn around time of the assay. This proofed to be too costly. Experiments were carried out to modify the IMB ELISA for antibody detection with the potential to be used for monitoring vaccine response. The proof of principle was established but the full development and validation of such a test were not possible within the frame of this project. Experiments were also carried out at AAHL to evaluate the use to determine CSF antigen in blood samples from experimentally CSF infected pigs with a special view to diagnose chronically infected pigs with reproductive failure. This proved to be difficult because of the "stickiness" of porcine blood. Further experiments, e.g. treatment of specimen, e.g. centrifugation are necessary to further develop and validate such a modification and could not be completed within the scope of this project. Another constraint was the IP for the monoclonal antibody, which basically excludes this reagent to be used in a commercial context.

5.3 Field application

Relative performance characteristics were established using the Ag detection ELISA as a standard of comparison. Repeatability and reproducibility was assessed using internal controls and proficiency testing rounds with different operators and at different laboratories in Lao PDR. Training was provided to national laboratory staff and 8 provincial staff from 4 provinces. This included troubleshooting exercises in Vientiane and at one provincial lab. Laboratory consumables, e.g. conjugate stabilizer, beads and equipment, e.g. 4 microcentrifuges, pipette were purchased to support these activities.

Results were published by Mr. Conlan in form of his thesis entitled "Improved diagnostic and management of Classical Swine Fever in Laos.", a publication in Trop Anim Health Prod, 2008 "Development and evaluation of a rapid immunomagnetic bead assay for the detection of classical swine fever virus antigen", and as a poster presentation at XIII WAVLD, Melbourne in November 2009 (see also 10.2 List of publications produced by project).

2. Establishment & validation of a system to apply locally produced CSF vaccine in Lao villages.

Investigations were conducted by Jamie Conlan to look into the efficacy of the local CSF vaccine when applied under field conditions. The CSF virus neutralization test (NPLA) was introduced to measure antibody in vaccinates and showed reduced protection rates in vaccinated pigs. The stability of the local CSF vaccine on storage at 4 °C and -20 °C was investigated. Results showed that the vaccine was unstable at 4 °C and that it maintained its potency for only 4 months and not for 12 months at -20 °C. A vaccination trial with village pigs with optimally stored and delivered vaccine demonstrated good neutralizing antibody responses. Serology showed that either the NPLA or Ceditest ELISA (E2 antigen) must be used for antibody monitoring in vaccinated pigs – not the CTB-ELISA (p80 antigen). The recommended procedures for CSF vaccination of village pigs have been extended to village veterinary workers and district staff. The vaccination strategy has been developed for various age and classes of pigs. However the strategy of first vaccination of piglets at 1-2 week of age is at variance with procedures elsewhere with endemic CSF. Most farmers use first vaccination at 5-6 weeks of age when maternal antibodies don't interfere with vaccination. Some countries use 3 and 6 weeks age vaccination. CSF vaccination campaigns have been conducted in 16 villages in Bolikhamxay Province.

Baseline and longitudinal studies were carried out to assess pig production and health from all pig farmers in 16 villages. Data were collected and recorded every month by village veterinary worker. This included collection of age specific production and health information. Key production indicators were: reproductive performance, inputs and outputs to pig production unit and CSF incidence and impact. Extension and education on CSF control has been provided to villagers. Procedures to monitor CSF vaccination in pigs in the above villages were established.

Ms Vitesnik visited 5 villages in Bolikhan district on 4 occasions and carried out 120 individual interviews to obtain information village pig farmers (mainly women) about their attitude to pigs, motivation to become a pig farmer, diseases and vaccination. Ms Vitesnik extended Mr. Conlan's experiment by storing the CSF vaccine for 1 and 2 months at 4C.. The aim of the experiment was to assess the locally produced CSF vaccine at the village level without the complication of an ineffectual cold chain. Temperature loggers were bought to monitor the cold chain over the entire process. For that purpose 30 vials of vaccine that had been stored for 2 months at NAHC were transported to two villages in Bolikhamxay. All pigs within the villages were vaccinated and blood samples taken at 0, 35 and 70 days post vaccination. Serum was tested at NAHC by CTB-ELISA and Cedi ELISA and NPLA at AAHL. 100% of the village pigs seroconverted in the Ceditest and NPLA but only 50% in the CTB-ELISA. From these results it could be concluded that after 2 months storage at -20C. and appropriate transport and delivery the locally produced lapinised C-strain vaccine was able to produce a protective antibody response in pigs.
Findings regarding the suitability of the NPLA and CEDI test and unsuitability of the CTB ELISA to monitor vaccine induced antibodies will be published.

3. Evaluation of the impact of the CSF vaccine program in the village pig production system.

The impact and success of the village vaccination program is most clearly demonstrated by the dramatic decrease in mortalities associated with CSF outbreaks that have occurred in villages in which the project is working. For example three outbreaks of CSF have occurred in 2 of the 16 villages between July 2005 and October 2006; the first in Houana in September 2005 and the second in Houana and Phontong villages in May 2006. From the production and health survey data collected in these villages, in total, 6 young pigs died during the 3 outbreak periods. These mortalities did not exceed the average monthly mortality for young pigs in either village. Before the vaccination programs began during this project, CSF associated mortalities were far greater. During a CSF outbreak in April & May of 2003 in Houana village, greater than 60 young pigs died in a single month, exceeding the average monthly mortality number

for the village by almost 40 times. The impact of these decreases can not be underestimated, e.g. the loss of 60 pigs is equivalent to 500-1000 $US depending on the weight and the average income of a rural family is in the range of 200$US/year. A qualitative description of the production cycle for village pigs in Lao PDR was undertaken by the project team assisted by specialist pig consultant, Dr Ross Cutler in December 2003. Limits to production and suggestions for capacity building were provided in their report (Cutler, 13 Dec. 2003). Pig production and health surveys were conducted in 2 districts in Bolokhamxay province and these identified and demonstrated the risks of spread of CSF by trading of pigs of unknown health status, the sell-when-sick practices of farmers and lack of quarantine when introducing new pigs to villages. Village surveys were conducted four times in 5 villages in Bolikahn district, Bolikhamxay in 2006 to obtain views of village pig farmers in relation to their farming system, pig disease (CSF in particular) and on CSF vaccination. As well as reduction in CSF cases the Cutler report identified parameters such as increased forage planting and improved housing that can be measured as indicators of enhanced disease control, especially CSF control in villages. Presentations at the completion workshop in 2006 showed the social and economic advantage of increased forage planting and improved pig housing in Lao PDR. They noted that widespread investment to make these changes will only occur if CSF is under control. Other constraints identified for pig production in villages in Lao PDR other than good quality forage with adequate protein and better housing systems included adequate daily supply of good quality water and better management of breeding to prevent in-breeding.

A report about the socio-economic impact of CSF control in Lao villages was produced by the Lao Women Union and published in October 2005.

4. Monitoring the epidemiology of FMD and CSF in Lao PDR.

The project has been collaborating with the SEAFMD and FAO-ADB campaigns for the control of transboundary animal diseases in the Upper Mekong countries. This involved the undertaking of FMD and CSF serological surveys in 5 provinces in the north, Oudomxay, Luang Prabang, Houaphan, Xayabouly and Phongsaly. Serological surveillance for FMD was carried out using a non-structural-protein antibody ELISA, approximately 2% of cattle and buffalo in Luang Prabang and Xayabouly provinces were positive, no positive samples were detected in the other three provinces. Investigations of virus subtype and on possible source of introduction were conducted on new outbreaks of FMD in Lao PDR in 2003-2005. No new outbreaks reported in 2006. Two papers about the molecular epidemiology of FMD in Lao PDR and a review of recent outbreaks were published by Khounsy et al. 2009 and 2008 respectively. Another 50 CSF and 100 FMD isolates are ready to be analysed to continue this study. The first paper about FMD seroprevalence using structured sero-surveillance and abattoir surveys was published by Blacksell et al. 2008. This included investigations to assess the risk of FMD outbreaks from persistent virus infections and transit animals. Bovine sera obtained from FMD outbreaks in Lao PDR were used to validate the 3 abc ELISA developed at AAHL.

CSF surveillance resulted in findings similar to those found in the previous ACIAR project AS1/1994/038, greater than 20% of pigs surveyed were positive for antibodies to CSF virus. CSF outbreaks and investigations from passive surveillance in Lao PDR for 2003-2006 were reported at the international project workshop in Vientiane in November 2006.

5. Communication of project findings to extension staff and animal health and production scientists in national, regional and international networks.

The mulitdisciplinary character of the project has facilitated a network between animal health and production/nutrition, e.g. in conjunction with the international workshop in November 2006 a 2 days pig health and production and 2 days laboratory diagnosis

workshop were held in Vientiane. Publications targeted villagers, governments, NGOs and other development organizations and scientists.

Annual reports and study visit reports relating to the project were produced and a summary is available on the ACIAR website. The project has communicated the findings of the FMD studies and activities undertaken by the project to the Annual meeting of the SEAFMD program each year. A completion workshop presenting the findings from the project and other livestock research pertaining to pig production in Lao PDR was held on 20-21 November 2006 with national and international attendees. The workshop also included two training courses, one on the IMB ELISA and other laboratory techniques for CSF diagnosis and the other on pig health production and housing in collaboration with staff from Forage and Livestock Systems, CIAT. The papers and presentations from the workshop have been collated into an ACIAR Proceedings booklet No 128 entitled " Management of Classical Swine Fever and Foot-and-Mouth disease in Lao PDR". A CSF resource booklet was produced in English language and still need to be translated into Lao and then introduced to interested parties, such as pig farmers, village veterinary and other field workers, NGOs and others. At the end of the international workshop in November 2006 an interactive session was organized to discuss the future research directions for CSF and FMD by capturing skills and experience of workshop delegates. In summary the three areas of importance and improvement were vaccination, human resource and public awareness of disease control measures (Cutler & Conlan "Future research directions for CSF and FMD in the Lao PDR, ACIAR proceedings 128, 2008).

Mr Conlan had his MSc thesis entitled “Improved diagnostic and management of Classical Swine Fever in Laos” submitted at the University of Melbourne. Ms Vitesnik had her thesis entitled "Pig Health and Production in the Lao People’s Democratic Republic" submitted for her degree of Bachelor of Animal Science at the University of Melbourne. Kristina Osbjer submitted results from her work for her Master of Veterinary Science at the Swedish University of Agriculture Sciences.
Further publication details are available under 10.2 List of publications produce by project.

An earlier version of this report identified 10 additional objectives (objectives 6-15) as extensions of the five main objectives listed above. Subsequently these were condensed and reported against as part of the 5 main objectives.

6 Achievements against activities and outputs/milestones

Objective 1: To develop, evaluate and implement a simple, rapid diagnostic test for CSF

no.	activity	outputs/ milestones	completion date	comments
Sub-objective 1 a and 1 b as pointed out under 4) Objectives are addressed below, e.g. a) Undertaking experiments to validate the IMB-ELISA and develop a system to introduce the test into the mainstream of CSF diagnosis nationally and regionally. b) Supply CSF IMB ELISA reagents and internal quality controls as a near-to-field diagnostic tool to other interested countries in the region, e.g. Vietnam, China, Indonesia and in Europe. Sub-objective 1 c is addressed in 4.11 below c) Investigate options for other diagnostic platforms for IMB CSF ELISA such as point-of-care rapid test.				
1.1	To develop a simple diagnostic test for CSF in Laos (A, PC)	Simple test format to provide diagnosis of CSF	June 2004	The IMB-ELISA for CSF virus was developed and compared to an antigen capture (AC)-ELISA. Initial estimates of diagnostic specificity and sensitivity show 100 % agreement with the AC-ELISA and the IMB-ELISA has up to 64-fold greater analytical sensitivity. The rapid test is a highly robust and stable test format and much simpler to perform than the AC-ELISA. The IMB-ELISA has the added advantage that the test can be read by eye, lending it to the possibility of adaptation to a near-to-field test with minimal equipment needed. The new test is comparable in cost to the current AC-ELISA; however it can be performed in approximately 3 hours upon receipt of specimens compared to 1 to 2 days for the AC-ELISA. The constraints on transfer to other laboratories were lack of reagent stability of the coated beads and availability of a stable activated substrate. This could not be confirmed when IMB CSF kits were repeated at AAHL using fresh buffers and need further investigation.
1.2	To validate the diagnostic test in Laos (PC field validation, A laboratory validation)	Test validated using experimental and field specimens	June 2005	The test was validated in the laboratory in Lao and AAHL and in the field. Results were published in Jamie Conlan's Master thesis and papers and posters.

1.3	To implement the diagnostic test in field laboratories in Laos (PC)	Test protocol developed suitable for field laboratory staff	Nov 2006	A detailed test protocol entitled: "Kit preparation, quality testing and users guide for the detection of classical swine fever antigen from spleen and tissue specimens" with flowchart and figures is available. Turnover of reagents in a provincial laboratory is important and currently there may not be enough samples in regional laboratories to avoid excessive reagent expiry.
1.4	Organize and provide training (PC, A)	Staff in field laboratories trained to undertake test	Nov 2006	8 staff from 4 provincial laboratories were trained in the test and intra- and interoperator variation assessed. 7 staff from Cambodia (1), China (2), Lao (2), Thailand (1) and Vietnam (1) were trained at an international training workshop in November 2008.
		Information about the cost of the test system		The cost if the test are low when compared to commercially available test kits, such as the CEDI test. If the kit can be produced at large scale, for example > 100 kits then costs can even drop more.
1a) 1.5	Conduct experiments to further develop the IMB-ELISA and determine its application for detecting viral antigen in blood (A)	- Prepare kits and test on archived blood samples at AAHL - Analyse data	Feb/May 2008	The project extension started with a delay of 4 month (April 2007). That had an impact on the completion dates. Blood samples were tested at AAHL in the first half of 2008. Experimental findings indicated that the reliability of test results for blood samples is compromised because of interference of fibrin clots with magnets. Further experimental testing is required but could not be finished within the timeframe and budget of this project, e.g. treatment of samples to remove fibrin.
1a) 1.6.	Conduct experiments to determine diagnostic sensitivity and specificity using 'gold-standard' comparator tests (A)	- Experiments completed - Analyse data	Feb-May 2008	Specificity Samples from animals experimentally infected with Bovine viral diarrhoea (BVD) and border disease (BD) were tested at AAHL and showed no cross reaction = good specificity. Sensitivity Positive results in the IMB ELISA were obtained as early as 5 days post infection. Samples from spleen, ileum, tonsils and brain and positive controls resulted in the highest OD values in the IMB ELISA and were confirmed by standard Ag ELISA and higher viral genetic load = good sensitivity.

1b) 1.7	Develop a system for the routine supply of reagents and kits through a regional network (PC, A)	Supply of reagents and test kits to regional partners for routine use in CSF diagnosis	May-Oct 2008	Reagents for approx 50-100 kits were produced and consumables, such as dropper bottles, magnets and beads were purchased. The IMB-ELISA has continued to be used at the diagnostic laboratory in Vientiane Capital and the transfer of technology to the provincial laboratories was initially successful with the laboratory in Luangnamtha using the test. There is interest for the use of IMB CSF kits in the region.
1.8	Provide continued training and quality assurance testing (ring tests conducted regionally and in Lao PDR) (PC, A)	Ring test trials showing that test results fall within acceptable limits	2007/8	Ring tests were performed in Lao. Training provided to project staff and international laboratory staff from Myanmar, China, Cambodia and Vietnam in November 2006 workshop by Jamie Conlan and Chris Morrissy. Jamie Conlan visited AAHL in June 2007 to train lab staff in production of reagents and use of IMB ELISA

PC = partner country, A = Australia

Objective 2: To establish and validate a system to apply locally produced CSF vaccine in Lao villages

no.	activity	outputs/ milestones	completion date	comments
General comment: Longitudinal study of pig production and health ~ 3.5 years was done in two districts in Bolikhamxay province and baseline data were established. As a result it could be shown that with the delivery of a well planned vaccine delivery program (good quality vaccine, new batch) significant improvement for CSF is achievable.				
2.1	Organize and provide training (PC, A)	Staff trained in techniques to introduce vaccine to farmers	Nov 2006 ongoing	Outputs 2.1 and 2.2 have been achieved with the FLSP in 8 villages in 2 districts in Xiengkhouang. Province. In Bolikhamxay 8 new project villages were enrolled and the farmers given information about pig production in general and CSF control in particular. Concerning output 2.1, in March 2004 a planning workshop was held with the FLSP in Luang Prabang to discuss the animal health inputs and the vaccination program to be carried out in the FLSP villages in Xieng Khouang. The training was provided to the FLSP staff and the vaccination program implemented during the current work period. A training workshop was held 23-24 November 2006 with > 20 staff from Bolikhamxay, Luang Prabang and Xieng Kuang.
2.2	Undertake vaccination experiments and analyse results (PC)	Protocol for immunisation of pigs in villages established	Nov 2006	Recommendations for suitable CSF vaccination program are summarized in papers from Conlan et al, Khounsy et al, in ACIAR proceedings 128 and in a CSF resource booklet. Under ideal field conditions vaccine stored at -20C for 2 months and transported at temperatures less than 1C elicited an immune response in 89% of vaccinated pigs by day 35 and 100% of pigs by day 70 post vaccination.

2.3	Write guide and review and translate into Lao (PC, A)	Extension packages to inform farmers about the impact of disease and how to control CSF	Dec 2007	The first draft of the booklet: "CSF, A guide to improving disease control at the village level in Lao PDR" was completed in mid 2007. The booklet needs to be translated into Lao.
2.4	Organize vaccination program and monitor program (PC)	CSF vaccination program active and under evaluation in villages	Dec 2007	The CSF vaccination was carried out twice in all of the 24 villages in the project. Two vaccinations were used as there was some lingering concern about the potency of the vaccine in the field and it was important to reduce the risk of vaccine failure. Sera were collected from a sample of vaccinated pigs to assess antibody responses. No further work was undertaken with the local vaccine.
2.5	Organize survey and analyse results (PC)	Herd immunity dynamics determined	Nov 2006	See also, 2.2. Findings from vaccination experiment indicated that CTB ELISA is not suitable to monitor vaccine induced antibodies (paper in preparation). Results were used to redefine the CSF vaccination schedule.

PC = partner country, A = Australia

Objective 3: To evaluate the impact of the CSF vaccine program in the village pig production system.

no.	Activity	outputs/ milestones	completion date	comments
3.1	Obtain baseline data about pig production at the village level (PC)	A qualitative description of the production cycle for village pigs in Lao PDR	2007	Survey of 16 villages in Bolikhamxay was conducted in June 2007 to assess the risk factors for the introduction of CSF into a village. Major risk factors include pigmeat traders; pig populations greater than 150; and being in Bolikhan district. From the limited number of villages surveyed, risk of disease was similar in villages with penned or free range pigs. Results have been presented at a regional trans-boundary animal disease meeting in Bangkok in January 2008 and were also useful for a new project proposal entitled: “Improved control of pig diseases in Lao PDR AH 2005 084”. Baseline data for pig production at the village level were established by Mr. Conlan in his Master thesis. This included identification of risk factors such as mobile meat-traders and sell-when-sick practice. See papers from Conlan et al and Vitesnik et al in ACIAR proceedings 128, 2008.
3a) 3.2	Design a protocol (PC)	A protocol to measure the impact of CSF control in the villages	2007/8	A formal protocol to measure the impact of CSF control in the village was not produced but Mr. Conlan's longitudinal study and Ms. Vitesnik's study have provided baseline information for a protocol.

3a) 3.3	Evaluate impact of CSF vaccination (PC)	Information about the social and economic impact of CSF control in the villages	2007/8	A report about the socio-economic impact of CSF control in Lao villages was produced by the Lao Women Union and published in October 2005. Results about smallholder attitudes to pigs, diseases and vaccination were published in Tess Vitesnik's thesis, who had visited 5 villages in Bolikahn district in Bolikhamxay on four different occasions and carried out 120 individual interviews. Baseline data from Jamie Conlan also provided information about the socio-economic impact of CSF. Ross Cutler's report from 2003 gives an overview of pig production, nutrition and health including some cost benefit examples.
3.4	Assess other factors which impact on pig production at village level (PC)	Information about other constraints on pig production in villages in Lao PDR	2007	Section 1, Pig production and extension of ACIAR proceedings 128 gives an overview of current pig production and extension practices, limitations and recommendation for improvement.
1a) 3.5	Adapt and preliminary validate the IMB-ELISA into a blocking format for the sensitive detection of antibodies to CSF virus (PC)	Conduct experiments to develop test Test characterised serum to validate test Collect blood onto filter paper and test Assess tests ability to detect vaccinated sero-positives	2007	Experiments were carried out in Vientiane. Proof of principle in microfuge tubes was successful but adaptation to plate format was unable to be completed due to beads adhering to the plate and impacting on the test results. Serum controls were compromised by maternal antibodies present in 8 week old unvaccinated pigs
1a) 3.6	Apply the IMB-Ab-ELISA to monitor herd immunity in villages where vaccination has been conducted (PC)	Identify target villages Vaccinate pigs Collect sample and test Report results back to village and district office	Oct-Nov 2007	This activity was not progressed because 7.1 was not successful.

3a) 3.7	Conduct experiments to measure the decay curve of maternally derived antibodies to CSF virus (PC, A)	Assemble samples and send to AAHL Test by NPLA in AAHL Report results	June 2007	Because of the unsuitability of the IMB Ab ELISA these experiments were conducted with the Neutralizing Peroxidase Linked Assay (NPLA), a commercial CEDI ELISA and a complex trapping blocking (CTB) ELISA at AAHL using ~300 serum samples from a maternal antibody transfer experiment. In these samples maternal antibodies were found to persist for greater than 12 weeks in pigs housed at the National Animal Health Centre. The Cedi ELISA and the CTB-ELISA showed very low sensitivities (69.5%; 50.9% in relation to the NPLA) and therefore are not suitable for testing of maternally derived CSF antibodies . Very low sensitivities were observed with an NPLA cut-off of 1:8. A paper is in preparation. Results are presented and discussed in paper from S. Khounsy et al. "Recommended vaccine programs for village based pig production systems in Lao PDR" in ACIAR proceedings 128. See also 10.2 list of publications produced by project.
3a) 3.8	Conduct participatory problem diagnosis in upland villages to identify incentives to incorporate CSF vaccination in the village production system (PC, A)	Identify target villages Prepare questionnaires and interviews Conduct field work Analyse results and report back to villages	2007/8	This activity was not progressed because of time and budget constraints. The possibility to investigate this topic in the new multidisciplinary project should be considered. Initial discussions were held with the Australian project leader.

PC = partner country, A = Australia

Objective 4: To monitor the epidemiology of FMD and CSF in Lao PDR

no.	**activity**	**outputs/ milestones**	**completion date**	Comments
All 4 sub-objectives as pointed out under 4) Objectives are adressed below, e.g. a) To maintain a diagnostic capability for FMD by providing FMD reagents b) Supply diagnostic and control reagents for CSF and FMD, update standard operating procedures (SOPs) and c) Deliver training in their application and in trouble shooting in order to maintain a diagnostic capability for these diseases in Lao PDR. d) Alternatives for continued long-term supply of diagnostic reagents are to be investigated. e) Initiate molecular epidemiology analysis of selected sets of archived CSF and FMD samples at CSF reference lab in Hannover, FMD-WRL, Pirbright, UK and AAHL.				

4.1	Ongoing testing of FMD and CSF samples (PC)	A semi-quantitative description of the epidemiology of CSF in village pigs	2007/8	CSF surveillance resulted in findings similar to those found in the previous ACIAR project AS1/1994/038, e.g. the average CSF sero-prevalence in pigs was >20%. Molecular epidemiology showed that all CSF isolates belonged to the genogroup 2. Viruses from Lao PDR grouped on a geographical basis with the majority of northern/central isolates falling into subgroup 2.1 and southern/central isolates falling into subgroup 2.2. (FMD see 4.3)
4.2	A semi-quantitative assessment of risks to village pig health and development of methodologies to reduce or eliminate such risks from the village production system (PC)	Identification of risks impacting on pig health and evaluation of methods to prevent these risks	2007/8	After establishing baseline production data and trade patterns for pigs in 16 villages the impact of disease was assessed. There was a significant difference between the two districts, e.g. Bolikhan district 30 outbreaks/100 village years vs 5 outbreaks/100 village years in Pakading district. Villages experience regular often yearly or biannually, epidemics in which the reported death rate of pigs varied from about 20 to almost 100%. The major risks identified to be responsible for the spread of CSF are trade of pigs of unknown health status (mobile pig traders), the “sell-when-sick” practices of farmers and the lack of quarantine when introducing new pigs to villages and higher stocking densities, e.g. >150 pigs per village. Results are summarized in the papers from Conlan et al. "Pig production and health in Bolikhamxay Province, Lao PDR" and Vitesnik et al. "Pig production and disease management: a village perspective." ACIAR proceedings 128.

4.3	Receive field samples and provide laboratory diagnosis (PC)	Information about the epidemiology of new outbreaks of FMD in Lao PDR	Ongoing	For the first time structured sero-prevalence studies were conducted. Diagnostic results obtained for the period 1996 to 2005 showed that cattle had the highest percentage of antibodies and that serotype O was the most prevalent serotype in southern and central Lao was in cattle. Serotype Asia 1 was also prevalent in central Lao and serotype O in the north, e.g. Louangphrabang. No FMD outbreaks were reported between 1998-2002 but serotype O was reported in 2003, 4 and 5. A major serotype A outbreak occurred in 2006 in Vientiane Capital. Papers about sero-prevalence and recent review of outbreaks and lessons learned were published in the OIE Scientific and Technical Review, 2008. Results from a study about the molecular epidemiology of FMD viruses from South East Asia with a focus on Lao 1998-2006 has confirmed findings from seroprevalence studies and were published by 2009 by Khounsy et al in Veterinary Microbiology. See also 10.2 list of publications produced by project.
4.4	Receive field samples and provide laboratory diagnosis (PC)	Information about the risks of FMD outbreaks from persistent virus and transit animals	Ongoing	Transboundary movement of infected animals and movement of infected animals through local trade together with elevated livestock densities were confirmed to be the major causes for FMD transmission in Lao PDR. Surveillance, vaccination and movement control are suitable measures for the control of FMD. The role of the Asian buffalo as a carrier needs to be further investigated.
4.5	Receipt and testing of FMD post-outbreak sera at NAHC and AAHL (PC, A)	Validation of 3abc FMD ELISA developed at AAHL	2007/2008	Commercial 3abc FMD antibody ELISA kits were bought to monitor activity of FMD virus in Lao PDR. AAHL has received over 300 serum samples from FMD vaccinated pigs and post-outbreak cattle from Lao. The bovine sera were taken during and after a serotype O FMD outbreak in 2003. These sera were tested at AAHL by a 3 abc FMD ELISA using the commercial CEDI test as standard of comparison. Results were used to validate the 3 abc ELISA, especially to estimate its diagnostic sensitivity. A paper and a SCAHLS validation template are in preparation.

4.6	To supply diagnostic reagents for the diagnosis of FMD serotype specific ab and ag ELISA and FMD specific NSP ELISA (CSIRO/AAHL) (A, PC)	- To provide critical FMD reagents - To test samples at the Lao lab following SOPs incl. qa/qc - Be prepared to diagnose FMD in case of an outbreak - Ability to undertake serosurveilllance and outbreak investigation	2007/8	NAHC participated in a proficieny testing exercise for FMD AAHL has received over 300 serum samples from Lao. These are bovine sera taken during and after a serotype O FMD outbreak in 2003. These sera were tested at AAHL by the AAHL 3abc ELISA using the commercial CEDI test as standard of comparison. Results are used to further validate the AAHL 3 abc ELISA, especially to estimate its diagnostic sensitivity.
4.6	To design a sampling frame and take field samples to adequately monitor the epidemiology of FMD in Lao (DLF)	To have sample frame ready for use - To take samples (field staff) - To send samples to central lab in a timely manner	2007/8	Samples were submitted to NAHC from outbreak investigations, e.g. between June 2007 and May 2008 69 samples (cattle, buffalos, pigs and goats) were received from 9 districts (mainly Vientiane and Xayabuly province) for FMD diagnosis and serotyping (41 positive, 28 negative All positive samples were of O type except one which was diagnosed as ecthyma contagiosum). During the same period 99 samples (6 pos 93 neg) were received for CSF diagnosis from 8 provinces.
4.8	To report results to national (DLF) and regional authorities (SEAFMD)	Analyse data - Produce report with adequate epidemiological information	2007/8	Results were communicated to the National Animal Health Centre (NAHC) and DLF in the yearly report. 2 FMD papers were submitted to OIE (2008), 1 paper to Veterinary Microbiology (2009)
4.9	Supply diagnostic and control reagents for CSF and FMD Assist Lao lab staff to update standard operating procedures (SOPs) Provide training in application of SOPs and in troubleshooting (A)	Maintain diagnostic capability for CSF and FMD in Lao PDR - operational diagnostic reagents for CSF and FMD in place - updated Standard Operating Procedures for FMD and CSF diagnosis - 1-2 trained staff - QC/QA in place	June 2009	Kim Newberry visited NAHC for 2 weeks. SOPs and control are updated. Laboratory staff need to analyse internal quality control results and troubleshoot procedure if necessary.
4.10	Supply CSF IMB ELISA reagents and internal quality controls (PC, A)	International availability of near-to-field diagnosis for CSF - use of CSF IMB ELISA kits in Vietnam, China, Indonesia and Europe	May 2009	There is interest for the use of the IMB ELISA in the region. AAHL has received positive and negative reference material to produce kits.

1c) 4.11	Investigate options to transfer CSF IMB ELISA to other diagnostic platforms such as POC Rapid Test (A)	Alternative near-to-field diagnostic test for CSF	April 2009	This option was pursued but at this stage IP issues prevent mab to be used for commercial purpose.
4.12	Investigate alternatives for continued supply of diagnostic reagents, e.g. CM to contact WRL about discount options for SEAsia and explore options for support through AUSAID (A, PC)	Alternatives for continued supply of crucial diagnostic reagents for FMD and CSF beyond project involvement - Maintain diagnostic capability for CSF and FMD	June 2009	Diagnostic reagents can be obtained through regional network.
4.13	Initiate molecular epidemiology analysis of selected sets of archived Lao CSF and FMD samples at CSF reference lab in Hannover, Germany, FMD-WRL, Pirbright, UK and AAHL (A, PC)	Improved Molecular epidemiology of CSF and FMD in Lao PDR	May 2009	~ 100 FMD isolates and ~ 50 CSF isolates obtained between 1998 and 2009 are available at NAHC for molecular diagnosis and sequencing. Isolates have been prepared (c-DNA) and have been shipped to AAHL for analysis.
4.14	Prepare and submit manuscripts 1) The reduced sensitivity of CSF CTB ELISA, in comparison with NPLA and CEDI tests, in detecting maternal antibodies in CSF vaccinated piglets. 2) Results from molecular epidemiology studies for CSF and 3) Results from molecular epidemiology studies for FMD (A)	Publication of project results	2009-2010	Data for CTB ELISA need to be analysed and manuscript produced. Isolates need to be prepared, RNA extracted, c-DNA produced and material shipped to AAHL for analysis. Isolates are on their way to AAHL and on behalf of an invitation of AAHL Dr. Khampouth will spent one week at AAHL for analysis in 2010.

4.15	Review and update SOP for sample collection and submission and disease investigation (Workshop for provincial and district staff recommended if sufficient funds are available during project extension) (PC)	Harmonized protocol for sample collection and submission and disease investigation with existing documents	2009	Kim Newberry visited the laboratory in Vientiane and worked with lab staff for 2 weeks. SOPs and other protocols were reviewed and training in quality control and troubleshooting procedures provided.

PC = partner country, A = Australia

Objective 5: To communicate project findings to extension staff and animal health and production scientists in national, regional and international networks

no.	activity	outputs/ milestones	completion date	Comments
The 2 sub-objectives as pointed out under 4) Objectives are adressed below, e.g. a) Develop and distribute an education package for the control of CSF at the village level b) Produce manuscripts for publication about important project results, e.g. 1) reduced sensitivity of CSF CTB ELISA to detect maternal antibodies in CSF vaccinated piglets in comparison with NPLA and CEDI tests, 2) Results from molecular epidemiology studies for CSF and 3) Results from molecular epidemiology studies for FMD				
5.1	Analyse results and produce manuscripts (PC, A)	Information packages about disease control for farmers and policy makers	2007 2005 Ongoing/update	CSF, A guide to improving disease control at the village level in Lao PDR. This booklet is ready for translation and distribution. Gender Resource Information and Development Center (GRID), Lao Women's Union, October 2005, "Management of CSF and FMD at the village level in Lao PDR" Standard operating procedures (SOPs) for CSF and FMD diagnostic tests are updated and available. More attention needs to be given to quality control, troubleshooting and corrective actions.
5a) 5.2	Analyse results and produce manuscripts (PC, A)	Reports of project activities for ACIAR, CIAT and CSIRO publications	2003-2009 2008	Annual and technical reports (ACIAR) Management of Classical Swine Fever and Foot-and-Mouth disease in Lao PDR, ACIAR proceedings 128, Conlan J.V., Blacksell, S.D., Morrissy C.J. and Colling A. (eds.) 2008 (ISBN 978 1 921434 98 3 (print), ISBN 978 1 921434 99 0 (online) http://www.aciar.gov.au/publication/PR128 See also 10.2 list of publications produced by project

5a) 5.3	Organize workshop (PC, A)	Workshop on project findings and application of diagnostic tests	20-21 November 2006	The following national and international workshops were organized: Completion workshop for ACIAR project AS1/2003/001Management of CSF and FMD at the village level in the Lao PDR. Over 40 participants from Lao and neighbouring countries such as Cambodia, Thailand, Myanmar, Vietnam and China attended.
			20-21 November 2006	Use of the IMB ELISA for local staff and from neighbouring countries (7 participants from Cambodia (1), China (2), Lao PDR (2), Thailand (1) and Vietnam (1).
			23-24 November 2006	Pig Health and Management Training Program for Lao District Staff with > 40 participants.
			2006 and 2007	Ongoing training of staff from provincial laboratories in the use of the IMB CSF test was provided by Mr. Conlan including proficiency testing
			14-15 June 2007	ACIAR project workshop Canberra
5.4	Analyse results and produce manuscripts (PC, A)	Scientific publications and MSc thesis	2003-2007	Kristina Osbjer (MVS), Jamie Conlan (MSc), Tess Vitesnik (BAnSc), see also under 10.2 List of publications produced by project, e.g. Conlan et al, 2008 Development and validation of a rapid IMB assay for the detection of CSF antigen and poster presentation at the WAVLD in Melbourne in November 2007
5.5	Complete the analysis of village surveys conducted during the first phase to better understand farmer's perception of disease and disease control (PC)	Complete data analysis Report results	June 2007	Survey of 16 villages in Bolikhamxay was conducted in June 2007 to assess the risk factors for the introduction of CSF into a village. Major risk factors include pigmeat traders; pig populations greater than 150; and being in Bolikhan district. From the limited number of villages surveyed, risk of disease was similar in villages with penned or free range pigs. Results have been presented at a regional trans-boundary animal disease meeting in Bangkok in January 2008 and initiated a new project proposal entitled: "Improved control of pig diseases in Lao PDR AH 2005 084".
5a) 5.6	Develop education and communication material for CSF with the support of communications specialists (PC, A)	Compile English version of book Engage communications expert ad graphic designer Translate into Lao Produce books and field test Evaluate and amend where necessary	2007/8	The first draft of the book was completed in mid 2007 following a writing session attended by Jamie Conlan, Axel Colling and Syseng Khounsy, and sent to collaborators for review. The booklet has been edited and need to be translated into Lao for distribution.

5.7	Disseminate information to district level extension officers and farmers through a network of livestock development agencies and NGO's (PC, A)	- Hold workshop - Train district staff - Engage livestock stakeholders and NGO's to disseminate booklet	June-Dec 2008	Workshop was not held due to time and budget constraints. Booklet still to be translated into Lao and distributed.
5b) 5.8	Analyse data about reduced sensitivity of CSF CTB ELISA to detect maternal antibodies in CSF vaccinated piglets in comparison with NPLA and CEDI tests	Manuscript	2009 - 2010	Manuscript needs to be produced in conjunction with paper from Conlan et al in ACIAR proceedings 128 "Application of immunomagnetic bead technology for improved diagnosis of classical swine fever virus"
5b) 5.9	Analyse results from molecular epidemiology studies for CSF	Manuscript	2009 - 2010	Manuscript needs to be produced
5b) 5.10	Analyse results from molecular epidemiology studies for FMD	Manuscript	2009 - 2010	Manuscript needs to be produced in conjunction with papers from Khounsy et al and Blacksell OIE 2008 and Khounsy et al Vet Mirobiology 2009
5b) 5.11.	Produce IMB ELISA Manual	IMB ELISA Manual ready for international training course	October 2006	This manual was used for national and international training on the use of IMB ELISA. It includes a brief section about terminology and some definitions, an introduction to CSF and a clear description of the IMB ELISA test methods including a flow chart with an illustration. Participants and users found this very useful information to perform the test correctly.

PC = partner country, A = Australia

7 Key results and discussion

7.1 Summary

Classical swine fever (CSF) was identified as a major cause of pig deaths in Lao village pig raising systems. Sero-surveillance conducted during this project showed that CSF infection was endemic at varying levels throughout Lao PDR. Without effective control of this high mortality pig disease it will be difficult to convince village farmers throughout Lao PDR to invest in improvements to nutrition and animal management strategies that can give a significant enhancement in village pig production. An immunomagnetic bead ELISA (CSF IMB ELISA) was developed, validated and field tested during this project and found to be an ideal near to field diagnostic test in a low technology setting. Improved control of CSF depends on developing and delivering an effective CSF vaccination program to village pigs and this in conjunction with simple quarantine measures such as penning is seen as key technical requirements to improving pig health management for the rural poor. While the cold chain remains to be a problem results indicate that the incidence of CSF can be substantially decreased if a sustainable vaccination strategy is put in place and farmers are well informed. The CTB antibody ELISA was found to be unsuitable to monitor vaccine induced antibodies. Other tests such as a commercially available CSF antibody ELISA (CEDI) or Neutralising Peroxidase Linked Assay (NPLA) Linked are more suitable for this purpose.

The project maintained and improved a diagnostic capability of OIE recommended serological and antigen detection tests at NAHC in Vientiane including internal and external quality assurance and quality control and training. This facilitated and improved Lao's contribution to the regional OIE FMD control program and the generation of national reports which assisted to establish control policies, strategies and activities. Post-outbreak sera from Lao PDR were critical for the evaluation of an AAHL developed 3abc ELISA.

This project successfully used improvements in husbandry and disease control as an entry point to conduct research on the implementation and impact of CSF vaccination in the village pig production systems. The linkage with the CIAT/AusAID Forages and Livestock Systems Project (FLSP) enhanced the successful introduction of the CSF vaccination program to villages and implementation of an effective and practical strategy to maintain the level of herd immunity necessary to prevent outbreaks of disease.
A new mulitdisciplinary project: entitled " Increased productivity and reduced risk in pig production-market chains for improved livelihoods in Lao PDR" builds on this successful cooperation.

Specific key results and discussion

1) Key result IMB CSF ELISA
An IMB ELISA for CSF antigen detection was developed and evaluated. This test has been shown to have equivalent or better performance than the standard CSF antigen capture ELISA but it is less expensive, more robust and rapid and requires less sophisticated facilities and equipment than the standard test. Over 22 Lao staff were trained and 8 international participants attended a 2 days regional training course in November 2006. This test has potential application in similar settings at the regional level.

Discussion:
The IMB ELISA test has shown to be a reliable and robust test when used in a low technology setting with minimal laboratory infrastructure. The lifetime of reagents limits

its use to 2-3 months. This may become a problem if there are only very little numbers of samples to be tested and over an extended period. Samples should be sent to the NAHC as a routine to confirm any results obtained by this test.
Further experiments were carried out to modify this test into an antigen detection test for blood samples to detect sub-clinically infected pigs and into an antibody detection format, e.g. to monitor success of vaccination and results showed proof of principle. Commercial point of care (POC) and more sophisticated test systems are available for detection of CSF antigen in blood and CSF antibodies in serum. The availability of crucial reagents used for the CSF IMD ELISA such as the monoclonal antibody is restricted because of intellectual property rights, e.g. at this stage the mab can not be used in a commercial setting. And can These established test methods are more important for testing of large numbers, e.g. for surveillance studies and are of less urgency than the IMB CSF ELISA which purpose is to quickly provide a diagnostic result in the face of a CSF outbreak.

2) Other key results were obtained through experiments to investigate the reason for the poor performance of the locally produced CSF vaccine and to establish and validate a system to effectively vaccinate pigs using the locally produced CSF vaccine in Lao villages.
During field experiments it could be shown that CSF vaccine is able to produce a protective immunity if it is delivered under ideal conditions. Results showed that the vaccine was only stable for approximately 4 months at -20 °C compared to a recommended 12 month shelf-life and it was not stable at 4 ° C for extended periods of time. Further monitoring and analysis is needed to better understand the factors which impact on the quality of the vaccine and to make the delivery system more robust.

Discussion
In the Lao PDR, lapinized CSF vaccine is produced from live attenuated C-strain virus and is produced from homogenised rabbit spleen and mesenteric lymph nodes, which are freeze dried in the presence of stabilisers in a rubber stopped vial. Only a small proportion of pigs are vaccinated and vaccine failure has been 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 maternally derived antibodies. The research presented by Conlan et al. 2008 described specifically the second factor, vaccine stability during transport and storage. The vaccine study 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 ≥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 up to 12 weeks (Dahle and Liess, 1995; Terpstra et al. 1990; Terzic et al. 2003), this was observed during the study.

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 °C. It could be shown that storage at the provincial and district level is inadequate according to the manufacturer’s recommendations and the normal field conditions during the delivery process are not ideal. For example the National Vaccine Production Centre recommends that the vaccine be stored at -20 ºC for up to one year, however, the vaccine loses viability after four months storage under recommended conditions. The capacity of rural agricultural offices to hold vaccine at -20 ºC 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. Repeated freeze-thawing cycles and prolonged storage at 4 C and higher do occur under natural conditions and are likely to have an impact on the quality of the vaccine. Changing from lapinized to cell-culture production, introduction of rigorous quality assurance and quality control procedures including potency testing and aliquoting into

smaller, e.g. individual units will improve standardization and quality. A number of high quality reports regarding the production and quality control of the CSF vaccine have been produced by several experts (Dr. Sithong 20 May, 2000 Report on Vaccine Production Improvement, Dr. Sithong May 1998 General Outline for Production and Testing of Veterinary Vaccine, Report from Mr. Jettteur 1998, Report from Mr. Guilloteau 2002, Report from Colin Wilks 2005) and should be considered for future vaccine improvement.

The study also highlighted the importance of having the capacity to monitor vaccination success. The only antibody detection test routinely available for CSF in Lao PDR is the complex trapping blocking or CTB-ELISA and 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). The Ceditest and NPLA are expensive tests in comparison to the CTB-ELISA, and could not be introduced in to the mainstream of laboratory testing without the continued support of foreign donors. Further 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.

The Lao government has emphasized the importance of a national independent vaccine production centre and a new ACIAR project entitled "Improved supply and quality of livestock vaccines in Lao PDR" AH 2005/084 was developed. It was found that a buiseness model need to be developed to improve the vaccine quality in the long term.

3) Results from baseline and longitudinal investigations allowed to better understand the production cycle for village pigs in Lao PDR including main production & husbandry practices, trading practices, nutrition, disease control strategies and proximity to markets, transport routes and other villages.

Results from the baseline survey (carried out in 8 villages in Bolikhamxay province) showed that a) there was poor quality feed and limited water supply, b) village farmers had 2 to 4 hours each day to spent to collect and prepare feed, c) farmers from all villages reported disease as a problem and d) pigs were sold if disease was recognised.

Results from the longitudinal survey (carried out in 16 villages in Bolikhamxay province) showed the following reproductive performance in sows:

Litters-per-sow-per-year:	0.8	(0.2 to 1.6)
Litter size:	5.9	(5.0 to 7.3)
Pre-weaning mortality:	2 %	(0 to 15.3 %)
Sow/boar ratio:	22:1	(6 to 83)

Off take and intake of pigs at the village level
Over 80% of the pigs raised in the villages were sold and the remaining 20 % were used for home consumption or died. Almost 80% of the sold pigs were 0-3 months old and the remaining 20 % were 4-6 months or older or sows.
Over 60% of pigs entered the production unit by way of birth (0-3 months), e.g. 7644 by birth & 1494 young pigs by purchase.

CSF incidence & impact:
7 outbreaks of CSF in 6 villages occurred since the start of the survey in May 2002 resulting in an overall incidence of 18 outbreaks per 100 village years and 30 per 100 village years in Bolikhan district and 5 per 100 village years in Pakading district.

It was concluded that a) husbandry and management was basic with poor nutrition, b) the reproductive performance was poor, c) pigs were sold at very young age due to nutritional limitations and fear of disease and d) CSF outbreaks had a major impact on village pig population.

4) Identifying the risks of CSF
Results obtained during the baseline and longitudinal surveys identified the risks of

spread of CSF by trading of pigs of unknown health status, e.g. through mobile pig salesmen, the “sell-when-sick” practices of farmers and the lack of quarantine when introducing new pigs to villages.

Discussion
Change of established selling practices such as sell-when-sick and mobile tradesmen require continuous education of village farmers and village veterinary workers about the epidemiology of CSF infection in pigs. It is important to highlight these risks by explaining and demonstrating the early signs of CSF in pigs, diagnosis and mode of transmission to the target groups. Practical demonstrations, workshops and the CSF booklet were crucial in this process and were successfully emphasized and included in this multidisciplinary project.

5) The project maintained and improved a diagnostic capacity for FMD and CSF and continued to monitor and conduct investigations of CSF and FMD outbreaks in Lao PDR. Dr Khounsy and Inthavong have been collaborating with the SEAFMD and FAO-ADB campaigns for the control of transboundary animal diseases in the Upper Mekong countries. This has involved undertaking FMD and CSF serological surveys in 5 provinces in Lao PDR. Results were published in OIE Scientific Review (2) and Veterinary Microbiology (1). Approximately 50 CSF and 100 FMD isolates have been collected and are stored at NAHC at the moment for further processing, e.g. c-DNA and sequencing.

Discussion
The project was able to maintain and improve the diagnostic capability for these diseases at NAHC but it is important to increase the awareness of quality control and quality assurance and troubleshooting of laboratory staff. That includes the use and analysis of internal controls and the participation in external proficiency testing rounds.

6) Project findings have been communicated to ACIAR in annual reports and reports from visiting scientists and summaries are available on the ACIAR website and through a number of papers (see also 10.2 list of publications produced by project). A highlight was the organization of an international project workshop in November 2006, followed by two 2 days training courses on pig production and diagnosis of CSF. Results were published in 2008 as ACIAR proceedings 128 entitled "Management of CSF and FMD at the village level in the Lao PDR". Production of information packages for farmers (Report of Lao Women's Union 2005) and a comprehensive resource booklet about CSF control for farmers, village veterinary workers, provincial and district livestock staff and NGO’s working on livestock activities in Lao PDR were produced. The CSF booklet has been edited and still need to be translated into Lao.

Discussion

It is important to translate this booklet and make it available to all interested parties in Lao PDR. It would be of great assistance if results could be disseminated through the Pig Systems Learning Alliance established by CIAT.

7) A sample submission network was maintained and extended and training courses were organized for village farmers and veterinary village workers. This included training in the detection of clinical signs of FMD and CSF and epidemiology, vaccination, taking, packaging and submission of samples and administrative procedures. This improved local capacity for diagnosis, surveillance and outbreak investigation of FMD and CSF.

8) With the support of the project during the longitudinal study funds were created at the villages for a sustainable vaccination program beyond the project. The project improved pig genetics at the village level by introducing cross breed pigs to villages following vaccination trials in Vientiane.

Summary of external review 2006

The project was reviewed in November 2006 by Trevor Ellis who made the following comments:

"One clear message coming from this information was that CSF was a serious and major disease of village pigs in Lao PDR and it had a major impact on whether village farmers would or could invest in other activities like improved forage systems, housing and other improvements in pig production systems that could increase pig production and provide valuable cash flow.

Another major output has been to investigate the reason for the poor performance of the locally produced CSF vaccine and to establish and validate a system to effectively vaccinate pigs using the locally produced CSF vaccine in Lao villages."

"Other major activities included investigations to understand the production cycle for village pigs in Lao PDR and to identify and demonstrate the risks of spread of CSF by trading of pigs of unknown health status, the “sell-when-sick” practices of farmers and the lack of quarantine when introducing new pigs to villages. The project has continued to monitor and conduct investigations of CSF and FMD outbreaks in Lao PDR and has been collaborating with the SEAFMD and FAO-ADB campaigns for the control of transboundary animal diseases in the Upper Mekong countries and this has involved undertaking FMD and CSF serological surveys in 5 provinces in Lao PDR. Project findings have been communicated to ACIAR in annual reports and study visit reports and these have been made available on the ACIAR website. Production of information packages for farmers and a comprehensive resource booklet about CSF and FMD control for provincial and district livestock staff and NGO’s working on livestock activities in Lao PDR is planned.

Conduct a further review of the technical aspects of the CSF vaccine production storage and distribution to understand why this live attenuated lapinised C-strain vaccine is not able to be stored at 4°C as similar vaccines elsewhere can be freeze-dried and successfully stored at 2-8°C. (This should be a priority if the business review of the local CSF vaccine production recommends continuing production of this vaccine).

Strengthen linkages with CIAT groups to extend CSF vaccination into their study areas using the optimized vaccination strategy". (End of T. Ellis quotation)

8 Impacts

8.1 Scientific impacts – now and in 5 years

Results from this project were published as ACIAR proceedings, 128 "Management of CSF and FMD at the village level in Lao PDR" and in a number of papers and presentations/posters at national and international conferences (see also 10.2 List of publications produced by the project)

Baseline data and results from longitudinal study for pig production and health were obtained over 3.5 years from 16 villages of two districts in Bolikhamxay province. Results indicated that the reproductive performance of sows were low compared with tropical commercial pig production, e.g. median litter size 6 (4.6-6.8) and the medium number of litters/year/sow was 0.8 (0.5-1.5). Piglets were traded out of the village production units early, with 76% of all sales comprising piglets between 0-3 months of age.

CSF had a major impact on village production units, affecting farmer confidence and sales patterns and resulting in substantial piglet mortality. The overall CSF incidence was 21 outbreaks per 100 village years, but it differed markedly in the two districts with 34 and 4 outbreaks per 100 village years in Bolikhan and Pakading district respectively.

A coordinated CSF vaccination program was however able to impact on CSF incidence in the target villages. As an important result it could be shown that with the delivery of a well planned vaccine delivery program (good quality vaccine, new batch, good application) significant improvement for the control of CSF is achievable.

One key finding was that the CTB ELISA is not suitable to measure CSF vaccine induced antibodies. Other tests such as the NPLA and commercial ELISAs such as CEDI test are suitable for this purpose. A paper is in preparation.

Pig sales during disease outbreaks is a problem and facilitates disease spread within and around villages.

A review of recent FMD outbreaks analysed the history of FMD outbreaks between 1997-2006 and discussed the major causes of FMD outbreaks as 1) the introduction of infected animals through transboundary movement, 2) movement of infected animals through local trade and 3) stocking densities. A strategic FMD vaccination program with a bivalent vaccine Asia 1 Shamir and O1 Manisa was carried out in 1999 and was assessed by screening of 359 pre- and 182 post-vaccination sera with the Liquid Phase Blocking ELISA for serotypes O, A and Asia 1. 9/10 herds samples had herd immunities of greater than 80% when tested six months after the vaccination indicating that the strategic vaccination was successful. But immunities against individual serotypes varied considerably, e.g. for serotype O = 85.2%, Asia 1 = 57.1%. As a possible reason for the lower immunity for Asia 1 pre-vaccination exposure is discussed. These findings were presented in Khounsy et al., OIE Technical Review 2008.

Using the Liquid Phase Blocking ELISA seroprevalence estimates for antibodies against serotype O from surveillance and abbattoir studies between 1996-2005 showed percentages of seropositive cattle and buffalo ranged from over 60% in Vientiane capital to very low levels <34% in other provinces and very low levels in pigs in general. Species and age had a significant impact on seroprevalence. These results were published by Blacksell et al. in OIE Rev Sci, 2008.

The molecular epidemiology of FMD virus in Lao PDR and neighbouring countries was described using isolates samples between 1998-2006. Serotype O was the dominant serotype followed by A and Asia 1 during this period. Outbreaks with serotype A

occurred in 2003 and 2006 and interestingly the 2006 was identical to the 2003 outbreak virus type. These findings were published in Khounsy et al. Veterinary Microbiology, 2009.

Approximately 100 FMD and 50 CSF isolates obtained between 1998 and 2009 are expected to be sent to AAHL for sequencing.

The FMD survey work undertaken in cattle and buffalo in Savannakhet province has demonstrated that it is very unlikely that FMD is endemic in this significant bovine population. This finding has a significant impact on the understanding of the epidemiology of FMD in the region.

8.2 Capacity impacts – now and in 5 years

With the support of this project the National Animal Health Centre NAHC was able to undertake FMD outbreak investigations and report results at OIE SEAFMD Sub-Commission and Upper Mekong FMD Control Zone grouping of countries within the SEAFMD program.

The project has increased the capacity of Lao laboratory and field staff to diagnose CSF and FMD, including taking and submission of samples, to conduct vaccination and analyse vaccination experiments and to communicate results to in a simple and relevant form. Village farmers and village veterinary workers have been trained in key issues related to pig health and production, e.g. early recognition of sick pigs, understanding of major risks of disease transmission with a focus on CSF, use of simple quarantine measures such as penning to prevent the spread of infectious diseases and better knowledge how to achieve improved productive performance, e.g. through high quality food such as Stylo. Farmers and village vet workers were trained in a range of pig health and production interventions. This has improved their understanding about the limitations of pig production and capacity to introduce these interventions in their own target areas.

These experiences and new, knowledge based motivations of target groups are fundamental for the success of a new multidisciplinary ACIAR project entitled: "Increased productivity and reduced risk in pig production-market chains for improved livelihoods in Lao PDR" (AH 2009/001). This project is estimated to run 2010-2014.

8.3 Community impacts – now and in 5 years

Pigs provide important sources of supplementary income, unperpin cultural festivals and weddings and provide clearly demonstrable ways for farmers (mainly female) to further contribute to family income and to accumulate discretionary cash.

A key issue for this project was that farmers were able to see and evaluate the impact of pig health and improved management on the production of their pigs. The project benefited smallholder farming communities through changes in the number of people growing pigs and the number of pigs sold. Increased sale of pigs has and will increase household income for women, who are mostly responsible for pig production and they are likely to benefit most. Improved production techniques may lead to changes in village layout, and reduce in the number of people engaged in shifting cultivation.

8.3.1 Economic impacts

The holistic approach to the pig health in the project villages has had significant impacts on production outputs. For example the collection of production data highlighted that poor reproduction efficiency had a significant impact on income generation. In some instances there were very few suitable male pigs of breeding age and quality and inbreeding was a problem. The Lao project leader used animals produced in the project vaccination trials to supply suitable breeding males to these

villages. It was evident that the villagers developed an appreciation of the impact of inbreeding and readily accepted the need for the introduction of new genetic resources to the village program.

The project used vaccination of cattle and buffalo against haemorrhagic septicaemia as an adjunct activity to attract farmers' interest where serum samples have been collected for surveillance purposes. In the routine project activities in Borikhamxay the application of vaccines has been used to establish a revolving fund for farmers in the village to use a bank to finance vaccinations at times of cash shortage. This fund is managed within the village by the project contact and the village chief. It is likely that this system has resulted in more consistent use of vaccine and application at the most appropriate time in the production cycle. The Lao Women's Union undertook an attempt to evaluate the project's social and economic impacts. The report provides a number of illustrative examples how farmers spent cash return (e.g. purchase of a tractor) obtained through marketing their pig but recommendations are not always conclusive.

The Cutler report 2003 provided some good examples of a typical village pig production scenario, e.g. "a weaner pig could be purchased for about 80,000 kip. At about 15kg it would be worth about 100,000 kip. A 40kg pig was worth about 300,000 kip and an 80kg pig worth about 600,000 kip (1 AUD=7500 Kip). The pigs are growing at a rate of about 65kg in 700 days or more – an ADG of about 93 grams/day compared with about 650 grams in modern production. Based on the price of rice bran a total feed cost per pig is estimated to be about 409,500 kip. The profit then on an individual pig is about 100,000 kip over the course of 2 yrs – or about $13 AUD – nearly 3 weeks salary. Another example is given from a village farmer, who grew his own foods. If he produced 4-6 piglets per year he would get a return of about 640,000-960,000 kip ($85-128 AUD/year).

The profitability of this form of pig production can't be compared with intensive production. It will never compete with mainstream production in Laos, Vietnam or Thailand. However, it will provide discretionary income and access to cash otherwise not available. Pig production at the village level is not a long term option but it could be a viable short term way of generating cash and other improvements in nutrition or living standard. Pigs provide a way of turning otherwise unused resources (time, cassava, rice bran, fermented rice) into very useable cash. The production enterprise is, however, only profitable if the people put little or no value on their time."

In the future changes in international pig production and sales will lead to an increased pressure from neighbouring countries on pig production in Lao PDR, e.g. in 2003 Vietnam was the eight highest pork producing country in the world producing 1.795 Mio tonnes of pork from its 23 Mio pigs. Imports from Vietnam will likely place downward pressure on Lao pork prices, that is villagers will get less for their pigs (Ross Cutler & James Conlan 2008)

8.3.2 Social impacts

The focus of this project were the village farmers, who belong to the poorest in Lao's rural environment. In particular women are in charge of managing pig production. The knowledge, skills, attitudes of the village farmers in relation to key management indicators such as disease, feeding, housing and genetics were captured through interviews and questionnaires. Additional income and availability of cash is likely to raise the status of the person who manages this process.

In one of the villages where there had been an outbreak of CSF one of the risk factors for spread was the high contact rate between the mostly free ranging pigs. As a result of input from the Lao project leader the management of the pigs has been altered to confine pigs in pens. The resultant change in the village environment has been dramatic.

Since the implementation of the expanded village program there has been a decrease in outbreaks of CSF in the vaccinated villages. It is apparent from the classification of the project by the provincial staff as 'Village Animal Health Project' and the approach is one that has engaged the interest and commitment of the animal health staff on the ground.

An inspection of the animal production data in the villages showed that at one point a large number of animals were purchased when the avian influenza outbreak occurred. It was likely that such importation was a risk for the introduction of other diseases of pigs. Under such circumstances it is difficult for proper quarantine measures and biosecurity to be applied.

8.3.3 Environmental impacts

In the mid-long term it is unlikely that Laos PDR will become a major force in pig production because of the limited supply of feed grain and genetics. No major environmental impacts are to be expected. However improving ways to produce pigs will have an impact on the practice of shifting cultivation as villagers increase their profitability through more intensive systems of raising animals. More intensive village pig production systems require higher stocking densities with the potential to facilitate the spread of infectious diseases. Some land need to be cultivated for high quality food, e.g. Stylo. It is easier to make use of effluents in an intensive system, e.g. as a fertiliser for crops and forages.

8.4 Communication and dissemination activities

Results have been published as simple technical resource booklets for village farmers, veterinary village workers, NGOs and other interested parties. Results from the development, validation and application of diagnostic tests for CSF and FMD and epidemiological studies were published in scientific journals such as OIE and Veterinary Microbiology and presented at regional and international conferences, e.g. SEAFMD, FAO, ADB, WAVLD.

Results from surveillance studies and outbreak investigations of CSF and FMD were analysed and published in yearly reports for national authorities such as Ministry of Agriculture and Forestry.

Training was provided to laboratory staff from Lao and neighbouring countries (China, Vietnam, Thailand and Cambodia) in the use of the IMB ELISA.

A summary of the project outcomes was presented and regional CSF and FMD control strategies were discussed at an international project conclusion workshop with over 40 participants in Vientiane in November 2006. Key results were published in ACIAR proceedings 128 "Management of Classical Swine Fever and Foot-and-Mouth disease in Lao PDR". 2008

It would be of great assistance if results could be disseminated through the Pig Systems Learning Alliance established by CIAT.

Bell et al. 2005 evaluated the effectiveness of different knowledge dissemination interventions on the mastitis knowledge of Tanzanian smallholder dairy farmers and came to the conclusion that "diagrammatic handout", "village meeting and video", and "diagrammatic handout, village meeting and video" were the most effective dissemination interventions.

Further details are given under 10.2 List of publications produced by the project.

9 Conclusions and recommendations

9.1 Conclusions

The livestock diseases Classical Swine Fever (CSF) and Foot and Mouth Disease (FMD) are major constraints to village livestock production systems in Laos PDR.

A review of ACIAR project AS1/2003/001 2003-2008 entitled: “Management of CSF and FMD at the village level” confirmed that CSF was a serious and major disease of village pigs causing high pig mortality and economic hardship in Lao PDR. The findings from this review were confirmed through a facilitated session at an international project closing workshop in 2006 where participants were invited to provide inputs to assess project achievements and future directions. The following factors were identified as crucial for a successful CSF control under the endemic situation in Laos:

1) fast and reliable diagnosis and precise epidemiological knowledge including knowledge about the risk of introducing or reactivating CSF through animal movement, e.g. through commercialization and marketing,
2) 2) use of simple biosecurity measures, e.g. quarantine to avoid the spread of disease after animal movement or in case of an outbreak
   3) a quality and robust vaccine and appropriate knowledge and infrastructure for suitable vaccine application and supply chain.

The project has delivered a validated, sensitive, specific and robust IMB ELISA, which is an ideal diagnostic tool for the low technological setting of a provincial lab in Lao.

It was possible to show that under ideal conditions the locally produced CSF vaccine was able to produce an immune response in pigs, e.g. the project has successfully achieved to introduce a CSF vaccination program at the village level together with husbandry strategies to maintain herd immunity against future disease outbreaks, e.g. a total of 2286 pigs were vaccinated in 16 villages in Borikhamxay and 1107 in 8 villages in Xiengkhouang. Experimental results raised concerns regarding the efficacy and stability of the lapinised CSF vaccine. In addition vaccination is not applied in a consistent and systematic manner. The high contact rate between mostly free ranging pigs was identified as a major risk for the spread of a CSF outbreak in 2005. As a consequence pigs were kept confined in pens. Also the project has used vaccination of cattle and buffalo against haemorrhagic septicaemia as an adjunct activity to attract farmers interest where serum samples have been collected for surveillance. The sample submission network was maintained and further extended.

A number of technical and management issues surrounding the quality of vaccine production, supply, cold chain management and vaccine use were identified, which led to two ACIAR projects, AH/2006/155 and AH/2007/125 respectively. Recommendations from these projects in turn provided inputs into a new project proposal entitled: " Increased productivity and reduced risk in pig production-market chains for improved livelihoods in Lao PDR" AH/2009/001.

The project also created baseline data for pig production and health including trade and marketing practices which lead to basic understanding of risk factors associated with CSF outbreaks, e.g. pig traders, sell-when-sick practice and a concentration of more than 150 pigs. Understanding of these circumstances led to simple biosecurity measures such as quarantine of new piglets after purchase as powerful tools to avoid the spread of CSF.

9.2 Recommendations

The top priority within the Laos Agriculture and Forestry Development Plan 2006-2010 is to stabilize and enhance food security and food production by developing pig and poultry industry. Pig production is the leading livestock species with approximately 1.750.000 pigs estimated in 2005. Continuous improvement of control of pig diseases including zoonotic diseases together with enhanced management (reproduction and genetics, nutrition and production) and marketing strategies were identified as the mayor goals to improve the living conditions of the village farmers in Lao PDR. These recommendations have become the basis for a new multidisciplinary proposal entitled: "Increased productivity and reduced risk in pig production-market chains for improved livelihoods in Lao PDR" AH/2009/001.
This new project draws together the outcomes and continuing research priorities of three current more narrowly defined ACIAR projects in Laos (AH/2003/001 Animal Health, AH/2004/046, AH/2006/161), uniting these into a project that researches the marketing chain in a more holistic approach.

The project has made considerable progress in developing diagnostic capabilities and disease surveillance but the endemic character of CSF at the different smallholder production systems, uncertainties regarding viable strategies for improved and consistent vaccine quality and delivery and marketing habits, which facilitate the spread of disease remain major challenges for the future. Under these circumstances future attention should be focused on interventions and education that will help to physically interrupt the chain of transmission of infection, such as changes in marketing practices (sell when sick, mobile pig tradesmen and simple quarantine measures like isolation or penning of recently bought or "sick" pigs .

The critical shortage of Lao veterinarians and animal science specialists remains an important issue that should be addressed by future projects. 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.

10 References

10.1 References cited in report

- Kamakawa, A., Viet Thu H.T., Yamada, S., Epidemiological survey of viral diseases of pigs in the Mekong Delta between 1999 and 2003, Veterinary Microbiology, 118 (2006), 47-56
- Bell, C.E, French, N.P., Karimuribu, E., Ogden, N.H., Bryant M.J., Swai, E.M., Kambarage D.M., Fitzpatrick, J.L. The effect of different knowledge dissemination interventions on the mastitis knowledge of Tanzanian smallholder dairy farmers, Prev Vet Med 72, 2005, 237-251.
- Classical Swine Fever and Emerging Diseases in Southeast Asia, ACIAR proceedings 94, S.D. Blacksell, 2000 (ISBN:1 86320 286 2) http://www.aciar.gov.au/publication/PR094
- OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, 2009 Chapter 1.1.4 .PRINCIPLES OF VALIDATION OF DIAGNOSTIC ASSAYS FOR INFECTIOUS DISEASES http://www.oie.int/eng/normes/mmanual/2008/pdf/1.1.04_VALID.pdf
- Chapter 2.1.5 . FOOT AND MOUTH DISEASE http://www.oie.int/eng/normes/mmanual/2008/pdf/2.01.05_FMD.pdf
- Chapter 2.8.3 .CLASSICAL SWINE FEVER, (hog cholera) http://www.oie.int/eng/normes/mmanual/2008/pdf/2.08.03_CSF.pdf
- Bell, C.E, French, N.P., Karimuribu, E., Ogden, N.H., Bryant M.J., Swai, E.M., Kambarage D.M., Fitzpatrick, J.L. The effect of different knowledge dissemination interventions on the mastitis knowledge of Tanzanian smallholder dairy farmers, Prev Vet Med 72, 2005, 237-251.

10.2 List of publications produced by project

Journal articles

- S. Khounsy, J.V. Conlan, L.J. Gleeson, H. Westbury, A. Colling, D. Paton, N. Ferris, J.F. Valarcher, J. Wadsworth, N. Knowles, SD Blacksell (2008) Molecular epidemiology of foot-and-mouth disease viruses from South East Asia 1996-2006: The Lao perspective, Veterinary Microbiology, 137 (2009), 179-183
- S. Khounsy, J.V. Conlan, L.J. Gleeson, H.A. Westbury, A. Colling, D.J. Paton, N.J. Knowles, N.P. Ferris & S.D. Blacksell. Foot-and-mouth disease in the Lao PDR: I. A review of recent outbreaks and lessons from control programs, Rev. sci. tech. Off. Int. Epiz. 2008, 27 (3), 839-849
- Stuart D. Blacksell, Syseng Khounsy, James V. Conlan, Laurence J. Gleeson, Axel Colling, Harvey A. Westbury. Foot-and-mouth disease in the Lao PDR: II. Sero-prevalence estimates using structured surveillance and abattoir surveys;, Rev. sci. tech. Off. Int. Epiz. 2008, 27 (3), 851-859
- Conlan, J.V, Khounsy, S., Blacksell, S.D., Morrissy C.J., Wilks, C., Gleeson, L. (2008) Development and evaluation of a rapid immunomagnetic bead assay for the detection of classical swine fever virus antigen, Trop Anim Health Prod 41: 913-920 http://www.springerlink.com/content/q340680270965705/fulltext.pdf

- Blacksell, S.D., Khounsy, S., Boyle, D.B., Greiser-Wilke, I., Gleeson, L.J., Westbury, H.A. and Mackenzie, J.S. (2004a) Phylogenetic analysis of the E2 gene of classical swine fever viruses from Lao PDR, Virus Res 104, 87-92.
- Blacksell, S.D., Khounsy, S., and Westbury, H.A. (2004b), The effect on sample degradation and RNA stabilization on classical swine fever virus RT PCR and ELISA methods, J Virol Methods 118, 33-7.
- Blacksell, S.D., Khounsy, S., Boyle, D.B., Gleeson, L.J., Westbury, H.A. and Mackenzie, J.S. (2005) Genetic typing of classical swine fever viruses from Lao PDR by analysis of the 5' non-coding region. Virus Genes 31, 349-55.
- Blacksell, S.D., Khounsy, S., Phethsouvanh, R., Newton, P.N. (2006), A simple and inexpensive container for the transport of biological specimens in limited resource situations, Transaction of the Royal Society of Tropical Medicine and Hygiene, 100, 1084-1086.
- Khounsy, S., Gleeson, L.J., Van Aken, D., Westbury, H.A., Blacksell, S.D., (2007) Diagnosis of classical swine fever virus in a limited resource setting: The influence of pig breed on methodology and sample selection, Trop Anim Health Prod 39, 21-25
- Blacksell, S.D., Khounsy, S., Van Aken, D., Gleeson, L.J., Westbury, H.A., (2006). Comparative susceptibility of indigenous and improved pig breeds to Classical swine fever virus infection: Practical and epidemiological implications in a subsistence-based, developing country setting. Trop Anim Health Prod, 38, 467–474

In preparation

- Development and validation of a 3abc ELISA antibody ELISA
- Reduced sensitivity of CSF CTB ELISA to detect maternal antibodies in CSF vaccinated piglets in comparison with NPLA and CEDI tests,
- Results from molecular epidemiology studies for CSF and FMD

Conference proceedings/presentations

- James Conlan, Syseng Khounsy, Manivanh Phruaravanh, Vilayvan Soukvilai, Christopher Morrissy, Axel Colling, Stuart Blacksell, Colin Wilks and Laurence Gleeson "Application of Immunomagnetic Bead Technology for Improved Diagnosis of Classical Swine Fever in a Low Technology Setting", Poster at WAVLD, Melbourne November, 2007
- Other presentations and didactical material produced by the project
- Management of Classical Swine Fever and Foot-and-Mouth disease in Lao PDR, ACIAR proceedings 128, Conlan J.V., Blacksell, S.D., Morrissy C.J. and Colling A. (eds.) 2008 (ISBN 978 1 921434 98 3 (print), ISBN 978 1 921434 99 0 (online) http://www.aciar.gov.au/publication/PR128

Contents:

- Foreword, Director ACIAR
- Introduction to proceedings, Laurence J. Gleeson and Axel Colling

Section 1 Pig production and extension

- International pig production and implications for Lao PDR, Ross Cutler
- Smallholder Pig Systems in Luangphabang and Xiengkhuang Provinces: Current Feed Situation and Potential of Forage Legumes for Improving Pig Productivity, Phonepaseuth Phengsavan

- Extension models for pig production in Lao PDR: the art of how to best use limited resources, John G. Connell and Ounkeo Pathammavong
- Improving Pig Production Systems in the Integrated Upland Agricultural Research Project Target Area – Pak Ou District, Luangprabang Province, Lao PDR, Gavin Varney
- Pig production and health in Bolikhamxay Province, Lao PDR, James Conlan, Syseng Khounsy, Lapinh Phithakhep, Manivanh Phruaravanh, Vilaywan Soukvilai, Axel Colling, Colin Wilks and Laurence Gleeson
- Pig production and disease management: a village perspective, Tess Vitesnik, Hongxay Bansalith and Laphin Phithakhep

Section 2 Classical Swine Fever and Foot and Mouth disease country reports

- Classical Swine Fever and Foot and Mouth Disease in Yunnan Provience, People's Republic of China, Li Le, Gao Huafeng
- Country Report of Myanmar for Classical Swine Fever and Foot and Mouth Disease, Cho Cho Htun and Mya Mya Oo
- Country report on CSF and FMD in Cambodia, Dr. Bun Chan
- The Classical Swine Fever and Foot and Mouth Disease Situation in Viet Nam, Dr. Nguyen Thu Ha
- Country Report on Classical Swine Fever and Foot and Mouth Disease in Thailand Dr. Nopphavanh Maiya and Dr. Wichittra Wannawoharn
- Classical Swine Fever and Foot and Mouth disease: Country Report for Lao PDR Syseng Khounsy and James Conlan

Section 3 Diagnosis and vaccination

- The molecular epidemiology of classical swine fever viruses from Lao PDR and Asia: A brief review, Stuart D. Blacksell
- Diagnostic Tests for the control of Classical Swine Fever and Foot and Mouth Disease in South East Asia: an overview, Chris Morrissy, James Conlan, Lynda Wright, Winsome Goff, Axel Colling, Jef Hammond, Michael Johnson, Stuart Blacksell and Peter Daniels
- Application of immunomagnetic bead technology for improved diagnosis of classical swine fever virus, James Conlan, Syseng Khounsy, Manivanh Phruaravanh, Vilaywan Soukvilai, Christopher Morrissy, Axel Colling, Stuart Blacksell, Colin Wilks and Laurence Gleeson
- Classical swine fever virus vaccine stability in the Lao PDR, James Conlan, Tess Vitesnik, Syseng Khounsy, Colin Wilks and Laurence Gleeson
- Recommended vaccine programs for village based pig production systems in Lao PDR
  Syseng Khounsy, Tess Vitesnik and James Conlan
- Future research directions for classical swine fever and foot and mouth disease in the Lao PDR: A facilitated session to capture the skills and experience of workshop delegates
  Ross Cutler and James Conlan
- Maximising Training Outcomes in Diagnostic Laboratories: A Two Way Process Chris Morrissy, Lynda Wright, Winsome Goff, Axel Colling, Greer Meehan, Michael Johnson, Stuart Blacksell, Laurence Gleeson and Peter Daniels

Other didactical material, thesis and published interviews produced by the project continued:

- Gender Resource Information and Development Center (GRID), Lao Women's Union, October 2005, "Management of CSF and FMD at the village level in Lao PDR"
- CSFRapid, Kit preparation, quality testing and users guide for the detection of classical swine fever antigen from spleen and tissue specimens, Lao National Animal Health Centre, Vientiane, Lao PDR, 2006
- Classical Swine Fever Virus: A guide to improving disease control at the village level in Lao PDR, 2006, Lao Australian Animal Health Research Project, ACIAR Project Number: AS1/2003/001
- Stuart Blacksell produced a large photographic library consisting of over 100 photos of livestock in Lao PDR and donated these to the project. Many of these are photos of acute FMD infected animals showing typical lesions.
- James Conlan, Improved Diagnostics & Management of Classical Swine Fever Virus in Laos, A thesis submitted in total fulfilment of the requirements of the degree of Master of Science, March, 2006, School of Veterinary Science, The University of Melbourne
- Tess L. Vitesnik, Pig Health and Production in the Lao People's Democratic Republic, A thesis submitted in total fulfillment of the requirements of the degree of Bachelor of Animal Science, December, 2006, School of Veterinary Science, The University of Melbourne
- Kristina Osbjer, 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.
- Animal Magnetism checks disease, ACIAR Partners Magazine, Spring 2006, http://www.aciar.gov.au/node/736
- CSIRO Horizon newsletter, 30 September 2005, Issue No 12 International snapshot, L. Gleeson

Project documents and annual reports

Management of CSF and FMD at the village level in Lao PDR, AS1/2003/001
Final project document prepared by L. Gleeson

Annual report for period 1 July 2003- 30 June 2004, Author: Laurence Gleeson

Annual report for period 1 July 2004- 30 June 2005, Author: Axel Colling

Annual report for period 1 July 2005- 31 October 2006, Author: Axel Colling

Review report Management of CSF and FMD at the village level in Lao PDR, AS1/2003/001, Author: Trevor Ellis, 20-22 November 2006

Project extension 1 January 2007 - 31 December 2008: Management of CSF and FMD at the village level in Lao PDR, AS1/2003/001, Author: Axel Colling

Annual report for period 1 June 2007- 31 May 2008, Author: Axel Colling (submitted 21 July 2008)

Project extension 1 January - 30 June 2009: Management of CSF and FMD at the village level in Lao PDR, AS1/2003/001, Author: Axel Colling

Consultant visits and reports

Greer Meehan, Laboratory diagnosis of FMD and CSF (Dec 2005)

Kim Newberry, Laboratory diagnosis of FMD and CSF, March 2009

Ross Cutler, December 2003, June 2006

L. Gleeson, 1-4 August 2004

Colin Wilks, 31 July - 8 August 2004, 19-27 February 2005

Axel Colling, 10-17 June, 18-25 November 2006, 17-18 September 2009

Chris Morrissy, 18-25 November 2006, 17-18 September 2009

Axel Colling, project discussion at ACIAR in Canberra, 23 March 2006

Axel Colling, ACIAR project workshop in Canberra, 14-15 June 2007

Axel Colling and Peter Durr, Lao PDR 1-5 September, 2008 AH 2005 084 “Improved control of pig diseases in Lao PDR”

Axel Colling, Chris Morrissy, Jim Parsons, Ross Cutler, a combined ACIAR project conclusion and new project proposal workshop was held at the Ministry of Agriculture & Forestry, DLF Regional Office in Luang Prabang, Lao PDR, 12-13 December 2008.

11 Appendixes

11.1 Appendix 1

Management of CSF and FMD at the village level in the Lao PDR:

Opening Address	Lao government official
Opening address	Mr. Michael Hasset: AusAID
Official opening of the workshop	Senior Lao government official
Overview and scope of the project	Dr. Axel Colling, CSIRO, AAHL
ACIAR strategic perspective in SE Asian region	Dr. Peter Rolfe, ACIAR
Pig production: a global perspective	Dr. Ross Cutler
Pig production systems in the Lao PDR	Mr Niwat, Technical division, DLF
Improving feeding systems	Phonepaseuth Phengsavanh, NAFRI
Extension models for pig production in Lao PDR	John Connell, CIAT
Improving pig production systems	Gavin varney
Pig production and health in Bolikhamxay	James Conlan
Pig production and disease management	Tess Vitesnik
Recommendations for improved pig production	Dr. Ross Cutler
CSF and FMD situation in Yunnan Province	
CSF and FMD situation in Thailand	
CSF and FMD situation in Myanmar	
CSF and FMD situation in Cambodia	
CSF and FMD situation in Vietnam	
CSF and FMD situation in Lao PDR	Dr. Syseng Khounsy
Molecular epidemiology of CSF virus in Lao	Dr. Stuart Blacksell
CSF and FMD diagnosis	Chris Morrissy, CSIRO, AAHL
IMB-ELISA technology for improved diagnosis	James Conlan
Maximising training outcomes in laboratories	Chris Morrissy, CSIRO, AAHL

Vaccine stability, storage and delivery in Laos	James Conlan and Tess Vitesnik
Recommended vaccine programs	Dr. Syseng Khounsy

Status of CSF and FMD in Laos. General discussion of project outcomes and future directions

11.2 Appendix 2

Pig Health and Management Training Program for Lao District Staff

Dates: 23rd-24th November 2006

Venue: Meeting room at National Animal Health Centre, Vientiane

Timeline and content:

Tues 22nd November- half day discussion with organizers, presenters and translators to ensure a cohesive program is presented

Wed 23rd November- AM- pig health

PM- pig nutrition

Thurs 24th November- AM- pig reproduction and genetics

PM- pig housing and management

Participants:

8 district staff from Bolikhamsay Province (2 from each of Paksane, Bolikhan, Pakkading and Thaphbath districts)

10 district staff from Luang Prabang and Xieng Kuang Provinces (to be recommended by CIAT)

1 staff from the National Animal Health Centre

1 staff from the Technical Division of the DLF

Presenters:

Dr. Ross Cutler (ACIAR)- pig reproduction and genetics, and pig health

Dr. Syseng Kounsey (NAHC)- pig health

Mr Gavin Varney (ACIAR)- pig housing and management

Mr Viengsavahn Phimphachanhvongsod - pig nutrition

Translators:

Dr. Syseng Khounsey

Mr. Lapin, LAAHRP staff

Mr. Hongxai, LAAHRP staff

Outline

Issues raised in brains storming session with Laos animal health staff

The training course will be given 23-24 November. It will be preceded by a meeting on Wed 22 to make sure that we are covering the appropriate bases in the appropriate way.

I) Pig production training course, 1 day (course facilitator Ross Cutler)

Morning session 1,5 h
Brainstorming and involve farmers

Key needs and interests
Response and present results

1) Pig Health 0.5 days
a) diseases and disease reporting, sample taking and submission,
b) disease spread/ epidemiology principle, disease reporting
CSF pig to pig, hygiene, worms, eggs
c) principles/concepts of quarantine, Epidemiology spread of disease (paint pig with red chalk and let it run around to illustrate spread of disease, e.g. CSF, understand mechanisms of disease spread, antiparasite treatment, husbandry system
d) vaccination program e.g. CSF, when to vaccinate, e.g. piglet after 1 week then after 1 month then 6 months then every year, always vaccinate when pig is newly introduced,
cold chain, doses, syringes and needles, injection

2) Pig Feeding (Vieng CIAT,)

Subject to Vieng's interest but might include:

Understand the proportion\composition of food, e.g. protein, energy, fibre etc, e.g. Stylo 40kg or bucket, Cassava (get skin off/cyanide) 20kg, Sweet potato 10kg, Rice bran 20kg, Maize 5 kg
Uncooked meat,
a) Young pigs stylo/protein/energy
b) Sows stylo/protein/energy
c) as much water as they want

3) Pig housing (Gavin)
Matters for consideration in housing pigs in Laos villages
Better production rates, better control, 80% of piglets which die in first 3 days are crushed
a) separate pigs from faeces
b) stop roaming
c) stop disease spread
d) control breeding

4) Pig reproduction and genetics

a) x-breed grow best (Duroc x village, or village x village e.g. Moo Lat x Moo Dong)
b) x-breed sows have bigger litters, produce more and wean more and bigger pigs

but only if they are well fed
c) sows come into heat about 3-14 days after weaning. They are on heat for about 24-48 h (signs of heat). They should be served twice by the boar.
d) sows wean more pigs they are provided with straw and leaves to make a nest

Other matters that might arise
c) castration ? castrated pigs grow faster have less fat and are more efficient, uncastrated pigs have a strong taste, also important for breeding management, castration is normally done in the first week in Laos

how is castration done ? problems, bleeding, infection

e) why cut teeth in piglets? (Not recommended any more as it does not improve growth but can induce infection)

f) colostrum management

List participants attend on training “Pig Health and Management for Lao District and Provincial Staff “

In Vientiane on 23 to 24 November 2006

No.	Name and surname	Organisation	Responsibility
1	Mr. Phonexay Khoulavong	National Animal Health Centre	Technical Staff
2	Ms. Thongphoon Theungpachanh	National Animal Health Centre	Technical Staff
3	Ms. Somphayvanh Chanthavong	National Animal Health Centre	Technical Staff
4	Mr. Intha Phoangsouvanh	Vaccine Production Centre	Technical Staff
5	Mr. Souksakhone Wiwasan	Vaccine Production Centre	Practice student
6	Mr. Bounxuang Yhasothorn	Vaccine Production Centre	Practice student
7	Ms. Phitsamai Sotsamany	Vaccine Production Centre	Practice student
8	Ms. Litsamai Khamsouvanh	Vaccine Production Centre	Practice student
9	Mr. Damduan Phommachack	Livestock and Fishery Section of Capital	Technical Staff
10	Mr. Somvang Vilayphanh	Livestock and Fishery Office of Oudomxay Prov	Technical Staff
11	Mr. Xayyhasith Vilayvong	DAFEO of Phakbang Dist	Technical Staff
12	Mr. Somsack Phanthavong	DAFEO of Hoon Dist	Technical Staff
13	Ms. Keola Phathana	Livestock and Fishery Section of Luangprabang Prov	Technical Staff
14	Mr. Somsack	Livestock and Fishery Section of Luangprabang Prov	Technical Staff
15	Ms. Chansouk Chanthanon	DAFEO of Phak Ou Dist	Technical Staff
16	Mr. Thongkham Vongphalad	DAFEO of Phak Ou Dist	Technical Staff
17	Mr. Somvanh	DAFEO of Xienghean Dist	Technical Staff
18	Mr. Sengphet Sysouk	DAFEO of Xienghean Dist	Technical Staff
19	Ms. Phoutsady Syfongxai	Livestock and Fishery Section of Xiengkhong Prov	Technical Staff
20	Mr. Khouthao	DAFEO of Nonghat Dist	Technical Staff
21	Mr. Vong Philavong	DAFEO of Nonghat Dist	Technical Staff
22	Ms. Davanh Duongthanousone	DAFEO of Phank Dist	Technical Staff
23	Mr. Keo Anong	DAFEO of Phank Dist	Technical Staff
24	Mr. Thongphout Phewthongvongsa	Livestock and Fishery Section of Borlikhamxay Prov	Technical Staff
25	Mr. Chantha Souksavath	DAFEO of Phakson Dist	Technical Staff
26	Mr. Khaophone	DAFEO of Thaphabath Dist	Technical Staff
27	Mr. Khammao Xaiyavong	DAFEO of Thaphabath Dist	Technical Staff
28	Mr. Dam Phearnchit	DAFEO of Phakkading Dist	Technical Staff

29	Mr. Thongsavanh	DAFEO of Phakkading Dist	Technical Staff
30	Mr. Sengsamai	DAFEO of Borlikhan Dist	Technical Staff
31	Ms. Sonthala	DAFEO of Borlikhan Dist	Technical Staff
32	Mr. Phouth Inthavong	Lao Œ Australian Animal Health Research Project	Technical Staff
33	Mr. Laphinh Phithacthep	Lao Œ Australian Animal Health Research Project	Technical Staff
34	Mr. Hongxay Bansalith	Lao Œ Australian Animal Health Research Project	Technical Staff
35	Mr. Nithiphon Somsanit	Lao Œ Australian Animal Health Research Project	Practise Student
36	Ms. Tess Vitesnik	Lao Œ Australian Animal Health Research Project	Volunteer of Project
37	Mr. Thongvanh Chanphaserth	Micro Community Development Project/Luangprabang Prov	Technical Staff
38	Mr. Khamsing	Micro Community Development Project/Luangprabang Prov	Technical Staff

Notice:

Prov: Province

Dist: District

DAFEO: Department of Agriculture and Forestry Extension Office

11.3 Appendix 3

IMB Training course participants NAHC, Vientiane, Lao 23-24 November 2006

Participants received a course booklet: "CSFRapid, Kit preparation, quality testing and users guide for the detection of classical swine fever antigen from spleen and tissue specimens, Lao National Animal Health Centre, Vientiane, Lao PDR, 2006"

Participants
Lao
Manivanh Phruaravanh
Vilaywan Soukvilai
wiwanvet@yahoo.com

Thailand
Wittchittra Wannawoharn
wichittraw@dld.go.th

China
Gao Huafeng
Ms, Li Le
kmghf@hotmail.com
htmlile@hotmail.com

Vietnam
Nguyen Thu Ha
phuongmouoc2006@yahoo.com

Cambodia
Soklay
chinsoklay@yahoo.com

Myanmar
Dr. Mya Mya Co
lbvd@mptmail.net.mm

Australia
Jamie Conlan
Chris Morrissy
Axel Colling
jvconlan@hotmail.com

Chris.morrissy@csiro.au
Axel.colling@csiro.au

11.4 Appendix 4

Report of Training Course: IMB-ELISA test for rapid CSF diagnosis

ACIAR Project AS1/2003/001:

Management of CSF and FMD at the village level in Lao PDR

3 day course: October 30, 31 and November 1, 2006

Objectives of training course:

Train staff from 4 provincial laboratories in Lao PDR: Luang Namtha, Luang Prabang, Oudomxay and Champassack

Train staff from the National Vaccine Production Centre (NVPC) and the National Animal Health Centre (NAHC)

Work through the test and pin-point and correct any problems with the test format

Plan for second training course in December

Staff attending training: 2 provincial staff from each provincial laboratory
2 staff from the NVPC
3 staff from the NAHC

Trainers: Mr. Jamie Conlan, Ms. Manivan Phouaravanh and Miss Vilaywan

Day 1:

Morning

Introduction and short background discussion about CSF virus and diagnosis and control

IMB-ELISA method and kit components

Jamie performed test with participants looking on

Participants practiced using dropper bottles and pipettes before doing the test

Afternoon

All participants tested 3 control samples (C+++, C++ and C-)

Almost all participants performed the test well but some confusion evident regarding washing IMBs and transferring to a new tube before adding TMB substrate (this step will need to be clarified and improved before next training course)

Some background noise evident in negative control after 10 minute incubation

Day 2:

Morning

Biosafety and sample preparation demonstration and discussion

Participants were divided into 7 groups of 2 and prepared 2 samples each (4 samples per group)

Afternoon

In groups of 2 all participants tested 4 samples and controls that were prepared in the morning session

Again, majority of participants got correct results for the samples tested but background noise evident after 10 min TMB incubation in the negative controls

Confusion was evident if a small amount of colour was evident in the sample or negative control (washing step needs to be improved and the incubation time for the TMB should be decreased to 5 min)

Day 3

Morning

Participants retested samples prepared on day 2 but used a 5 min incubation period for TMB instead of 10 min

Much less confusion and much less background noise in negative control

Results were improved upon compared to afternoon of day 2

Note that all positive samples were positive with decreased TMB incubation time

5 min incubation should be adopted

Afternoon

Each participant tested 5 randomly selected samples that had been prepared earlier

Majority of results were correct, only 3/14 people had a small number of errors

Note again that all positives were detected (but some false positives detected)

Some background noise still evident but will be improved with stricter wash steps

Some issues arose relating to the accurate recording of sample numbers. On several occasions tube labels were mixed up or numbers were written down incorrectly. This will potentially have repercussions down the track.

General notes:

Overall very positive outcomes

Participants very keen to continue with further training and to use the test in the provincial labs

Kits will be prepared and sent to the labs in the next 2 weeks

Further training in early December

Some participants stressed the need to make stronger links with diagnostics and control programs

Need to change method according to points outlined above – 5 min TMB incubation and rework the wash method

It will be important to develop strong QA/QC protocols that are easy to follow to ensure the results coming from the provincial labs are correct (or more to the point, that the test sensitivity is high)

It will also be important to have regular follow-up training sessions in Vientiane – at least once a year and possibly once every 6 months (this will be particularly important if new staff assigned to the labs)

11.5 Appendix 5

Summary of other related projects:

AH 2009/001 Increased productivity and reduced risk in pig production-market chains for improved livelihoods in Lao PDR (Stage 1, 25 October 2009)

ACIAR project AH/ 2006/125 (Kennedy/Bevans)
Improved quality assurance and control for livestock vaccines in Lao PDR (Mr. Bevans)
This project aims at improving the quality assurance and control of vaccine manufacture at the AVMSC and is being currently developed by RE & HO Bevan Consulting Pty Ltd..
Objective 2 Improved quality and supply of effective vaccines for pig diseases of this project proposal is directly linked to the outcomes of this project.

AH/2006/155 (Kennedy, AusVet)
Vaccine business development in Lao PDR
Vaccines for animal diseases in Lao PDR have been produced locally, however the sustainability is limited under present arrangements. This activity will investigate, in cooperation with the Laos government, the options for the future management, marketing and supply of vaccine and provide a preferred option that can be implemented by government over time. The outcome of that project was a business model that was endorsed by the Lao Government in November 2007, comprising nine key elements:

The establishment of a single Animal Vaccine and Medicine Supply Centre (AVMSC) that would be responsible for supplying the animal vaccine and animal medicine needs of Lao PDR.
- Improved financial management.
- A review and probable increase in prices.
- Discontinuing production of some unprofitable vaccines.
- Recovering long-standing debts.
- Improving quality in production and introducing Good Manufacturing Practice.
- Better marketing and brand identity.
- Appointing a skills-based AVMSC Advisory Board.
- Establishing a Veterinary Medicine Regulatory Authority (VMRA) to regulate of domestic and imported vaccines and medicines.

Workshops to initiate these changes were held in November 2007 and some changes have been made in management of the production role of the AVMSC. Mr Bevan's project will assess progress in other areas during a visit in late 2007-08.

Objective 2 Improved quality and supply of effective vaccines for pig diseases of this project proposal is directly linked to the outcomes of this project.

ADB (Syseng Khounsy)
This project is concerned with baseline surveys to improve livestock production in the northern part of Lao PDR. Dr. Syseng Khounsy, the former Lao project leader has expressed interest to collaborate in this project and assist Dr. Phout Inthavang in his duties as new Laos project leader.

ACIAR AH/2004/046
Forage legumes for supplementing village pigs in Lao PDR (Stur, CIAT, and UQ)
Rearing pigs is a widespread smallholder livelihood activity in the northern mountainous regions, but productivity is low due to poor nutrition. The introduction of forage legumes into the farming system offers the opportunity to improve pig nutrition and to reduce the time spent by women in gathering and preparing feed. The project will initially document
existing pig feeding and production systems to help identify entry points for new

practices. It will use the scaling-up of a promising forage with at least 1000 farmers to learn how they adapt and integrate that feed into their farming systems. It will also introduce and valuate new forages for their suitability as pig feeds. Broader adoption of these technologies will be achieved through the network of agencies in Lao, the development of guidelines for use by other groups in scaling up this innovation and through pending large development programs that involve CIAT and the Lao partner organisations.

ACIAR AH/2006/161
Management of pig associated zoonosis in the Lao PDR (Conlan, Murdoch).
Livestock production, especially pig raising, is becoming increasingly important for food security in Lao PDR. Demand for pig meat has increased as a result of avian influenza outbreaks decreasing chicken meat availability, but pigs raised in the smallholder sector are susceptible to a broad range of medically important pig-associated zoonoses (diseases that infect both animals and humans). Some examples are leptospirosis, hepatitis E virus, salmonellosis, cryptosporidiosis and toxoplasmosis. The central aim of this project is to establish the evidence base regarding the presence of pig-associated zoonoses in Lao PDR and their socioeconomic impact, also to identify and implement appropriate and sustainable veterinary public health interventions.

ACIAR AH/2006/025
Understanding livestock movement and the risk of spread of transboundary animal diseases, (Hawkins, AgWA)
Transboundary animal diseases in Southeast Asia (including foot-and- mouth disease and classical swine fever) cause significant losses. Such diseases cross national borders and spread primarily to new areas through livestock movements. This project, involving Cambodia, Lao PDR and Australia, seeks to provide a detailed, timely, quantitative understanding of livestock movement patterns and their influence on the spread of disease, enabling researchers to quickly pinpoint high-risk areas. This will enable preventative actions to be taken and also aid the development of new strategies to minimise the risk of disease spread. Technology transfer workshops also involving Thailand, Vietnam, alaysia, Myanmar, and China will promote the development of an integrated regional approach.

AusAID CARD project Vietnam FMD 2005 – 08 (Chris Morrissy, CSIRO)
The objectives of the project are: To establish an effective laboratory network for the diagnosis and control of FMD by the provision on resources and training of staff in required methods and quality assurance and 2) To provide accurate data to explain failure of vaccination to control FMDV and to develop new effective vaccine application strategies. Lessons learned from the control of FMD and CSF in Vietnam can be applied to the Laos PDR and viceversa and cooperation and coordination of control efforts between neighbouring countries is required for an improved sustainable approach.

12 Abbreviations:

A: Australia
AAHL: Australian Animal Health Laboratory
AC: Antigen Capture
ACIAR: Australian Centre for International Agriculture Research
ADB:Asian Development Bank
ADG: Average Daily Growth Rate
AUD: Australian Dollar
AYAD: Australian Youth Ambassador for Development
BD: Border Disease
BVD: Bovine Viral Diarrhoea
CARD: Centre for Agriculture Research and Development
CIAT/AusAID: Centre for International Agriculture Technology/Australia Aid
CSF: Classical Swine Fever
CSIRO: Commonwealth Scientific Industrial and Research Organization
CTB: Complex Trapping Blocking
DLF: Department of Livestock and Fisheries
ELISA: Enzyme Linked Immunosorbent Assay
FAO: Food and Agriculture Organization
FLSP: Forages and Livestock Systems Project
IMB: Immunomagnetic Bead
FAO: Food and Agriculture Organization
FMD: Foot-and-Mouth Disease
Lao PDR: Lao Peoples Democratic Republic
Mio: 1.000.000
MSc: Master of Science
NAFRI: National Agriculture and Forestry Research Institute
NAHC: National Anima Health Centre
NGO: Non-governmental Organization
NPLA: Neutralizing Peroxidase Linked Assay
OIE: Office International des Epizooties
PC: Partner Country
POC: Point of Care
QA: Quality Assurance
QC: Quality Control
SEAFMD: South East Asia Foot-and-Mouth Disease
SOP: Standard Operating Procedure
WAVLD: World Association of Veterinary Laboratory Diagnosticians
WRL: World Reference Laboratory