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Australian Centre for International Agricultural Research

Final report

Project

Improving the production and competitiveness of Australian and Philippines pig production through better health and disease control

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Contents

1 Acknowledgments

A focus of the project involved on-farm and slaughter-house studies in Regions IV and V. The co-operation and assistance of the farmers and management and staff of the slaughterhouses was essential in the performance of these field-based studies.

In the Australian based studies, the technical assistance of Dr Henrik Christensen of the University of Copenhagen in Denmark for the provision of expertise in taxonomy and the formalities associated with the naming of a new bacterial species is gratefully acknowledged.

2 Executive summary

This project was undertaken to achieve both a scaling out and a scaling up from a prior ACIAR Project (AH-2009-022) that had focussed entirely on improving the diagnosis and control of porcine respiratory disease. The scaling out component of the project established improved and integrated laboratory and field diagnostic services in two new Regions, IVA and V. The laboratory SOPs implemented in this project were all targeted to be low cost and sustainable for use in the regional laboratories. This approach provides low cost, rapid responses at the regional level with high-cost, high level back-up available from the national laboratory in Manilla. The successful completion of a small-scale research study, one in each new regions, provided clear evidence of the achievement of this aim.

The remaining aspects of the Philippines based activities of the project had full stakeholder involvement in two full cycles of knowledge generation, action and reflection. A range of interventions (e.g. nipple drinkers and creep boxes) were identified in a participatory process and then applied by the research team. The feedback provided from the small-holder farmers, indicated strong support for the interventions. The same participatory approach was also used in the work on the incidence and economic impact of disease on small holder pig farms in San Simon. This aspect of the project focussed on the only disease syndrome identified by the farmers as being of importance - piglet diarrhoea. The research allowed the development of an understanding of the overall economics of small-holder pig production in San Simon as well as an understanding of the economic implications of piglet diarrhoea was developed. One major outcome of this project is the development of a simplified record keeping notebook that will allow far better economic analysis in future studies.

In the Australian-based activities, work focussed on the development of technologies and diagnostic assays that are highly relevant and immediately applicable to the Australian pig industry and achieved a series of important practical and relevant outcomes. A previously unrecognised bacterium that is linked to porcine respiratory disease has been formally described as *Glaesserella australis*. Novel tools for the typing of key bacterial pathogens that will allow the Australian pig industry and the associated vaccine companies to improve the efficacy and coverage provided by the current vaccines for porcine pleuropneumonia and Glässer's disease. Significant new knowledge on the nature of PCV-2 infection in Australia has been gained – particularly the understanding that high levels of the virus can occur in herds with no obvious PCV-2-associated disease, suggesting potential production impacts in the absence of frank clinical disease.

The impact adoption study has clearly demonstrated that the laboratory and field aspects of AH-2009-022 and AH-2012-066 have achieved marked progression along the adoption pathway. Indeed, there are key laboratory innovations that have clearly reached the end of the adoption pathway. Project AH-2012-066 had a strong focus on assisting small-holder farmers with five (nipple drinkers, creep boxes, market price information, record keeping and biogas utilization) of six innovations developed by the project being perceived as being useful by the farmers, although only three (nipple drinkers, creep boxes, market price

information) achieved a strong adoption. The results of the impact study indicate that these three innovations have moved from demonstration farmers to the level of “Next Users” (small holder pig farmers in San Simon). Additional strategies to support the other innovations that were perceived as being useful by the farmers but were not widely adopted are provided to guide future activities.

3 Background

This project targets major diseases of pigs in Australia and the Philippines. Pork is an important meat in both countries, particularly the Philippines, where pork accounts for about 60% of the meat produced and consumed in the country. About 98 million Filipinos rely on the pig industry as one of their main sources of animal protein food. In 2011, the Philippines pig industry produced just over 24 million pigs that yielded over 1.5 million metric tonnes of meat (FAO Statistics). The Australian pig industry consists of around 2.35 million pigs and 253,000 sows (2018 ABARES figures) and had an estimated annual production value of $1,375 million (2019-20 ABARES estimate). The Australian industry exports about 10% of the total production, with an estimated export value of about $95 million in 2019–20 (2020 ABARES estimates).

In both countries, respiratory diseases pose a significant challenge to the pig industry. Around 50% of all pig mortalities in the Philippines pig industry are caused by respiratory diseases (E. Villar, pers. comm., BAI statistics). Respiratory diseases have disrupted pig production in the Philippines for many years, reducing sector competitiveness, smallholder incomes, domestic market availability and stability of the price of pork products. In Pampanga province, where this project has had a 'scaling up' focus, heavy losses between 2007 and 2009 were associated with epidemics due to typical porcine respiratory and reproductive syndrome (PRRS) virus in 2007, and then HP-PRRS (highly pathogenic PRRS) virus in 2008–2009. In May 2009, PRRS affected 1,900 farms in 20 of the 21 municipalities, with 31% mortality in 9,930 cases (BAI figures). In terms of enteric disease, an outbreak of porcine epidemic diarrhoea occurred in 2010 in the province of Batangas (Region IV of Philippines) killing 67% of 17,115 sick pigs, causing a loss of PhP 9.13 m [$(Aus) 0.22 million] (all at 2010 rates) (Batangas report). AH/2009/022 provided some solid baseline data on the level of problems associated with respiratory disease. As an example, the lung score study showed 51% of pigs presented for slaughter in Pampanga and Bulacan provinces had acute lesions of cranioventral pneumonia and 27% had chronic lesions. The high prevalence of lesions of cranioventral pneumonia found in AH/2009/022 suggests that a high proportion of animals is exposed to major respiratory pathogens throughout the production cycle and close to slaughter.

In the Philippines, communities, including small-holder farmers, are organised in two distinct units – barangays[1] and puroks[2]. These formal organised units play a critical role in the planning and implementation of government policies and are also key features of how local communities are organised and interact.

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[1] *According to Section 384 of the Local Government Code of the Philippines, the barangay "serves as the primary planning and implementing unit of government policies, plans, programs, projects, and activities in the community, and as a forum wherein the collective views of the people may be expressed, crystallized and considered, and where disputes may be amicably settled." Accessed from http://www.chanrobles.com/localgov3.htm#.XONN6MgzbIU on May 15, 2019.*

[2] *A* purok *is a government unit smaller than a barangay. It was introduced in the 1950s to bring education to the masses. A* purok *consists of 50 to 100 households geographically close to each other (Rappler 2014). Accessed from https://www.rappler.com/move-ph/issues/disasters/preparedness/59060-camotes-island-purok-system-yolanda-zero-casualty on May 16, 2019.*

Respiratory diseases remain a major challenge to the Australian industry. Estimates submitted to the peak industry funding body, Australian Pork Limited (APL), indicate that respiratory diseases alone cost between $55 to $71 per sow per year. This project has contributed to the strategic goal of the ACIAR–Philippines cooperation agreement, specifically the undertaking to 'assist in increasing productivity, marketability and international competitiveness for Philippines agricultural products, taking into account the impacts of trade liberalisation'. It also aligns well with the Science and Technology (S&T) agenda of the Philippines through the PCAARRD's Industry Strategic S&T Plan (ISP), which considers pigs as one of the most important commodities in agriculture and specifically identifies a need for government intervention in animal health.

The ACIAR–Philippines cooperation agreement also aims 'to address food security by supporting research that would provide smallholder farmers and traders with increased cash income, supporting the purchase of staple foods.' Somewhere between 60% and 80% of all pork production in the Philippines remains in the hands of smallholders, meaning that the proposed project can have a significant impact on the smallholder sector. There is thus good alignment between the interests of the industry as a whole (providing economic growth and potential exports) and the smallholder sector (providing food security).
The Australian pig industry is fully aware of the importance of disease as a major cost of production. APL has identified that, even though Australia has a relatively good herd health status, pig diseases have a major impact on the cost of production. Hence improved disease control is a key strategy in the Core Objective 2 (Viable Productive Farms) in the 2010–15 Strategic Plan of APL[3]. The Cooperative Research Centre (CRC) for High Integrity Australian Pork (Pork CRC) also recognised the importance of disease and has a subprogram that focuses on alternative therapies, products or strategies to improve pig production efficiency and reduce mortality of all growth phases[4].

The Australian Pork Industry National Research, Development and Extension (RD&E) Strategy[5] recognised the need to enhance investment of RD&E funds from sources other than the traditional ones of the APL and the Pork CRC. This proposal links closely with this National Strategy, providing funds to support an organisation (UQ) that already has strong ongoing support from both APL and the Pork CRC and a long-term commitment to pig research.

This project was designed to align with the relevant industry and government priorities for the pig industry in both countries. The synergies of working on diseases in both countries in a single project include diagnostic testing in populations with different disease backgrounds and the training of Philippines and Australian veterinarians in detection and control of major endemic and exotic diseases of pigs.

The research strategy adopted for this project was the same as that used successfully in AH/2009/022 with the addition of an approach driven by using the six principles of Ecohealth research – systems thinking, transdisciplinary research,

[3] See: http://www.australianpork.com.au/pages/page130.asp

[4] See: http://www.porkcrc.com.au/html/program_2.html

[5] See: http://www.australianpork.com.au/pages/page188.asp

participation, sustainability, gender and social equity and knowledge to action[6]. The outcome of this type of approach is a project that is highly collaborative and consultative and which is working at the human-animal-environment interface. Importantly, we aim to link the two key arms of a diagnostic service (field and laboratory), two levels of service (national and regional), and two countries (Australia and the Philippines). This needs to be achieved within the context of also drawing in support and involvement of industry (particularly smallholder farmers). The project strategy (scaling up and scaling out) was adopted as it draws on the strengths and achievements of the past project and provides maximum benefits to the pig industries of both countries.

The strategy draws on the strengths of a multidisciplinary team that includes experienced laboratory-based diagnosticians (veterinarians, bacteriologists, virologists, pathologists), field-based veterinarians, and skilled epidemiologists, building on the skills and expertise within the Philippines team acquired as a result of AH/2009/022. A key achievement of AH/2009/022 was the creation of a highly skilled research and diagnostic team at both the national level (PAHC and BAI) as well as the regional level (RADDL III and the Region III field staff). The Philippines team gained skills in both the laboratory and field aspects of disease diagnosis, in study design and in performing complex, integrated field and laboratory studies. Hence, this project used this highly motivated Philippines research team to 'scale out' by being the main delivery of training to other regional staff. In addition, the motivation and drive of the Philippines team means that a 'scale up' in the level and application of diagnostic skills in the field staff in Region III, as well as the laboratory staff at both the regional (RADDL III) and national level (PAHC), can be achieved at the same time as 'scaling out' to Regions IVA and V.

[6] See: Charron, DF (2013) Ecohealth: origins and approach. In *Ecohealth Research in Practice: innovative applications of an ecosystem approach to health* (ed. DF Charron), pp. 1–30. Springer, New York, and International Development Research Centre, Ottawa.

4 Objectives

Overall aim
To improve the efficiency and competitiveness of the Philippines and Australian pig production through improved diagnosis, better health and enhanced disease control practices.

Objectives and subsidiary activities

1. To improve the diagnostic systems for respiratory disease in two selected Regions of the Philippines.
   - 1.1 Identify two regions where improved diagnosis of respiratory disease will be of most benefit
   - 1.2 Enhance regional laboratory capacity
   - 1.3 Enhance field investigation
   - 1.4 Introduce a lung scoring program

2. To identify, document and address constraints to the use of good disease prevention practices by smallholder pig farmers in Region 3
   - 2.1 Conduct training workshop in the principles of ecohealth research and participatory epidemiology for field staff
   - 2.2 Identify constraints through semi-structured interviews and group discussions
   - 2.3 Cycle through knowledge generation, action and reflection, assessing progress in overcoming constraints on an ongoing basis

3. To estimate the incidence and economic impact of two disease syndromes of importance to smallholder farmers.
   - 3.1 Identify two disease syndromes of highest priority to smallholder farmers
   - 3.2 Implement any additional systems required for diagnosis of agents associated with identified disease syndromes
   - 3.3 Develop decision support system
   - 3.4 Cycle through knowledge generation, action and reflection, assessing impact of disease syndromes on an ongoing basis
   - 3.5 Estimate incidence and economic impact of disease syndromes and agents

4. Develop new diagnostic tests for, and undertake applied epidemiological research on, pig diseases important to Australia.
   - 4.1 Identify novel *Actinobacillus*-like bacteria and understand their potential role in disease
   - 4.2 Rep-PCR typing of *Pasteurella multocida* established, validated and used
   - 4.3 Validate the use of MALDI-TOF to serotype *P. multocida*
   - 4.4 Evaluate the use of bead-based PCR to serotype *Haemophilus parasuis*
   - 4.5 Improve the understanding of the role of PCV-2 in pig disease in Australia

5. Conduct an assessment of the adoption and emerging impacts of project outputs from AH/2009/022 and AH/2012/066.
   - 5.1 Describe the outputs produced by the projects – both technical and capacity-building
   - 5.2 Describe the extent of adoption of outputs from both projects and identify the factors that influenced adoption
   - 5.3 Describe solutions, strategies and methods for further adoption in the future through the identification of adoption pathways

5.4 Assess the impact that the projects have made to date in both the Philippines and Australia and at the broader international level

5.5 Recommend actions to maximise impacts in the future and are expected to make

5.6 Identify any lessons for ACIAR that have been highlighted during the adoption study

5 Methodology

5.1 Objective 1: To improve the diagnostic systems for respiratory disease in two selected Regions of the Philippines

5.1.1 Identify two regions where improved diagnosis of respiratory disease will be of most benefit

This activity was undertaken by the Philippines team, who reviewed the respiratory disease diagnostic capabilities of 6 regions of the Philippines; Region IVA (Batangas), Region V (Albay), Region VI (Ilo-Ilo), Region X (Cagayan de Oro), Region XI (Davao) and Region XII (General Santos).

After a series of scoping visits and interviews with the Regional Animal Health Coordinators, the Chief of the laboratory and Provincial/City Veterinarians, two regions for the 'scale out' component of the project (Region IVA and V) were selected based on several criteria. The selection criteria was finalised by discussion with the full Philippines research team and were along the following lines:

A) Profile of the local pig industry
B) Capacity of the relevant Regional Animal Disease Diagnostic Laboratories (both staff and equipment) to adopt the laboratory technologies developed in AH/2009/022
C) Capacity of the relevant Provincial Veterinary Offices.

5.1.2 Enhance regional laboratory capacity

A review of the current protocols in use at the regional laboratories in Region IVA and V was completed by Philippines project staff, highlighting the need for specific training in the laboratory protocols specific for the identification of bacteria associated with pig respiratory disease. As a result, a five-day training workshop for the laboratory staff working in Regions IVA and V was undertaken (Sept. 28 to Oct. 2, 2015) by Philippines project staff in Region III. Laboratory staff were also trained on disease investigation and lung scoring to gain knowledge and appreciation of the activity of the field staff.

The review also highlighted the need for standard operating protocols (SOPs) and reference/quality control strains at both laboratories. As a result, the Philippines team, with input from the Australian team, developed SOPs for the isolation and identification of key respiratory bacterial pathogens and provided them to the Region IVA and Region V laboratories. As well, reference bacteria, as quality control strains, were provided. The regional laboratories were also provided with culture media and reagents for biochemical testing.

Confirmation of the effective implementation of the laboratory protocols was undertaken by the Region III research team visiting both Region IVA (June 2-3,

2016) and Region V (February 10-12, 2016; November 2016) for on site audits to confirm the ability of the two regional laboratories to apply the techniques and methods transferred via SOPS and workshops.

After the laboratory protocols were established and the staff trained in those protocols, project activities, described in other areas of this report, were undertaken using these laboratory protocols.

5.1.3 Optimise field investigation capacity

A review of the current protocols for field investigations in use in Region IVA and V was completed by Philippines project staff.

This review highlighted the need for specific training in field investigations and lung scoring at the slaughterhouse. The Philippines team in Region III trained veterinarians from the Regions IVA and V in a hands-on program that focussed on disease investigation and lung scoring (November 9-13, 2015). As well additional training was provided for Local Government Unit staff and Meat Inspectors – February 9-12, 2016 (Region V) and February 15-16, 2016 (Region IVA). A follow up “audit” style visit to confirm that the relevant regional staff were effectively performing disease investigations and were capable of lung scoring was performed on June 2-3, 2016 (Region IVA).

The two major relevant manuals produced in AH/2009/022 (Disease Investigation; Lung Scoring) were reviewed as part of this activity.

5.1.4 Introduce a lung scoring program

The research teams in both Regions IVA and V, with input from the rest of the Philippines research team and the Australian team, designed a study (one in each Region) that was focussed on the use of the newly acquired skills in on-farm disease investigation (Activity 5.1.3), lung scoring (Activity 5.1.3) and laboratory examination of lung samples (Activity 5.1.2) as reported above. The two regional programs were independently designed and performed.

In Region IVA, the study was undertaken by performing lung scoring in five abattoirs (Batangas City, Bauan, Lipa City, Nas and Tanauan City). In Region V, the selected abattoirs were Daraga, Ligo City, Polangui and Tabaco City.

The lung scoring system used in this study was based the system originally introduced in ACIAR Project AH-2009-022described by Straw et al. (1986). The percentage of the volume of each lobe of the lung with visual and palpable signs of consolidation was estimated in a sample of 560 lungs. The degree of consolidation was scored for each of the two apical lobes (0-10 for each lobe), each of the two cardiac lobes (0-10 for each lobe), each of the diaphragmatic lobes (0-10 for each lobe) and the cardiac lobe (0-5). Hence, the maximum possible score is 55. When lesions were present, they were classified as either acute or chronic. Pleurisy was scored on a 0 to 3 basis where 0 was no pleurisy, 1 was fibrous adhesions between the lung lobes, 2 was pleurisy lesions over the caudal and or cranioventral lobes and also pleuritic lesions on the rib cage and 3 was when the

lungs severely adhered to the rib cage and were difficult to remove without tearing the lung tissue. The presence or absence of lesions on the diaphragmatic (caudal) lobes consistent with *A. pleuropneumoniae* was recorded. Pericarditis was recorded as present or absent.

Bacterial culture of lungs collected for sampling was performed as per the project developed SOP (Bacterial Isolation and Identification of Respiratory Pathogens). In brief, lung tissue was first surface sterilised by ethanol flaming and a fresh cut made into the lung tissue. This fresh cut surface was then sampled using a sterile swab and the swab used to inoculate a blood agar plate and a McConkey agar plate. The blood agar plate was cross-streaked with a nurse culture of *Staphylococcus hyicus*. The inoculated plates were incubated in air at 37C. The plates were examined and relevant colonies identified as per the project SOPs.

In both Region IVA and V, 8 farms that provided pigs examined in the lung scoring component were selected for a follow up on-farm disease investigation. The on-farm investigations were undertaken as per the project SOPs.

To gain insight into the results of the lung scoring, a latent class analysis was undertaken using the Latent Gold (Statistical Innovations, Belmont, MA, USA). In this analysis, the variables were as follows:-

Lung Score (4 categories – 0; 1-6; 7-20; 21-55)
Pleurisy Score (4 categories – 0, 1, 2, 3)
Pericarditis – Yes or No
Pleuropneumonia – Yes or No

The co-variates were the province (Region IVA or V) and the farm type (commercial or small-holder).

5.2 Objective 2: To identify, document and address constraints to the use of good disease prevention practices by smallholder pig farmers in Region 3

5.2.1 Conduct training workshop in Ecohealth and participatory epidemiology for field staff

Much of the work proposed under Objective 2 had been structured to reflect an ecohealth type approach. In this approach, there are cyclic periods of (i) participatory design, (ii) knowledge development, (iii) implementation of an intervention strategy, and (iv) systematization (which includes adaptations of interventions, and policy uptake). A 3-day workshop was conducted to train regional field staff (from Region 3) in the principles of ecohealth research and participatory epidemiology. This covered key principles of ecohealth (systems thinking, transdisciplinary research, participation sustainability, gender and social equity and knowledge to action) and the key methods used in participatory epidemiology such as semi−structured interviews, proportional piling and matrix scoring. Fieldwork training was also be included.

5.2.2 Identify constraints through semi-structured interviews and group discussions

A baseline survey was conducted to have an overview of smallholder pig production in the San Simone municipality followed by semi-structured interviews, farm visits, and focus group discussions to further explore and define the constraints on pig production. A list of key definitions adopted for this survey is provided in Appendix 11.2.2. A key point was the definitions used for the farmers:- active farmers were currently raising pigs or has raised them within the last month and inactive farmers were those with the facilities to raise pigs but had not done so for at least one month.

5.2.3 Cycle through knowledge generation, action and reflection assessing progress in overcoming constraints on an ongoing basis

The general methodology used for this cycle and indeed the second cycle in this Objective are illustrated in Figure 5.2.1. Objective 3 shared these same cycles.

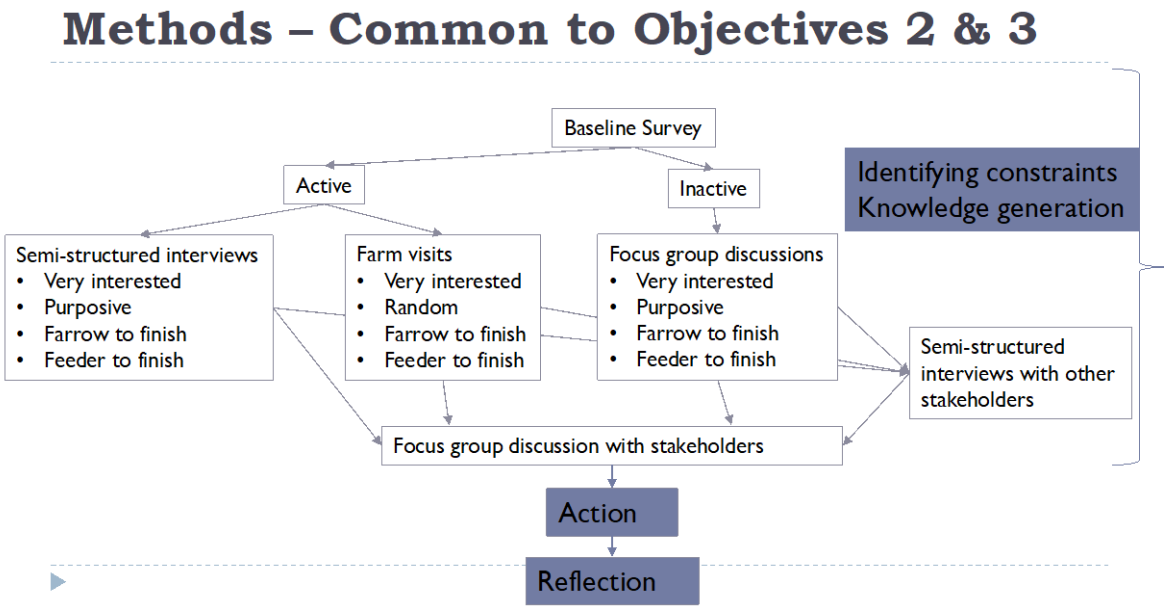

Figure 5.2.1. The common methodology used for the cycles of knowledge generation, action and reflection for Objectives 2 and 3.

The summary of the methods used and the activities undertaken in cycle 1 for Objectives 2 and 3 is provided as Figure 5.2.2 and that for cycle 2 in Figure 5.2.3.

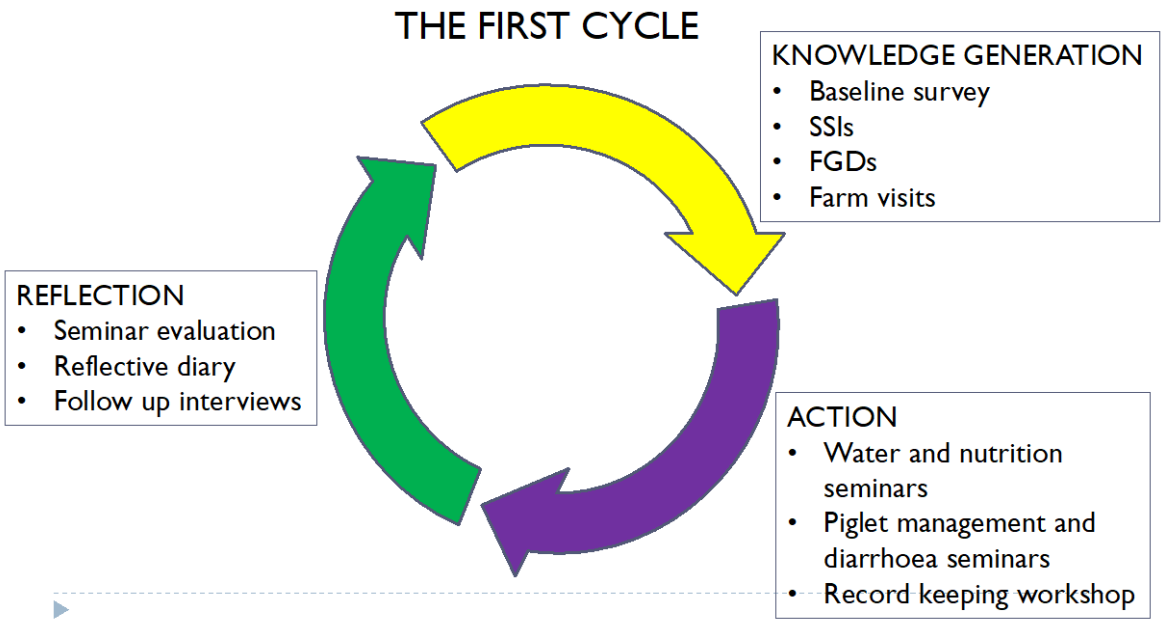

Figure 5.2.2. The first cycle of knowledge generation, action and reflection for Objectives 2 and 3. SSIs = semi-structured interviews, FGD = focussed discussion groups.

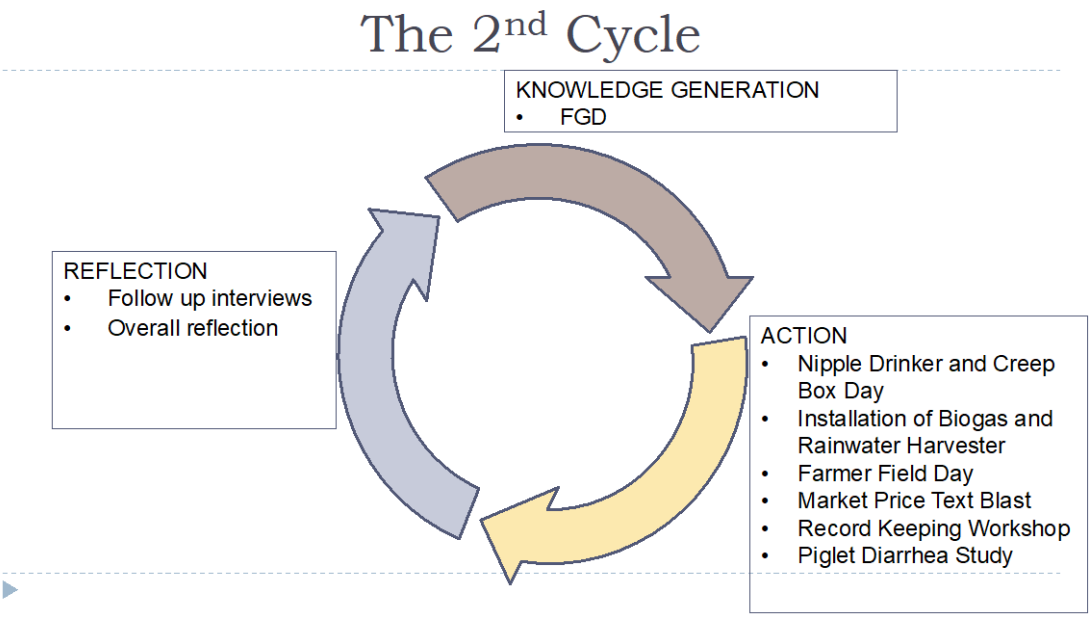

Figure 5.2.3. The second cycle of knowledge generation, action and reflection for Objectives 2 and 3. FGD = focussed discussion groups

5.3 Objective 3: To estimate the incidence and economic impact of two disease syndromes of importance to smallholder farmers

5.3.1 Identify two disease syndromes of highest priority to smallholder farmers

The base line survey, semi-structured interviews, focused discussion groups and farm visits (conducted under Objective 2) formed the basis on which disease syndromes of significance were able to be identified.

5.3.2 Implement any additional systems required for diagnosis of agents associated with identified disease syndromes

As detailed later in this Report, the only syndrome of significance identified by small-holder farmers was piglet diarrhoea. The required protocols for the detection and identification of the key bacterial pathogens associated with piglet diarrhoea were established at the RADDL III laboratory. As part of the support of the RADDL III laboratory, a set of agreed reports to explain the results obtained from typical porcine faecal samples (both normal samples as well as those associated with pathogenic bacteria *(Escherichia coli* and *Salmonella*) were developed (see Appendix 11.3.2 - Agreed Reports for Diarrhoea Study).

Other additional methods that were available and used for the purpose of the piglet diarrhoea study were as follows:

i) Parasitological examination (at RADDL III laboratory) for the presence and level of ova of internal parasites and protozoan oocysts;

ii) Real-time polymerase chain reaction assays (at ADDRL) for Porcine Epidemic Diarrhea virus (PEDV) and Transmissible Gastroenteritis virus (TGEV);

iii) Lateral Immunochromatographic assay based on a commercial kit (DipFit™) (at ADDRL) to detect Rotavirus in faeces;

iv) Loop-mediated isothermal amplification (LAMP) assays for Central Luzon State University (CLSU) for PEDV and *Lawsonia intracellularis*

5.3.3 Develop Decision Support System

A decision support system (DSS) intended for use by the Philippines research team was originally planned but was not developed during the course of the project. It was planned to be used to collect, store, and manage data that can be used for a number of purposes including descriptive and analytical epidemiological analyses, the design of disease surveillance strategies and development of risk analyses.

5.3.4 Cycle through knowledge generation, action and reflection assessing impact of disease syndromes on an ongoing basis

The first cycle of knowledge generation, action and reflection of this Objective was performed concurrently for Objectives 2 and 3. This involved the full ACIAR research team as well as stakeholders such as small-holder pig farmers, ag-vet store representatives, local government representatives and provincial government representatives.

As part of the second cycle, a piglet diarrhea study was conducted to explore the disease syndrome identified. Full details of the study design and the laboratory protocols are provided in Appendix 11.3.4 (Methodology of Piglet Diarrhoea Study). Brief details are provided below.

Objectives

1. Demonstrate ability of the RADDL III laboratory to detect the presence of range of potential pathogens in litters that have not experienced diarrhea in the two weeks prior to sampling and in litters with diarrhea at the time of sampling in the defined study population (litters prior to weaning in San Simon).
2. Establish the prevalence of potential pathogens in normal litters and in litters with diarrhea in the defined study population (litters prior to weaning in San Simon).

Sampling methodology

A cross-sectional study was conducted to estimate the prevalence of a range of potential pathogens in each of two target populations in San Simon: (i) pre-weaning litters where diarrhea has not been observed in the two weeks prior to sampling (healthy) and (ii) pre-weaning litters where at least one piglet has

diarrhea at the time of sampling (diarrheic). A sample size of 100 in each population was originally proposed, but subsequently 75 was deemed feasible. This sample size will allow estimation of a true prevalence of 50% with 11-12% precision and a true prevalence of 10% with 7% precision both with 95% confidence. The detailed inclusion criteria are provided in Appendix 11.3.4 (Methodology of Piglet Diarrhoea Study). Faecal material was collected from two piglets (bacteria, viruses and parasites), pen floor (for parasitology if needed to get the required sample volume of 3 g) and sows (parasitology and LAMP assays).

Laboratory methods

Full details of the methods for the detection of the agreed pathogens - *Escherichia coli* (haemolytic and non-haemolytic forms), *Salmonella, Lawsonia intracellularis,* PEDV, TGEV, rotavirus, internal parasites and protozoan oocysts are provided in Appendix 11.3.4 (Methodology of Piglet Diarrhoea Study).

5.3.5 Estimate incidence and economic impact of one disease syndrome and agents

To gain an understanding of the economic impact of piglet diarrhoea and the associated agents, an economic analysis of smallholder pig raising was performed. The overall economic analysis was achieved via the methodology shown in Figure 5.3.1.

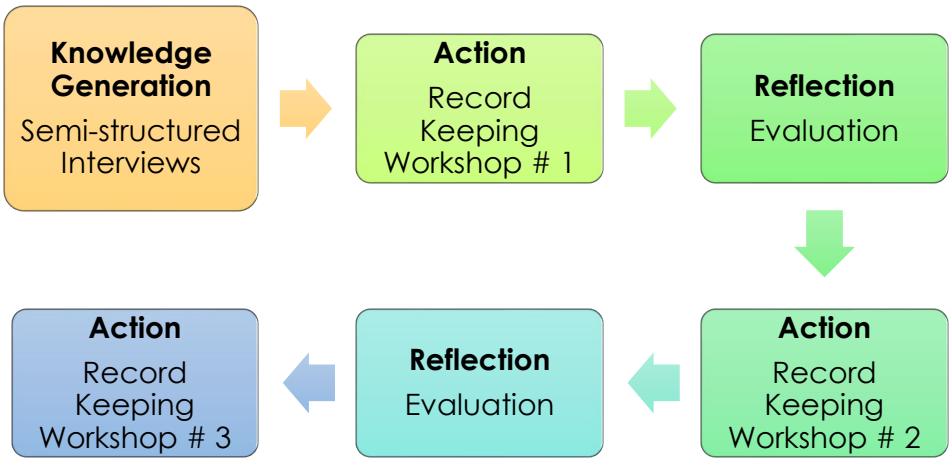

Figure 5.3.1. Methodology used to gather the data for an economic analysis of small-holder pig farming in San Simon.

The semi-structured interviews conducted involved 42 swine raisers, 6 traders, 4 agents, 2 wet market sellers, 3 agrivet store owners and the CEO of the Minalin farmer co-operative. The questions for the swine raisers focused on general questions and then some economic questions. The details of the questions are provided in Appendix 11.3.5 (Questions used in Semi-Structured Interviews with Small-holder Farmers). Similar questions, but modified to suit the interview group, were used with other stake-holders.

A total of three record-keeping workshops were held – with all three workshops having a strong emphasis on a participatory approach. The workshops focussed on the development – with strong farmer input – of a suitable record keeping notebook that would capture both production records as well as economic records. Details of the types of records that were the focus of these workshops are

presented in Appendix 11.3.5 (Areas of Focus for the Development of the Record Keeping Notebook).

The analysis of the incidence and impact of piglet diarrhoea was based on a number of project activities. Firstly, the baseline survey, undertaken as part of Objective 2, provided data on disease occurrence in sows, fatteners and piglets. The farm visits (n = 214) provided further data on diarrhoeal disease incidence. The semi-structured interviews (mentioned above) provided some economic data on the costs of production and the price of sold stock. A Progeny History method (Mariner and Paskin, 2000) was used in these semi-structured interviews. Progeny history is typically used with small-holder farmers who have an ability to recall the fate of animals across litters or batches, even when there are no or few formal records (Mariner and Paskin, 2000).

The lack of formal records prompted the research team – with input from farmers – to develop a Record Keeping Notebook. This notebook was developed and promoted via a series of workshops and then piloted with seven farmers. Data from this activity was also used to estimate the incidence of piglet diarrhoea.

5.4 Objective 4: To develop new diagnostic tests for, and undertake applied epidemiological research on, pig diseases important to Australia

This Objective was undertaken in Australia and consisted of four Activities. Details of the key methodologies are provided under each of the Activities below.

Explanatory Notes

At the time this research project was developed, the accepted name of the causative agent of Glässer's disease in pigs was *Haemophilus parasuis.* However, in recent times, the causative agent has been allocated to a new genus, *Glaesserella*, as a sole species - *Glaesserella parasuis* (Dickerman et., 2020). While this re-naming proposal is only very recent, the scientific community has broadly accepted the soundness of the concept. Hence, while the name *Haemophilus parasuis* has been used in the project documentation and will be retained for Objectives and similar headings, the scientific text areas of this Report will adopt the new terminology of *Glaesserella parasuis*.

5.4.1. Identify novel *Actinobacillus*-like bacteria and understanding of their potential role in disease

For the current study, 21 isolates originating from the lungs of clinically sick pigs or from lung lesions at slaughter were used. All the isolates had given negative reactions in two species-specific PCR reaction for *Glaesserella parasuis* (Angen et al., 2007; Olivera et al., 2001). Genomic DNA was extracted from 21 of the isolates (see Appendix 11.4.1 for full details). A conventional PCR targeting the 16S rRNA gene was performed as previously described (Turni and Blackall, 2011).

The three housekeeping genes (*recN, rpoA* and *thdF*) were amplified from the same 21 isolates according to the method of Kuhnert and Korczak (2006) with slight variations (see Appendix 11.4.1 for full details). The PCR products were sequenced with the same primers. Sequencing was done by Macrogen (Seoul, South Korea) or the Australian Genome Research Facility (Brisbane, Australia).

The sequences were aligned with the program Sequencher 4.9 (Gene Codes Inc., Michigan, USA). The sequences of the *rpoA*, *thdF* and *recN* genes of the relevant recognized species within the *Actinobacillus* genus, as well as *Glaesserella parasuis* and *Pasteurella multocida,* were acquired from the data bank of the National Center for Biotechnology Information (NCBI) for phylogenetic comparison and were the strains used by Kuhnert and Korczak (2006). Full details of the phylogenetic analysis are provided in Appendix 11.4.1. The 21 isolates were further analysed by phenotypic characterization assays (see Appendix 11.4.1).

Four isolates of the new species, (isolates HS 4420, HS 4509, HS 4607 and HS 4635) were subjected to whole genome sequencing by synthesis (Illumina sequencing). As well, HS 4635 was subjected to single molecule, real-time sequencing (PacBio).

5.4.2. Rep-PCR typing of *Pasteurella multocida* established, validated and used

Isolates

This study utilised 43 isolates from pigs (see Appendix 11.4.2, Table 11.4.2.1) and 41 isolates from poultry (see Appendix 11.4.2, Table 11.4.2.2) collected as part of the Department of Agriculture and Fisheries/Queensland Alliance for Agriculture and Food Innovation reference diagnostic services.

Identification and serotyping of P. multocida

The *P. multocida* isolates were identified by a species-specific PCR (Townsend et al, 1998) and then serotyped with the Heddleston serotyping scheme as described previously (Heddleston et al, 1972).
The LPS multiplex PCR, which targets the LPS outer core biosynthesis locus, was performed as previously described (Harper et al, 2016b) and assigned isolates to one of the eight LPS types (termed L1–L8) (Harper et al, 2016b).

Genotyping by rep-PCR

A rep-PCR assay was adopted from a previous study (Gunawardana et al, 2000) and commercial software was used to analyse the patterns. If two isolates had the same genomic fingerprint (i.e. an identical band pattern including size and intensity), they were assumed to be the same strain. Within each rep-PCR genotype found in the pig isolates, a representative isolate was subjected to MLST.

Genotyping by MLST

MLST genotyping was based on the RIRDC MLST scheme of sequencing 466–602 base-pair internal fragments of seven housekeeping genes and was performed on each representative isolate as previously described (Subaaharan et al, 2010). Each different sequence is assigned a distinct allele (a number) and the combinations of the alleles define the sequence type (ST). Isolates of STs that were shared across both pigs and chickens were re-examined by rep-PCR.

Analysis of the RIRDC database for P. multocida

The RIRDC MLST database (http://pubmlst.org/) was searched for isolates that had the same ST as the pig isolates and compared. The website is based at the University of Oxford and is funded by the Wellcome Trust.

5.4.3 Validate the use of MALDI-TOF to serotype *P. multocida*

***Reference strains of* P. multocida**

This study utilised the 16 serovar reference strains (see Appendix 11.4.3, Table 11.4.3.1).

LPS extraction

Several methods for extraction of LPS were evaluated. Full details of the methods that were evaluated are provided in Appendix 11.4.3 (Methods for Extraction of LPS).

Preparation for MALDI TOF-MS

A range of preparation procedures were applied (see Appendix 11.4.3 – Preparation for MALDI-TOF MS). These matrices were applied in a number of different ways, including co-spotting (mixing sample plus matrix solution) and spotting sample onto the dried matrix, the latter was typically better for these samples in terms of getting a response. We also tried thin layer preparations, but with less success as the thin layer was generally ruined during sample application. We investigated metal chelating agents, which were variable in their effect.

Final Method including MALDI-TOF conditions

A saturated solution of 0.5 M 2,5 dihydroxybenzoic acid (DHB) in acetone was diluted 1 in 10 with a solution of acetone:acetonitrile:water (6:3:1), and 1 µl of this dilution was spotted onto a polished steel target. After drying, 0.5 µl of sample was spotted over the top of the DHB crystals. Once the samples were dry, the samples were analysed in linear positive mode MALDI TOF using a Bruker Ultraflex III over a mass range of 700 – 5000 collecting 2,000 shots.

5.4.4 Evaluate the use of bead-based PCR to serotype *Haemophilus parasuis*

Bacteria

This study involved all 15 recognised serovar reference strains of *G. parasuis* (NR4, SW140, SW114, SW124, Negasaki, 131, 174, C5, D74, H367, H465, H425, IA-84-17 975, IA-84-22 113, IA-84-15 995) and 74 field isolates, taken from internal organs and nasal cavity of Australian pigs.

The 74 field isolates used in this study were recovered from the nasal cavity and internal organs and included all serovars recognised as being present in Australia in the last 10 years (Turni et al, 2018). These Australian field isolates came from farms in New South Wales, Queensland, South Australia, Tasmania, Victoria and Western Australia. All isolates had been previously confirmed as *H. parasuis* by the PCR and were serotyped by a conventional antisera based method as well as a molecular method.

DNA preparation

The DNA templates for the PCR amplifications were prepared by resuspending a 1 µL loopful of bacterial culture in 100 µL of sterile H_2O in a PCR tube. This

suspension was heated at 98ºC for 5 min after which it was held on ice for 5 min. The cell suspension was then centrifuged for five min at 13,000 X g. The resultant supernatant was stored at -20ºC until used in the PCR.

Luminex Micro-bead PCR

An alternative assay to the multiplex PCR assay, the Luminex microbead-based suspension array, was used in this work. These assays are rapid, high-throughput methods used for the detection of multiple analytes in solution and have been used for the identification and serotyping of bacterial species (Christopher-Hennings et al, 2013; Lin et al, 2013). The concept of the Luminex assay is a specific PCR amplifying a product of up to 300 base pairs with a reverse primer that is biotinylated on its 5' end and a forward primer that has a TAG sequence attached that is separated from the sequence by an internal spacer. A full explanation and an illustrative figure are provided in Appendix 11.4.3 (Luminex Micro-bead PCR and Figure 11.4.4.1).

PCR

The sequences of all the genes targeted in the multiple serotyping PCR by Howell et al (2015) were downloaded from the NCIB database (https://blast.ncbi.nlm.nih.gov). With a couple exceptions, the forward primer from Howell et al (2015) were used. New reverse primers were designed as the total length of the amplified product used in the Luminex based assay is recommended to be under 300 bp. The PCR was first optimised without the TAG sequence and once all bands were amplified the TAG sequence was added to the forward primer. Full details are provided in Appendix 11.4.4 (PCR).

Bio-Plex® 200 Systems

Full details of the bead detection system used in this study are provided in Appendix 11.4.4 (Bio-Plex® 200 Systems).

Analysis

The following equation was used to analyse the results:

$$\frac{Value\ for\ Isolate - \ Value\ for\ Negative}{Value\ for\ Signal\ Control - Value\ for\ Negative} = Result\ for\ each\ target$$

5.4.5 Improve the understanding of the role of porcine circovirus-2 (PCV2) in pig disease in Australia

A published qPCR method for detection of PCV2 DNA was established in the laboratory, and a plasmid containing the whole PCV2 genome was used to generate standard curves to allow for quantification of PCV2 virus load in serum samples.

To investigate the field epidemiology of PCV2, a longitudinal study was conducted on 24 pigs (part of the UQ (Gatton campus) pig herd). The 24 pigs examined at 4, 6, 10, 15 and 19 weeks of age for clinical status and weight, with blood samples collected for quantification of PCV2 virus load by qPCR and antibody levels by a commercial ELISA. As well, a PCV2 vaccination trial was conducted to explore the impact of PCV2 on viral parameters and weight gain. The clinical status and body weight of pigs was compared between 50 vaccinated and 50 non-vaccinated

pigs at 4, 9, 14 and 19 weeks of age, and the PCV2 viral load and antibody levels were determined in a subset of pigs from each group at each sampling time. At slaughter, lung scoring was performed and lymph nodes, serum and saliva were collected for viral load analysis.

A phylogenetic study was conducted to determine the PCV2 genotypes circulating in Australia. PCV2-positive samples (20 lymph nodes, 2 serum samples) from 22 pigs from 15 Queensland farms were subjected to conventional PCR and sequencing of the full PCV2 genome. Phylogenetic analysis of 17 of these sequences in relation to published PCV2 sequences was then performed and the genotypes were compared. Work was conducted to develop a rapid pen-side assay for detection of PCV2 DNA in oral fluid samples. A novel cellulose dipstick method was compared with a commercial kit for DNA extraction and two rapid DNA amplification methods (LAMP and mini-PCR) were assessed against qPCR for their ability to amplify PCV2 DNA.

5.5 Objective 5. Assessment of adoption and impact

5.5.1 Desktop assessment

A desk-top exercise approach was used to record the views of the research team on the impacts of both the current project (ACIAR-AH-2009-022) and the previous project (ACIAR-AH-2012-066).

5.5.2 Quantitative and Qualitative assessment stage

The objectives of this component of the project were as follows:

A) Determine the impact to date of the laboratory and field technologies introduced in both ACIAR AH-2009-022 and AH-2012-066.
B) Determine the change of practices of farmers and other stakeholders in relation to the innovations introduced by the project
C) Seek to assess the degree of support given to the innovations
D) Seek to evaluate any increased income flowing from the adoption of the innovations.

Laboratory and Field Innovations

The impact of the Philippines component of the project was evaluated by semi-structured interviews with key actors from the Phase 1 research team covering PCAARD, PVO Bulacan and Pampanga, ADDRL, RADDL III and CLSU.

The impact of the laboratory innovations driven in the Australian component of the projects was progressed by seeking independent expert overviews. Further analysis of the impact of the scientific impacts was undertaken by an evaluation of the metrics of the formal scientific publications arising from the projects.

Farmer survey

The adoption study focussed on six management practices recommended to farmers by the project: 1) water management through the nipple drinker; 2) piglet

management through the creep box; 3) record keeping; 4) market price information provision; 5) rainwater harvesting; 6) biogas production.

A key part of this stage of the project was utilization of the smallholder farmer database. This database was established at the early stages of ACIAR AH-2012-066 and was increased throughout this project. The farmers were allocated into three groups:

A - very involved (record keepers, won a creep box, have biogas generator and/or rainwater harvester;
B – involved – not in Group A but participated in at least three project activities
C – less involved –not in above groups but participated in at least 1 or 2 two project activities.

In addition to research team, five additional staff were employed to be field enumerators. After pilot testing and enumerator testing, the survey was undertaken from April 12 to May 6, 2019. Data was captured using tablets and Commcare software (Dimagi, Cambridge, MA, USA). Data analysis was undertaken via Stata 15 (Stata Corp, College Station, TX, USA) and Excel (Microsoft, Redmond, WA, USA). For the economic analysis aspects of this impact assessment, a partial budgeting that assessed the key innovations of this project was performed. As well, a cost-benefit analysis of a biogas digestor system was undertaken.

6 Achievements against activities and outputs/milestones

Objective 1: To improve the diagnostic systems for respiratory disease in two selected Regions of the Philippines

no.	Activity	outputs/ milestones	completion date	Comments
1.1	Identify two regions where improved diagnosis of respiratory disease will be of most benefit	Review of respiratory disease diagnostic capabilities throughout the Philippines (PC)	July 2015	Two regions (Region IVA and V) were identified after a series of scoping visits to six regions by Philippines project staff.
1.2	Enhance regional laboratory capacity	Review current methods in bacteriology, virology and pathology and their use in respiratory disease diagnosis in regional laboratories (PC)	January 2016	A review of the current protocols in use at the regional laboratories in Region IVA and V was completed by Philippines project staff.
		Train laboratory staff in application of the diagnostic test standards developed in Region 3 (PC)	July 2016	A five-day training workshop for the laboratory staff working in Regions IVA and V was undertaken (Sept. 28 to Oct. 2, 2015). It was run by Philippines project staff and was held in Region III. Laboratory staff were also trained on disease investigation and lung scoring to gain knowledge and appreciation of the activity of the field staff.
		SOPs and quality control materials provided to Regions (A, PC)	July 2016	SOPs for the isolation and identification of key respiratory bacterial pathogens were developed and provided to the regional laboratories. As well, reference bacteria, as quality control strains, were provided. The regional laboratories were also provided with culture media and reagents for biochemical testing.
		Validated methods are in operation (PC)	July 2016	Laboratory protocols well established and staff trained in those protocols. This was confirmed by on site visits of the Philippines research team from Region III to both Region IVA and V (to confirm effective implementation of the various trainings and SOPS). The protocols are now in routine use when required as well as in the project activities described in other areas of this report.
1.3	Optimise field investigation	Review of current approaches (PC)	January 2016	A review of the current approaches in use at the regional and provincial offices in Region IVA and V was completed by Philippines project staff.

		Train veterinarians in application of the standards developed in Region 3 (disease investigation, biosecurity, sampling) (A, PC)	July 2016	Training for the veterinary staff working in Regions IVA and V was completed. The workshops were run by Philippines project staff and were held in Regions III, IVA and V. Field staff were also trained on basic laboratory techniques to gain knowledge and appreciation of the activity of the laboratory staff.
		Revisions made to reference manual on farm biosecurity and disease control developed in AH/2009/022 to reflect regional issues (if required)	July 2016	There was an error in preparation of the original project document; a biosecurity manual was not developed in the previous project. However, the two major relevant manuals produced in AH/2009/022 were reviewed and there was no identified need for any revisions. It is worth noting that as part of other project activities, biosecurity leaflets, in Tagalog, were produced and distributed to smallholder farmers in San Simon.
		Disease investigations completed for representative set of farms in selected Regions (PC)	June 2017	This work was undertaken and is fully described under Objective 1.4
1.4	Introduce a lung scoring program	Train veterinarians in lung scoring (A, PC)	June 2016	Lung scoring training workshops for regional field staff were held in November 2015 and February 2016. The project team monitored the lung scoring activity of the field staff at Lipa City Slaughterhouse in June 2016.
		Conduct lung scoring for selected farms or at selected slaughterhouses (PC)	June 2017	A lung scoring study was conducted in five slaughterhouses in Region IVA between October and December 2016. A similar study involving four slaughterhouses in Region V was completed in April 2017.

PC = partner country, A = Australia

Objective 2: To identify, document and address constraints to the use of good disease prevention practices by smallholder pig farmers in Region 3

no.	Activity	outputs/ milestones	completion date	comments
2.1	Conduct training workshop in ecohealth and participatory epidemiology for field staff	Train field staff in ecohealth and participatory epidemiology (A, PC)	July 2015	A three-day workshop on participatory epidemiology and ecohealth research was held September 2015 with participation from the relevant Philippines and Australian team members.

2.2	Identify constraints through semi-structured interviews and group discussions	Identify participating stakeholders (A, PC)	October 2015	A baseline survey of 14 barangays in the selected region (San Simon) involved 1,082 respondents (629 active farms and 453 inactive farmers). A summary of the findings were presented to the Mayor. Reports were distributed to each barangay covering overall and barangay-specific results. The clear and strong support of the Local Government Unit (San Simon) and the Provincial Veterinary Office (Pampanga) have encouraged the participation of smallholder farmers.
		Constraints identified (A, PC)	April 2016	A series of semi-structured interviews (42 in total) and focus group discussions (8 in total) and follow-up farm visits (214 in total) have been conducted with farmers within San Simon. Additional semi-structured interviews and focus group discussion have been conducted with other relevant stakeholders. These have identified a number of constraints particularly in health and production management aspects of smallholder pig raising in San Simon, Pampanga.
2.3	Cycle through knowledge generation, action and reflection assessing progress in overcoming constraints on an ongoing basis	First cycle of knowledge generation, action and reflection completed (A, PC)	December 2017	Eight clusters of seminars on A) water and nutrition; B) piglet management and diarrhoea were conducted, followed by completion of farmer evaluation and a reflective diary, recorded by the trainers. Seminar presented to the 4Ps group (Pantawid Pamilyang Pilipino Program) Three workshops on record keeping were completed and farmer evaluation completed. Three sets of interviews were conducted with farmers to evaluate the technical aspects of improvised creep box, farmers' perception and the associated cost. Farmer engagement was encouraged through Barangay coordinators Overall perceptions of cycle one activities were gathered. Gaps and possible activities for cycle two were identified.

		At least one more cycle of knowledge generation, action and reflection completed. Constraints are overcome/reduced (PC)	June 2019	A community-focused nipple drinker and creep box day was held. Biogas units were installed on four demonstration farms and rainwater harvesters on two of those farms with farmer field days held to demonstrate the units and harvestors. A market price text blast was introduced. Two further record keeping workshops were held. A training needs analysis and follow on training for Agriculture Extension Workers (AEWs) were both undertaken. Extension materials such as the laeaflets were distributed to farmers. Videos were shown to farmers and Barangay coordinators for their feedback. Electronic copies of leaflets were provided to stakeholders. An end-of-project stakeholder workshop was held to facilitate reflection on the project as a whole and how momentum can be maintained going forwards.

PC = partner country, A = Australia

Objective 3: To estimate the incidence and economic impact of two disease syndromes of importance to smallholder farmers

no.	Activity	outputs/ milestones	completion date	Comments
3.1	Identify two disease syndromes of highest priority to smallholder farmers	Select participating smallholders (PC)	October 2015	The baseline survey identified a number of potential participating smallholder farmers. The clear and strong support of the Local Government Unit (San Simon) and the Provincial Veterinary Office (Pampanga) have encouraged the participation of smallholder farmers.
		Hold discussion groups (smallholders/stakeholders) to select two diseases for Activity 3.2 (PC)	April 2016	Through the baseline survey, semi-structured interviews and farm visits, the only disease syndrome that was of major concern was piglet diarrhoea. Hence, piglet diarrhoea was the selected disease syndrome for the remainder of this objective.
3.2	Implement any additional systems required for diagnosis of agents associated with identified disease syndromes	Review of diagnostic capabilities of regional and national laboratories (PC)	July 2017	A review of diagnostic capacities at both the regional and national level was completed in June 2016. This review identified the need for improved diagnosis of PED and *E. coli*-associated diseases. As a result the Australian partners provided protocols, training materials and reagents for the isolation and identification of potentially pathogenic *E. coli* at RADDL III. PED diagnosis will be performed at both the ADDRL (by PCR) and CLSU (LAMP)

		Establish methods for diagnosis of agents associated with identified disease syndromes as required (A, PC)	July 2017	The methods for diagnosis of piglet diarrhoea were established in the Philippine partner laboratories. A survey of both healthy piglets and piglets with diarrhoea was undertaken using those newly established protocols as part of Objective 3.4
3.3	Develop Decision Support System (A, PC)	Determine what functionality will be useful and usable (A, PC)	Not completed	There was limited progress under this Objective. Work was undertaken in Australia to develop a platform Decision Support System and a file server to run the system was purchased. Staff priorities with the Australian team limited the capacity of progress in this area. Interest in the concept remains strong in the Philippine partners.
		Design DSS (A, PC)		
		Integrate system with Phil-AHIS and use (A, PC)		
		Revise and fine tune system (A, PC)		
3.4	Cycle through knowledge generation, action and reflection assessing impact of disease syndromes on an ongoing basis (A, PC)	First cycle of knowledge generation, action and reflection completed (PC)	December 2017	The Objective 2 and Objective 3 cycles occurred concurrently. Hence, the comments provided for Activity 2.3 also directly apply to Activity 3.4 for cycle 1.
		At least one more cycle of knowledge generation, action and reflection completed (A, PC)	June 2019	A study on the prevalence of viral, bacterial and parasitic agents associated with the identified disease syndrome (diarrhoea in piglets) was undertaken.
3.5	Estimate incidence and economic impact of one disease syndrome and agents (A, PC)	Estimation of incidence and economic impact of syndrome and associated infectious agents completed	June 2019	The incidence of piglet diarrhoea (the identified disease syndrome under Objective 3.1) has been estimated from a number of sources – the initial baseline survey, farm visits, semi-structured interviews and finally via formal project developed record keeping notebooks. An analysis of the economics of smallholder pig farming was also undertaken.

PC = partner country, A = Australia

Objective 4: To develop new diagnostic tests for, and undertake applied epidemiological research on, pig diseases important to Australia

no.	Activity	outputs/ milestones	completion date	Comments

4.1	Identify novel *Actinobacillus*-like bacteria and understanding of their potential role in disease (A)	Identification and classification of novel bacteria completed	December 2015	The completed phenotypic and genotypic work confirmed that the isolates represented a novel species that has been proposed as *Glaesserella australis* (the formal naming has to await the formal re-naming of *Haemophilus parasuis* as *Glaesserella parasuis*). The field data linked to the confirmed *G. australis* isolates indicates that the organism is playing a role in porcine respiratory disease. Routine identification of *G. australis* is now provided to the Australian pig industry and veterinary diagnostic laboratories via the user pays reference services provided by the UQ.
4.2	Rep-PCR typing of *P. multocida* established, validated and used (A)	Rep-PCR scheme for sub-typing *P. multocida*	December 2016	Rep-PCR was validated for the genetic finger-printing of *Pasteurella multocida*. Assay found to be as informative as multi-locus sequence typing (MLST) – a sequence-based scheme that is widely accepted as a "gold standard" method. Rep-PCR is both a lower cost and a more rapid assay than MLST so the validation of the assay is an important step forward. The Rep-PCR assay is now provided to the Australian pig industry and veterinary diagnostic laboratories via the user pays reference services provided by the UQ.
4.3	Validate the use of MALDI-TOF to serotype *P. multocida* (A)	MALDI-TOF replacement for conventional serotyping established and validated	December 2017	Despite extensive work, a MALDI-TOF approach to serotype *P. multocida* could not be validated. While differences in the MALDI-TOF profiles were detected, the differences were too minor to form the basis of robust scheme to recognise the serovars of *P. multocida*.
4.4	Evaluate the use of bead-based PCR to serotype *H. parasuis* (A)	Bead-based PCR replacement for conventional serotyping established and validate	December 2018	A novel bead-based PCR assay to serotype *Glaesserella parasuis* was developed, validated with the reference strains and then applied to Australian field isolates. The evaluation showed that the assay functioned well for most serovars but that Australian isolates of serovars 4 and 14 could not be serotyped by the bead-based system. The bead-based assay is suitable for use but requires the additional use of a conventional PCR for serovars 4 and 14.

4.5	Improve the understanding of the role of PCV-2 in pig disease in Australia	Conduct field studies on PCV-2 infected pig farms including farms with and without PCV-2 associated disease	June 2019	A real-time PCR (qPCR) for PCV2 diagnosis were established. A longitudinal study of PCV2 infection showed a correlation between PCV2 antibody levels and a decline in average daily weight gain (ADG), both measures likely reflecting an effect of circulating virus in the preceding 2-3 week period. A vaccination trial demonstrated the effectiveness of PCV2 vaccine in eliminating virus load in serum. However PCV2 DNA was detectable in lymph nodes of vaccinated pigs at slaughter and there was no significant difference in the ADG of PCV2 vaccinated and unvaccinated pigs. Genotyping of PCV2 sequences revealed presence of PCV2b, PCV2d and PCV2f genotypes and an “intermediate” genotype. PCV2d and PCV2f have not been previously reported in Australia. For the pen-side diagnostic test, the novel cellulose dipstick method proved to be capable of effectively extracting DNA from oral fluids.

PC = partner country, A = Australia

Objective 5: Assessment of adoption and emerging impact

no.	**Activity**	**outputs/ milestones**	**completion date**	**Comments**
5.1	Desk-top Assessment	Identification of the differences that projects AH-2009-022 and AH-2012-066 have made to date (A, PC).	30 June 2019	A matrix to demonstrate the outcomes of the desk-top assessment has been developed. The scientific and industry impacts of the scientific publications of the two projects was evaluated by a review of the metrics of those publications. As well, independent reviews of the science and industry impact of the projects were obtained.
5.2	Quantitative and Qualitative assessment stage	Identification of current users, the next users and the final users for all outputs from projects AH-2009-022 and AH-2012-066 (A, PC).	30 June 2019	Several surveys, sets of semi-structured interviews (SSIs) and focus group discussions (FGDs) were undertaken
				Phase 1: • SSIs with key actors completed • Survey of participating stakeholders completed

				Phase 2: • Adoption of outputs by Farmers through survey • Adoption of outputs by Farmers through FGDs • Influence on Market price dissemination by farmers through FGDs • Dissemination of outputs through Survey of agricultural extension workers (AEWs) • Dissemination of outputs through survey of barangay coordinators
5.3	Final Impact assessment	Clear detailed analysis of the adoption pathway, impacts to date and potential for future impact for projects AH-2009-022 and AH-2012-066 (A, PC).	30 June 2019	An analysis of the adoption pathway to date has been performed. The impacts of the two projects to date are detailed and the potential future impacts identified.

7 Key results and discussion

7.1 Objective 1: To improve the diagnostic systems for respiratory disease in two selected Regions of the Philippines

This Objective was undertaken in the Philippines and consisted of four Activities. Details of the key results and a focussed discussion are provided under each of the Activities below.

7.1.1 Identify two regions where improved diagnosis of respiratory disease will be of most benefit

Results
The completed matrix used for the evaluation of the potential new regions for project activity is summarised in Appendix 11.1.1 (Table 11.1.1). On the basis of the evaluation, Regions IVA and V were selected as being those regions were the project would be active.

Discussion
The evaluation of the various possible regions for involvement in this project was undertaken by the Philippines team. This Philippines focus reflects the growing maturing and capacity of the Philippines team as a result of the involvement in the previous ACIAR Project (AH-2009-022). A key issue identified by the evaluation process was the lack of submissions to the Government veterinary diagnostic systems in all the regions. It was hoped that the involvement of the selected regions would encourage both small-holder and commercial pig farmers to make greater use of the government laboratory and field services. However, the outbreak of African Swine Fever immediately at the end of the project prevented any evaluation of the greater use of government services.

7.1.2 Enhance regional laboratory capacity

Results
The review of the laboratory capacity, performed by the Philippines research team, revealed the need for both “hands on” training as well as the need for Standard Operating Protocols to ensure that optimal diagnostic services were delivered to the backyard farmers accessing the government veterinary diagnostic laboratories.

As a follow up to the review, a five-day training workshop for the laboratory staff working in Regions IVA and V was undertaken (Sept. 28 to Oct. 2, 2015). The workshop was run by Philippines project staff and was held in Region III. The laboratory staff who attended were also trained in disease investigation and lung scoring skills to gain knowledge and appreciation of the activity of the field staff.

Standard operating protocols (SOPs) for the isolation and identification of key respiratory bacterial pathogens were developed and provided to the regional laboratories. The list of the SOPS developed for media were as follows: BA/SN (used for culture for *Actinobacillus pleuropneumoniae* and *Glaesserella parasuis*); 5% sheep blood agar (used for general culture of all pathogens), buffered peptone water (used general enrichment of bacteria), cryostorage medium, Eosin

Methylene Blue agar (used indicating presence of *Escherichia coli*), MacConkey agar (used for screening coliforms and *Salmonella*), Mueller Hinton agar (used for antimicrobial sensitivity testing), oleic-albumin supplement (component of BA/SN medium), phosphate buffered saline (used for dilution and suspensions); Rappaport Vassiliadis broth (used for selective enrichment of *Salmonella*), transport media (used to preserve bacteria while being transported to laboratory), Triple Sugar Iron Agar (used for characterisation of *Salmonella*), Urease agar (used for characterisation of *Salmonella*) and Xylose-Lysine Decarboxylase (XLD) agar (selective medium for *Salmonella*).

An SOP was also developed for the isolation (and subsequent identification) of the key bacteria[7] typically isolated from porcine respiratory sites. The following SOPs were developed for biochemical tests for identification of relevant bacteria: catalase, indole, Gram reaction by 3% KOH reaction, motility, oxidase activity and urease activity. An SOP for the performance of antimicrobial sensitivity testing was also developed.

Production of a summary table (Appendix 11.1.2: Table 11.1.2.1) to provide a method for identifying the key bacteria associated with respiratory diseases of pigs allowed for routine identification of these key pathogens in diagnostic laboratories with limited resources. To assist the regional laboratories in their other areas of disease diagnosis, similar brief identification tables were developed for the key bacterial pathogens associated with both avian and cattle respiratory diseases (Appendix 11.1.2: Tables 11.1.2.2 and 11.1.2.3). Reference bacteria were also provided as quality control strains, whilst regional laboratories were provided with culture media and reagents for biochemical testing.

The follow-up on site visits by the Region III research team, one follow-up visit per Region, confirmed that the laboratory protocols and SOPS were being effectively implemented. In particular, the impact of the training was shown by the fact that the Region IVA staff were able to isolate and identify isolates of *Actinobacillus pleuropneumoniae*, a demanding organism that requires optimal laboratory media and skills for isolation and identification.

Discussion

The enhancement of the capacity of the Region IVA and V diagnostic laboratories proceeded via hands-on training, development and implementation of SOPs and then follow-up “audit” style visits to ensure that the methods and techniques were in effective use. This aspect of the project was an outstanding success with both regional laboratories have considerably enhanced diagnostic capacities. Two key issues drove this success.

The first issue was the ownership of the training program by the research team in Region III. Rather than a reliance upon the Australian team, the Philippines Region III research team used their learnings and experience from the earlier

[7] *Actinobacillus pleuropneumoniae, Bordetella bronchiseptica, Escherichia coli*, *Glaesserella parasuis, Klebsiella pneumoniae, Pasteurella multocida*, *Proteus* spp., *Pseudomonas aeruginosa, Salmonella* spp., α haemolytic *Streptococcus* spp. (possible *S. suis*); ß haemolytic *Streptococcus* spp.

ACIAR project (ACIAR/AH/2009/022) to take on the training (both hands on in wet laboratories as well as theory training in workshops) and “auditing” aspects of this activity. This “ownership” bodes well for the long term impact of both projects. In particular, the production of an extensive catalogue of SOPs ensures that the gains made in these two projects can be embedded into the Department of Agriculture laboratory system and then passed outwards to all other laboratories in the other regions of the country.

The second issue that drove the success of this aspect of the project was the enthusiastic involvement of the relevant staff at the two regional laboratories. The willingness to engage in the training programs, the acceptance of new SOPs and then the commitment to ensuring the adoption and implementation of the new techniques and methods were essential contributions to the success of the capacity enhancement of this aspect of the project.

It is important to note that the SOPs and approaches implemented in this activity were all deliberately targeted to be sustainable and low cost. The intention was not to create a system with an exceedingly high level of confidence about bacterial identifications. The system was focussed on an approach that provides a low cost, sustainable diagnostic work flow that yields rapid results that are highly likely to be accurate. The system does not use expensive commercial kits or molecular reagents. The Philippines diagnostic system has a central laboratory (ADDRL, Quezon City) that provides this high level, high cost back-up identification options. The focus of the current system is to allow regional laboratories to achieve effective, diagnostic activities that can be backed up as required with support from the central laboratory at ADDRL.

7.1.3 Optimise field investigation

Results

The review of the laboratory capacity, performed by the Philippines research team, revealed the need for both “hands on” training for the regional staff involved in respiratory disease investigations. Training was also needed for veterinarians, LGU staff and meat inspectors in the lung scoring protocol developed in ACIAR AH/2009/022.

As a follow up to the review, three training workshops for relevant staff in Regions IVA and V were undertaken in November 9-13 2015, February 9-12 2016 and February 15-16 2016. The workshops were run by Philippines project staff. The training covered disease investigation and lung scoring. A monitoring visit to “audit” the effectiveness of the staff training was undertaken on June 2-3, 2016.

As noted earlier, there was an error in preparation of the original project document; a biosecurity and disease control manual was not developed in the previous project. Hence, a review of this manual in light of learnings from the work in Regions IVA and V was not possible. However, the two major relevant manuals produced in AH/2009/022 were reviewed and no need for revision was identified. The two manuals are as follows:

Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development. (2013) Field sample collection: A quick reference for swine

respiratory disease investigation and diagnosis. Los Baños, Laguna: PCAARRD-DOST (Information Bulletin No. 38/2013)
Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development. (2014) Disease outbreak investigation: A guide for animal health workers. Los Baños, Laguna: PCAARRD-DOST (PCAARRD Farm Primer No. 03/2014)

It is worth noting that as part of other project activities described under Objectives 2 and 3, a series of farmer-directed leaflets were produced. A biosecurity leaflet was produced in Tagalog and was distributed to small-holder farmers in San Simon (See Appendix 11.1.3, Figure 11.1.3.1).

7.1.4 Introduce a lung scoring program

Results

Table 7.1.3. Overview of the key activity areas undertaken components of the studies undertaken in Regions IVA and V.

Component	Region IVA	Region V
Number of pig lungs examined by lung scoring	260	300
Number of lungs examined by bacterial culture	40	16
Number of farms subjected to disease investigation	8	8

Table 7.1.4. Farm type for the pigs sampled in the lung scoring study in Regions IVA and V.

Farm Type	Region IVA	Region V
Commercial	61%	24%
Smallholder	38%	74%
Unknown	1%	1%

Table 7.1.5. Key results from the lung scoring study in Regions IVA and V.

Parameter	Region IVA	Region V
Lung Score		
Median	7	0
Interquartile range	2-19	0-2
Lungs with score $\geq$ 7	52%	14%
Lungs with pleurisy score $\geq$1	56.9%	5%
Lungs with pericarditis	24.6%	1.7%
Lungs with pleuropneumonia lesions	8%	0%

The results of the culture work performed in this study for Region IVA are presented in Table 7.1.6. In region V, there were very few lungs with lesions. Hence as a result only a small number of lungs were examined by culture (four lungs in Daraga, Ligo City and Tabaco City and seven lungs in Polangui). The only bacterial isolate of any significance was one lung that yielded *P. multocida*.

The latent class analysis recognised four classes of lungs that we have termed – healthy, mild respiratory disease, moderate pneumonia and multi-lesion. The four classes and the various indicators are detailed in Table 7.1.7. The allocation of the various classes with the province and farm type is shown in Table 7.1.8.

Discussion

This activity was undertaken to demonstrate the ability of the staff in the two selected Regions to apply the various techniques that were established in the earlier project (ACIAR/AH 2009/022) and then transferred to the various relevant staff in the current project. The results of the work provide clear evidence that the regional staff have developed high level skills in the various areas (lung scoring, bacterial isolation and identification, follow-up disease investigation). As an example, in Region IVA, the lung scoring identified a small number of lungs with typical lesions associated with porcine pleuropneumonia. When a subset of these lesions were examined, *Actinobacillus pleuropneumoniae* was isolated. This correlation of bacterial culture and lesion scoring provides clear evidence of the abilities and skills of the field and laboratory staff. As a further comment, these isolates of *A. pleuropneumoniae* are the first isolates of this species obtained in either this project or the prior project (ACIAR-AH-2009-022).

The lung scoring results, supported by the latent class analysis, showed a marked difference between the two regions – Region IVA had a level of lung lesions similar to that seen in the previous project (ACIAR-AH-2009-022) while Region V had very few lungs with any evidence of lung lesions. Region IVA also resembled the prior results from the prior project in that commercial farms had a greater number of lungs with lesions than the lungs of pigs from small-holder farms.

A possible explanation for the difference in lung lesions between the two regions may be the difference in swine density. As an example, in Region IVA, the province of Batangas has a swine density of 234 pigs/km^2. In contrast, in two provinces in Region V, the swine density is 79 pigs/km^2 (Albay) and 55 pigs/km^2 (Camarines Sur). The higher density of pigs in Region IVA would provide more available hosts and result in higher concentrations of challenge organisms. A further possible explanation is that the commercial farms in Region IVA may be larger in size or have a higher stocking density than the commercial farms in Region V. Unfortunately, it was not possible to obtain data on farm size or stocking density.

In terms of the different classes, it is clear that the groups have arisen because of exposure to different pathogens, possibly in terms of both diversity and concentration of those pathogens. A possible set of circumstances is set out below:

- healthy pigs – low pig density, few pathogens
- mild respiratory disease - low pig density but a mix of pathogens

- moderate pneumonia – dominance of infection with *Mycoplasma hyopneumoniae*
- multi-lesion - high pig density and a mix of pathogens

More detailed studies would be required to determine the explanation for the differences between the provinces, the differences between farm types and the explanation for the different classes detected in this study.

Table 7.1.6. Overview of the key activity areas undertaken components of the studies undertaken in Regions IVA

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Abattoir	Samples		Bacteria identified			
			Actinobacillus pleuropneumoniae	*Bordetella bronchiseptica*	*Pasteurella multocida*	*Streptococcus suis*
Batangas	11	0	1	3	2	
Bauan	5	0	0	2	0	
Lipa City	9	2	0	3	2	
Nas	7	0	0	1	0	
Tanauan City	8	0	0	2	2	
Total	40	2	1	11	6	

Table 7.1.7. Item response percentages in the latent class analysis.

Indicators	Classes			
	Healthy	Mild Respiratory disease	Moderate pneumonia	Multi-lesions
Lung Score				
0	77.2	28.4	6.7	2.0
1-6	21.8	53.4	38.9	21.8
7-20	0.9	15.3	34.4	36.3
21-55	0.0	2.9	20.1	39.9
Pleurisy Score				
0	99.7	76.3	93.4	3.2
1	0.3	23.0	6.5	36
2	0.0	0.8	0.1	45.3
3	0.0	0.0	0.0	15.5
Pericarditis				
No	100	96.4	92.2	56.8
Yes	0.0	3.6	7.8	43.2

Table 7.1.8. Class prevalence across Region and Farm type.

Region	Farm Type	Classes			
		Healthy	Mild Respiratory disease	Moderate pneumonia	Multi-lesions
IVA	Commercial	1.1	1.5	34.1	63.3
	Small-holder	3.6	55.5	1.2	39.7

V	Commercial	85.9	1.0	2.4	10.7
	Small-holder	72.5	2.3	24.1	1.1

7.2 Objective 2: To identify, document and address constraints to the use of good disease prevention practices by smallholder pig farmers in Region 3

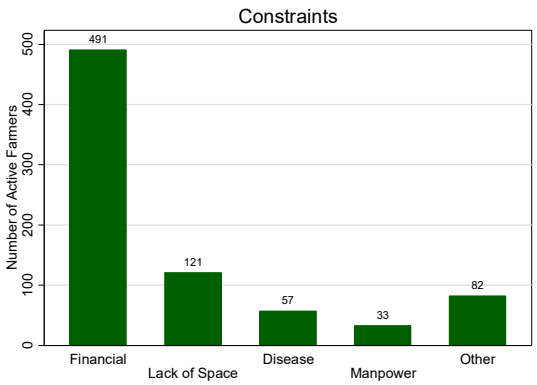
7.2.1 Conduct training workshop in ecohealth and participatory epidemiology for field staff

A three-day workshop on participatory epidemiology and Ecohealth research was held September 2015 with participation from the full Philippines research team (including Dr Ancog and his research associate Ms Corales, both the UPLB) and Dr Tamsin Barnes from the Australian research team. The training provided by Dr Ancog and Dr Barnes ensured that the Philippines team understand the principles of participatory epidemiology and eco-health as well having practical skills in planning and running semi-structured interviews and focus group discussions.

7.2.2 Identify constraints to the use of good disease prevention practises through semi-structured interviews and group discussions

Baseline Survey

A total of 1,082 smallholder pig farmers were surveyed during the baseline survey, of whom 629 (58.1%) were active and 453 (41.9%) inactive. A map showing the distribution of active and inactive farmers is shown in Appendix 11.2.2 (Figure 11.2.2.1). The greatest constraint identified by the smallholder pig farmers was finance. Disease was not perceived as a major problem in pig raising (Figure 7.2.1). Diarrhoea was the most common disease syndrome identified in piglets, fatteners and sows. A total of 70% of the farmers seek assistance when they observe a pig is diseased while 30% either treat their own pigs or do nothing. Farmers commonly seek assistance through government technicians (52%) and agri-vet supply store personnel (27%). Another constraint identified during the baseline survey was waste disposal, with the majority of the farmers sending pig waste into a canal, a lagoon or a river (Figure 7.2.1).



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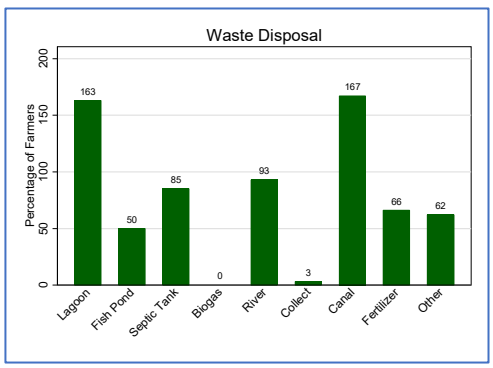

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Figure 7.2.1. A. Constraints identified by backyard pig raisers. B. Waste disposal methods used by backyard pig raisers

Semi Structured Interviews (SSIs) and Farm Visits (FVs)

A total of 214 SSIs and FVs were conducted from September 2015 to April 2016. Timelines were used to have an overview of the whole cycle of production, capturing the management practices, constraints and motivators, purchase and sale, use of credit, and income and expenditure. Another method used was progeny history – allowing the capture of sow productivity, disease, deaths, and purchase and sale of pigs. Body Condition Scoring was also done by the project veterinarian during FVs to assess the body condition of pigs specially the gilts and sows. The scoring reflected current conditions at the time of the FV (one visit per farm) and thus conclusions on the impact of seasonal or reproductive cycle cannot be drawn from this data.

Inadequate water supply to all pigs, especially to sows, was the most common problem identified. A possible reason was a local belief that giving more water to sows during gestation will cause the piglets to drown in-utero. It is also believed that giving to much water to piglets will cause diarrhoea, hence the reduced provision of water to the pigs. Inadequate water supply can lead to reduced milk from the sow which can cause poor growth of the piglets.

Another problem identified was inadequate nutrition for sows due to the high cost of feed and some farmers lacking knowledge on the proper feeding management of pigs. Poor body condition of sows can lead to poor fertility and reduced milk production during lactation. Other husbandry problems identified were piglets dying from ill-thrift and crushing, no or delayed heat, failure to conceive or abortion in sows, and lack of production and health records.

Farmers also lack economic records and thus cannot compute their income from backyard pig raising. Farmers either did not see the importance of keeping records, or they lacked knowledge in keeping economic records. Lastly, due to financial restrictions, farmers tended to buy less feed, or substitute with low- or poor-quality feed, and were thus unable to expand their operation.

Focus Group Discussion (FGD)

A total of 8 FGDs with inactive farmers were conducted from September 2015 to March 2016 to explore the reasons for stopping raising pigs, and what would encourage them to raise pigs again. The Proportional Piling method, in which the farmers piled counters in proportion to the importance of the reasons (Mariner and Paskin, 2000) was used to rank the responses of the farmers. A typical outcome is provided in Appendix 11.2.2.

Another FGD was conducted in April 2016 with multiple stakeholders to discuss the findings from various participatory activities that were conducted by the project staffs. Possible interventions were also discussed to address the constraints that were identified.

Identification of constraints and agreed interventions

The key constraints identified were all based around a lack of farmer understanding about key basic issues – water supply to pigs, nutrition of pigs, general piglet management, management of diarrhoea problems and a lack of records (in both economic and production areas). After the FGD, the interventions identified for the first cycle were the appointment of barangay coordinators, the conduct of water and nutrition, piglet management and diarrhoea seminars, and the introduction of a record keeping system suitable for backyard pig raisers.

7.2.3 Cycle through knowledge generation, action and reflection assessing progress in overcoming constraints on an ongoing basis

Cycle 1 – Knowledge generation

The knowledge generation stage was undertaken in Objectives 2.2 where key constraints were identified (see previous section).

Cycle 1 - Action

Barangay Coordinators

Barangay coordinators were selected for each of the 14 barangays of San Simon, Pampanga. They were tasked to assist the project team in conducting activities within their respective barangays and to monitor backyard pig raisers and report to the project veterinarian any problems or concerns observed or reported by the pig raisers. They were also responsible for organizing farmers for meetings and seminars. As a barangay coordinator, they were given a monthly compensation of ₱500 (=$(US) 11). Regular monthly meetings were also conducted by the project staff to update them of the current and future activities of the project. They were provided with a coordinator's notebook to be used in monitoring pig raisers within their assigned Barangay and were checked every meeting by the project veterinarian. The Barangay coordinators remained committed and active for the full life of the project.

Water and Nutrition Seminars

A total of 8 clusters (30 participants per cluster) of seminars were conducted on the topic of water and nutrition. Farmers who attended the seminars were given hard copies of the power point presentations together with leaflets that were developed by the project (Appendix 11.2.3). Farmers were taught the importance of giving clean, sufficient water for the pigs and the project team tried to eliminate the local belief on drowning of piglets in-utero. Proper feeding management were also discussed together with some economic computations for using the recommended feeding practises and comparing to their current practice. The use of an improvised nipple drinker system was introduced to make sure the pigs have clean access of water anytime. Two improvised nipple drinkers were raffled after the seminar for each of the 8 clusters. After the seminar, farmers were handed with evaluation forms to assess the content of the seminar and gather suggestions to further enhance the future seminars. Of the response forms completed, almost 50% were positive in nature (with indications that the farmers would attend more seminars (23%) or had learnt better ways to raise pigs (23%) (see Appendix 11.2.2). The project team also wrote a reflective diary to reflect on how they felt how the seminar went.

Piglet Management and Diarrhoea Seminars

Following the conduct of the first seminar series, another 8 clusters (30 participants per cluster) of seminars were conducted on the topic of piglet management and diarrhoea. Leaflets were also given to the participants together with the hard copy of the power point presentations. Proper piglet management from birth up to weaning was discussed and basic information about piglet diarrhoea. Farmers were also encouraged, as a general basic concept, to submit samples to the government laboratory (on a "fee for service" basis) to have proper diagnosis before treating the pigs. The use of a creep box was introduced to make sure that the piglets were kept warm and dry to minimize the cases of pre-weaning diarrhoea. An evaluation and reflective diary were also conducted after each cluster of seminars.

Record Keeping Workshops

The SSIs performed above revealed a lack of record keeping and thus all data gathered were very approximate. Thus, the concept of record keeping was introduced to (i) demonstrate to farmers the importance of record keeping to assess profitability, and (ii) improve farmer's financial literacy.

A participatory record keeping workshop was conducted among eight farmers (male=4; female=4) to develop a simple record keeping system to understand profits made through their business. Farmers also identified buddies in their barangays (villages) to share their learnings from the workshop. The production cost and income were based on litters (n=16) and batches of pigs (n=7). Feed cost was the main outlay in pig production which comprised 87% of the overall production cost. Examples of other input costs were: boar service, medication, vaccination, castration, electricity, water and gasoline. Total income per litter or batch was also recorded.

Cycle 1 Reflection

Nipple Drinker Follow-up Interviews

The project team interviewed a total of 34 pig farmers (male=21; female=13) from September 2016 to June 2017. Sensitizing the narratives with Rogers' Diffusion of Innovation Framework (Doyle et al., 2014),[8] qualitative data revealed that farmers reacted in an overall positive manner to the concept of using nipple drinkers (see Appendix 11.2.3 – Cycle 1 Nipple Drinker Follow-up Interviews).

Creep Box Follow Up Interviews

This series of interviews were conducted from November 2017 to March 2018. Sixteen farmers were interviewed from 10 barangays of San Simon, Pampanga. Out of this figure, eleven farmers were using the project's creep box prototype (or a similar one) and 5 farmers were using an improvised creep box. Details of the farmer views of the creep box are provided in Appendix 11.2.3 (Cycle 1 Creep Box Follow-up Interviews).

Costing of Improvised Creep Box

The cost of prototype creep box developed by the project was ₱670 (= $(US) 13), with the main component being the bamboo at ₱600 (= $(US) 11.50). The team

[8] *This framework characterises individuals into five types of adoptors – innovators, "early" adoptors, early majority, late majority and laggards. See Doyle et al (2014)*

also searched for other improvised creep box used by farmers in San Simon. The cost of materials used for making their improvised creep box was estimated by farmers themselves. Full details are provided in Appendix 11.2.3 (Cycle 1 – Estimates of Costs of Improvised Creep Boxes).

Cycle 2 Actions

Nipple Drinker and Creep Box Day

The Nipple Drinker and Creep Box Day, a community event to create awareness about the importance of using nipple drinkers and creep boxes, was attended by 159 farmers, 13 coordinators, 3 staff from ACIAR Philippines, 5 staff from DA Regional Field Office III, 13 feed company representatives, and 12 staff from the Local Government Unit of San Simon attended the event. Short presentations about the importance of using nipple drinkers and creep boxes were given. In addition, several farmers who were already using the technology gave their testimonies on their experience using it. A total of 150 nipple drinkers were given to the participants. Ten improvised creep box were also raffled together with 2 F1 gilts as the grand prize. Images from this event are presented in Appendix 11.2.3 (Cycle 2 Nipple Drinker and Creep Box Day).

Nipple Drinker Installation and Monitoring

During the Nipple Drinker and Creep Box Day, farmers were given a nipple drinker to encourage them to use the technology. The nipple drinkers that were given constituted a water container, a hose, and plastic pipes, all easily available components (see Figure 7.2.3). A total of 103 nipple drinkers were installed. To monitor the installation of the nipple drinkers, a checklist was developed by the project veterinarian. To facilitate the monitoring, the Barangay coordinators were trained to complete the monitoring form and take a photo of the installed nipple drinker. Full details of the monitoring, problems encountered and problem responses are provided in Appendix 11.2.3 (Cycle 2 Nipple Drinker Installation and Monitoring).

Figure 7.2.3. An example of improvised nipple drinker

Biogas and Rainwater Harvester

Biogas digesters breakdown organic matter in an anaerobic environment and produce various gas products including methane. This is beneficial for the farmers as the collected methane can be used as household gas and liquid fertilizer. This can also help decrease water and air pollution. A rainwater harvester can be used to collect and store rainwater during rainy season which can be used in cleaning their pig pens.

Two farmers were selected to become demonstration farm to showcase the biogas digester, rainwater harvester, and other interventions that the project introduced such as the use of creep boxes, nipple drinkers, and record keeping. The team collaborated with the Bureau of Animal Industry as they have an ongoing biogas project. The units were installed in February 2018 (Figure 7.2.4 – left and centre images). Two additional biogas units of a different design which is cheaper and more suitable to pig farmers with fewer pigs were constructed on additional demonstration farms (Figure 7.2.4 – right image).

Figure 7.2.4. The installed rainwater harvester (left image); the large biogas digester model (centre) and the smaller biogas digestor option (right image).

Training Needs Analysis and Upskilling Training for Agricultural Extension Workers

To identify the needs of the Agricultural Extension Workers (AEWs) servicing San Simon, a Training Needs Analysis workshop was conducted in November 28, 2016. Based on the needs identified in the analysis workshop, an Upskilling training workshop, conducted by Dr. Kit Parke, was held in January 2017. Best pig husbandry practices were discussed during the day 1 of the training and field work was conducted on the second day of the training.

Farmer Field Days

Two Farmer Field Days were conducted to create awareness about the project interventions to other farmers. The events were attended by 4 farmers, 14 barangay coordinators, 10 AEWs and 5 representatives from the Local Government Unit. Staff from the Municipal Environmental and Natural Resources (MENRO) also attended the activity and showed interest in the use of biogas digester.

Market Price Text Blast

The market price text blast is a system where the information can be easily disseminated among recipients through text messages. The market price text blast has been developed by the project as one solution to one of the key problems identified by the farmers - a lack of market price information. Access to market price information is an essential tool to increase profitability. The objective of the market price text blast is to inform enrolled farmers of current local price of per kilogram live-weight and to empower them in negotiating with traders as the buyers dictate the pricing system. A weekly market (every Monday) price update of commercial and backyard live-weight prices was delivered free of charge to all farmers in San Simon who registered for the service. The market price text blast commenced in October 2017. By the end of the project, 114 farmers were receiving the weekly blasts.

Record Keeping Workshop

Two additional workshops were conducted to enable farmers to use their records to calculate gross margin from pig raising and to identify opportunities to increase their profit. They were also taught how to compute their own income using a production cycle timeline.

The first workshop involved nine (five farmer participants and four farmer buddies) out of the initial 13 record keepers were able to continue keeping records (see Appendix 11.2.3). The reported reasons for not continuing to keep records were: stopped raising pigs (n=2), lack of time (n=1), and health problems of their relatives (n=1). Data gathered on production cost and income were based on litters (n=16) from four farmers and batches of fatteners (n=7) from five farmers. Feed cost was the main outlay in pig production and comprised 93% of the overall cost for litter production and 81% for the batch of fattener (Figure 7.2.5). Other input costs were: boar service, medication, vaccination, castration, electricity, water and gasoline.

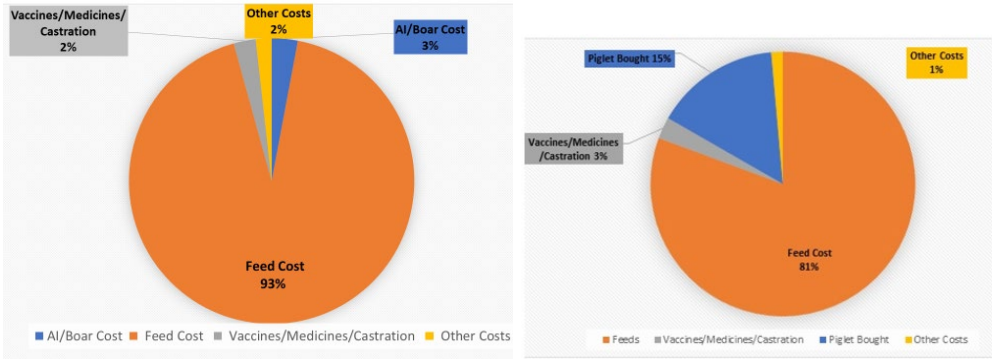


Figure 7.2.5. The distribution of cost for production of litter of piglets (left) and
batch of fatteners (right)

Ten farmers (six farmers participants and four farmer buddies) attended the follow-on workshop for record keeping to help farmers understand what some of them are failing to record, why all the data are important to be able to compute profit, and which part of the production cycle can be improved. Only variable and material costs were part of the record keeping as it was obvious that overhead and labour costs were simply too difficult to record. Through the timeline presented, the part of the production cycle which can be improved to be more profitable was easily determined (e.g. on-time breeding, sows getting pregnant on first mating). A sample timeline is presented in Appendix 11.2.3 (Figure 11.2.3.3). The data gathered in these workshops has been used under Objective 3 to help develop an estimate of the economics of small-holder pig farming in San Simon.

Cycle 2 Reflections

Semi-Structured Interviews on market price text blast

Semi-structured interviews were conducted among 12 randomly selected market price text blast recipients in May to July 2018. This was conducted to determine if receiving market price information allowed farmers to be proactive in making informed decisions in negotiating selling price. While only 7/12 farmers were

currently raising pigs, these 7 farmers used the market price information. Importantly, all 12 farmers indicated that the price information was valuable. The non-active farmers indicated they valued the information, passing it to neighbours or family. They want to receive the text blast on a regular (weekly) basis. Even those farmers that have not used the market price text blast, valued the information. All farmers indicated that they wanted the market price text blast to be a continuous program. Further specific farmer feedback is provided in Appendix 11.2.3 (Cycle 2 Reflections – Semi-Structured Interviews on Market Text Price Blast).

Focus Group Discussions on market text price blast

To further evaluate the impact of market price text blast, Focus Group Discussions (FGDs) were also conducted. The objectives were:

- To assess if the market price text blast has been useful to farmer recipients;
- To determine if market price allowed farmers to be proactive in making informed decision in negotiating selling price;
- To determine if farmers want this program to continue.

Two Focus Group Discussions (FGDs) were conducted in April, 2019. Each FGD was to be composed of eight farmers with one focussed at fattener operations and the other at feeder operations. These farmers were randomly selected from 114 farmers who are registered/enrolled in the text blast program. The list was divided into two groups: newly enrolled farmers and farmers enrolled in 2017 to 2018. The farmers were randomly selected and they were contacted beforehand to determine whether they are fattener or feeder operation. As with the SSI, these FGDs showed that farmers appreciated and valued the text blasts. The farmers indicated that they were willing to pay between 1 and 2 Philippine pesos weekly for the blasts. Full details of the FGDs are provided in Appendix 11.2.3 (Cycle 2 Reflections – Focussed Group Discussions on Market Text Price Blast).

The farmer support for the text blasts has resulted in a decision to transfer the operation of the blasts to the Department of Agriculture-RFO III Information Section. Mr. Alvin G. Allam, project team member responsible for the blasts, has transferred the expertise on the text blast methodology to the DA Information Staff who continued to provide the service after the formal closure of this project.

Semi Structured Interviews - Record Keeping

The study used semi structured interviews with open ended questions on Rogers' model of perceived characteristics of innovation (Rogers, 1962). Two series of interviews were undertaken between May to August 2017 and between July to August 2018. A total of 12 farmers were interviewed (nine female and three male) and consisted of both initial adopters who kept recording and another six who discontinued recording. While the farmers could see advantages (tracking performance) and agreed that the project record book was compatiable with their needs, they also indicated that they lacked time to keep records and were discouraged when they saw their performance. More details of the feedback are provided in Appendix 11.2.3 (Cycle 2 Reflections – Semi-Structured Interviews on Record Keeping).

7.3 Objective 3: To estimate the incidence and economic impact of two disease syndromes of importance to smallholder farmers

7.3.1 Identify two disease syndromes of highest priority to smallholder farmers

Data about disease syndromes were gathered in the base-line survey performed under Objective 2. This survey established that diarrhoea was the most common disease syndrome observed by the farmers, with a 31% occurrence in piglets and 33% in fatteners being recorded. To further explore the problem, farm visits (n=214) and semi-structured interviews (n=42) were conducted. The progeny history method was used to gather information on sow productivity, diseases, and pig deaths. The data gathered using this technique was mostly based on farmer recall as majority of the backyard pig farmers either do not keep records or do not keep sufficiently detailed records.

Of the 214 farm visits conducted, the Project Veterinarian was only able to observe 21 litters and little disease was noted. Skin lesions were commonly observed, and poor body condition were seen specially in sows. On the other hand, a total of 361 litters comprising 3,709 piglets were recorded using the progeny history method. Of the total piglets recorded, a total of 178 (6.1 %) were recorded with a case of diarrhoea between birth and weaning. A total of 366 (12.6%) piglets were recorded as dying between birth and weaning. Of the recorded deaths, 80 (21.9%) were due to crushing, 53 (14.5%) from diarrhoea, 194 (53%) from illthrift, and 39 (10.7%) from other causes. With regards to fatteners, a total of 1,112 fatteners from 243 litters and 145 batches were recorded. Fatteners were either piglets weaned from sows or purchased after weaning. A total of 20 (1.8%) were recorded with a case of diarrhea and 16 (1.4%) were recorded deaths from it.

Results from both the baseline survey and farm visits indicate that diarrhoea is a major concern to the farmers and can lead to production losses. To have a more accurate data on the production, a record keeping notebook was developed by the project and piloted to selected backyard pig raisers. Complete records were obtained for 26 litters from 7 farmers. A total of 292 piglets were born alive with a median of 12 per litter (interquartile range: 9-14, range: 5-16). Farmers recorded one or more cases of diarrhoea in 4 litters (15%) with 21 piglets (7%) affected. At least one piglet was reported as dying as a result of diarrhoea in 3 litters (12%) with 6 piglets (2%) dead. At least one piglet was reported as dying as a result of crushing in 5 litters (19%) with 5 piglets (2%) dead. At least one piglet was reported as being a runt and dying as a result in 8 litters (31%) with 16 piglets (6%) dead. In summary, all of the data gathering methods used in this project only identified one disease syndrome as being of any significance – piglet diarrhoea.

7.3.2 Implement any additional systems required for diagnosis of agents associated with identified disease syndromes

Following the identification of piglet diarrhoea, the suite of diagnostic assays necessary for the detection of the major pathogens (virus, bacteria and parasite) were identified. The identified pathogens of relevance was as follows:- Porcine Epidemic Diarrhea virus (PEDV), Rotavirus, Transmissible Gastroenteritis virus

(TGEV), haemolytic and non-haemolytic *Escherichia coli*; *Lawsonia intracellularis, Salmonella* spp, internal parasites and protozoa.

7.3.3 Develop Diagnostic Support System

As noted earlier, no significant progress was achieved on this objective.

7.3.4 Cycle through knowledge generation, action and reflection assessing impact of disease syndromes on an ongoing basis

The first cycle of knowledge generation, action and reflection of this Objective was performed concurrently for Objectives 2 and 3.

Diarrhoea Study

For the second cycle, a piglet diarrhoea study was undertaken. A summary of the key results is provided below.

Diarrhoea Study - Sample Size

A total of 65 litters were sampled, 26 of which were diarrheic and 39 were non-diarrheic. Collection of samples was done from December 2017 – February 2019. The team did not meet the 75 litters per population due to the low number of farrowing sows during the collection period and the fact that most sows did not have diarrheic litters. A total of 65 litters were sampled, 39 litters were healthy and 26 had suffered diarrhoea. An analysis of the litters sampled (age, litter size and management style is presented in Appendix 11.3.4 – Results of the Piglet Diarrhoea Study, Figures 11.3.4.3 and 11.3.4.4).

Diarrhoea Study - Bacterial isolation and identification

A total of 130 pig rectal swab samples were subjected to bacterial isolation. Out of these, 52 came from diarrhoeic litters and 78 were from piglets without diarrhoea. The identified microorganisms were: haemolytic and non-haemolytic *E. coli, Salmonella* spp*., Staphylococcus* spp*., Streptococcus* spp*., Enterococcus* spp*., Proteus* spp., and *Klebsiella* spp. There was no obvious or marked difference between the results of the litters with and without diarrhoea. Full details of the results for haemolytic *E. coli* and *Salmonella* spp. are presented in Appendix 11.3.4, Figure 11.3.4.5).

Diarrhoea Study - Faecal oocyst/egg count

A total of 102 faecal samples, 51 from piglets and 51 from sows, were collected for parasitological examination. Ova and oocysts were seen in both healthy and diarrheic populations with no marked difference between the litter types. The species identified were *Eimeria* spp*., Cystoisospora* spp*., Strongyloides ransomi, Oesophagostomum dentatum* and *Trichuris suis.*

Diarrhoea Study - qPCR detection of TGE and PED and dipstick detection of rotavirus

All rectal swab samples were subjected to quantitative real-time polymerase chain reaction (qPCR) for the detection of PED and TGE viruses. The results were all negative for both PEDV and TGEV. In addition, a dipstick method for detection of rotavirus was employed. Out of 119 rectal swab samples, four gave weak positive reactions.

Diarrhoea Study - LAMP detection of *L. intracellularis* and PED

A total of 166 samples (130 from piglets and 36 from sows) were examined for the presence of *L. intracellularis* by LAMP. The diarrhoea litters had an overall positive rate of 29% (37% for piglets and 14% for sows). Similar results were obtained for the litters without diarrhoea – overall positive rate of 21% (piglets 23%, sows 14%).

For the PED assay, the same 166 samples were examined. The diarrhoea litters had an overall positive rate of 21% (20% for piglets and 21% for sows). Similar results were obtained overall for the litters without diarrhoea – overall positive rate of 26%. A noticeable difference was that the healthy sows had a markedly high positive rate (41%), higher than the sows linked to diarrhoea (21%). The piglet positive rate (22%) in the healthy litters was very similar to that of the piglets from the litters with diarrhoea (20%).

Economic analysis

The record keeping activities allowed an analysis of the economics of pig production by small-holder farmers in San Simon. Details are provided below.

The gross margin (excluding labour) per litter (16 observations) ranged from ₱-2,955.00 to ₱21,547.00 ($US-57 to 414) with a median of ₱8,038 ($US155). For every batch of fatteners (7 observations), the gross margin ranged from -₱12,400.00 to ₱42,517.00 ($US -239 to 818) with a median of ₱2,594 ($US50). The maximum gross margin for the litter of piglets amounting to ₱21,547.00 ($US414) was achieved because of a large litter size (13 piglets) and a higher selling price, ₱2,250 ($US43) per piglet, compared to other litters where selling price ranged from ₱1,900 to ₱2,200 per piglet ($US36 to 42). It was also noticeable that one of the farmers linked to fattener production had a gross margin of negative ₱12,400.00 ($US239). Even though no mortalities were reported, the reasons for the negative profit were as follows:- A) piglets were stunted when bought, B) feed was given for a long period of time and C) the selling price amounted to ₱6,500 ($US125) per fattener, the same as other batches' sales.

As an alternative approach, the data gathered from SSIs with traders and agri-vet stores was supplemented with the knowledge of correct piglet nutrition and management (vaccines, iron injection, boar hire) costs to determine the theoretical cost (without labour costs) of producing a piglet (within a 9 piglet litter). These costs estimates were then balanced against the typical selling price of a batch of piglets. Details of the costs are provided in Table 7.3.1.

Table 7.3.1. Estimated costs expressed as Philippines pesos (and $US) of producing a litter of nine piglets through to 35 days of age.*

Item	Cost
Feed – Sow	₱6,958 ($134)
Feed – Nine Piglets (0.2 kg/day/piglet)	₱5,940 ($114)
Boar Hire OR Artificial insemination	₱700 ($13)
Iron injection (two)	₱360 ($7)
Hog Cholera vaccination	₱180 ($3.5)
TOTAL COST	₱14,138 ($272)
	₱1,571 ($30 per piglet)

*Based on the data gathered from the SSIs with traders, a selling price structure of ₱2,000 ($US 38) for a piglet with a further ₱100 ($US2) for every kilo over 10 means that the typical selling price for a nine piglet litter would be ₱2,200 ($US42). As the cost or producing the piglet in this scenario was ₱1,571 ($US30), each piglet provides a profit of ₱629 ($US12). On this basis, the loss of a piglet due to diarrhea results in the loss of a theoretic profit of ₱629 ($US12), although there may be reduced costs associated with the reduced need for piglet feed.

One farmer who had significant involvement in the project (barangay co-ordinator; site of some of the demonstration activities organised by the project) (Farmer E) provided sufficient detail to allow a full understanding of the costs and profit associated with the production of a litter of 13 piglets. Based on the actual records kept as part of the project initiative, the costs of producing this litter (200 day cycle; costs covered feed, breeding and so on) was ₱16,880 ($US325), which converts to ₱1,298 ($US25) per piglet. These costs are remarkably similar to those done by theoretic calculation in the above paragraph. This particular 13 pig litter was sold for a total of ₱25,400 ($US489), with 10 piglets sold for ₱2,000 ($US38) and 2 piglets for ₱1,800 ($US35) – again very similar to the theoretical calculations in the above paragraph. Hence, in this real-life example each piglet generated a profit of ₱655 ($US13) – again almost the same as the theoretic profit from the previous paragraph.

Overall, the data gathered in this project and analysed under this Objective was quite consistent and showed that a well managed system could generate a profit of around ₱630-650 (at the time of this project). A similar calculation was done using the real-life data gathered by Farmer E and indicated a profit of ₱1,548 ($US30) per fattener pig (full details in Appendix 11.3.4, Table 11.3.4.4).

7.4 Objective 4: To develop new diagnostic tests for, and undertake applied epidemiological research on, pig diseases important to Australia

This Objective was undertaken in Australia and consisted of five Activities. Details of the key results and a focussed discussion are provided under each of the Activities below.

7.4.1 Identify novel *Actinobacillus*-like bacteria and understanding of their potential role in disease

Results

The 21 isolates examined in this activity came from 14 farms ranging from free range farms to conventional farms. Most of the pigs from which the isolates were obtained showed no clinical signs or coughing, yet at the abattoir abscesses, lesion and pleurisy were observed. Only on one farm were isolates of the new species retrieved from animals that had succumbed to disease at 12 to 18 weeks of age.

The 21 isolates had identical sequences for the 16S rRNA, *recN, rpoA* and *thdF* genes. The strain HS4635 was selected as being representative of the 21 isolates. The 16S rRNA similarity between strain HS4635 and the type strain of *Glaesserella parasuis* was 97%, indicating that the isolates are a different species

within the genus *Glaesserella.* To further investigate this relationship, whole genome sequences were compared and used to estimate the degree of DNA-DNA renaturation (DDN). Between the genome of strain HS4635 and the type strain of *Glaesserella parasuis* (accession number MUXW01, CCUG 3712^T), 30.9% DDN was estimated indicating a unique species.

The phylogenetic tree for the 16S rRNA gene and the relatedness of HS 4635 (as the representative 21 Australian isolates) to other members of the family *Pasteurellaceae* is shown in Appendix 11.4.1. The phenotypic characters shared by the 21 isolates are fully described in Appendix 11.4.1. This pattern of phenotypic properties clearly distinguishes the isolates from the other currently recognized haemophilic organisms known to be present in pigs.

Discussion

Overall, the phenotypic and genotypic characterization of the 21 isolates examined in this study confirms that these isolates represent a novel species of haemophilic organisms that can be distinguished by phenotypic and genotypic methods from all currently recognized species within the family *Pasteurellaceae* known to occur in pigs. The novel species is most closely related to *Glaesserella parasuis* and [*Actinobacillus*] *indolicus.* The overall genomic and sequence analysis work clearly places the novel species within the genus *Glaesserella*. We propose the name *Glaesserella australis* (aʊsˈtraː.lis. N.L. masc. adj. *australis* southern, the pigs that first yielded this organism were from Australia, a continent originally named as *Terra Australis*) for this new species. The proposed type strain is HS4635^T. A full comparison of the phenotypic properties of all the currently recognized haemophilic members of the family *Pasteurellaceae* known to occur in pigs is presented in Appendix 11.4.1.

As this novel species has been isolated from the lungs of clinically sick pigs as well as from lesions in the lungs of slaughter pigs, the organism clearly plays a role in porcine respiratory disease. Whether that role is a primary one or a secondary role remains to be determined. Further studies on this organism are clearly needed.

7.4.2 Rep-PCR typing of *Pasteurella multocida* established, validated and used

Results

LPS typing

Of the eight recognised LPS genotypes, only four were detected in the pig and poultry isolates: L1, L3, L4 and L6 (see Appendix 11.4.2, Supplementary Tables 11.4.2.1 and 11.4.2.2). For the pig isolates, the LPS PCR assigned all isolates to an LPS type. There were two poultry isolates that could not be typed by the LPS PCR method.

Rep-PCR

The rep-PCR revealed 20 different profiles for the 43 pig isolates (47% diversity; see Appendix 11.4.2). The 21 isolates with the LPS L3 genotype displayed 10 different patterns, while the 20 isolates with LPS L6 genotype displayed 8 patterns and the 2 isolates of LPS L1 genotype displayed 2 patterns.

MLST

The 22 pig isolates examined by MLST had 16 STs, with 4 STs (specifically STs 50, 124, 167 and 185) having multiple isolates (see Appendix 11.4.2). The 41 poultry isolates consisted of 37 different STs, with only STs 7, 8 and 9 having multiple isolates (see Appendix 11.4.2). Four new STs were recognised in the pig isolates, being ST 326 (a member of clonal complex (CC) ST20), ST 327 (member of CC ST74), ST 328 (member of CC ST13) and ST 329 (member of CC 20).

There were five STs (STs 7, 11, 20, 24 and 58) shared between the poultry and pig isolates (Table 7.4.2). The isolates of the shared STs were always the same LPS PCR type within the ST across the two host species.

The rep-PCR analysis showed that all three isolates of ST 7 and the two isolates of ST 24 had the same pattern (Table 7.4.2). All other isolates of the three shared STs had slightly different rep-PCR patterns within an ST with only one band difference being detected (see Appendix 11.4.2, Figure 11.4.2.1).

Table 7.4.2. *Pasteurella multocida* isolates of chicken and pigs sharing the same sequence types (STs) assigned by multilocus sequence typing (MLST)*

Host	Isolate	ST	LPS	REP-PCR profile
Chicken	48	7	L3	A
Chicken	1434	7	L3	A
Pig	1812	7	L3	A
Chicken	49	11	L1	B**
Pig	1463	11	L1	C**
Chicken	1417	20	L3	D**
Pig	1475	20	L3	E**
Chicken	83	24	L3	F
Pig	1676	24	L3	F
Chicken	878	58	L1	G**
Pig	1732	58	L1	H**

* CC, clonal complex; LPS, lipopolysaccharide; Rep-PCR, repetitive element PCR fingerprinting assay; ST, multilocus sequence typing (MLST) sequence type.
**Isolates of the same ST differ by only one band in the rep-PCR (see Appendix 11.4.2).

The STs shared across pigs and poultry that were identified in our study (ST7, ST11, ST20, ST24 and ST58) mostly consisted of Australian isolates. The database for the *P. multocida* MLST (https://pubmlst.org/pmultocida/) reports ST7, ST11 and ST20 as only occurring in Australia, with all of them having been reported from poultry and pigs (see Appendix 11.4.2, Table 11.4.2.3). ST24 was reported from two chickens in Australia and one rabbit in Italy and ST58 was reported from one chicken and one pig from Australia, but also from a sheep in New Zealand and a partridge from Belgium (see Appendix 11.4.2, Table 11.4.2.3).

Of the 16 STs observed for the pig isolates of *P. multocida*, four were new STs and one ST (ST 167) has only been previously recorded from pigs in Australia according to the RIRDC *P. multocida* MLST database. One isolate of ST 185 had an unknown host, but all other 10 STs had other species listed (Table 7.4.3).

Of the 37 STs from the *P. multocida* isolates from poultry, 24 have only been observed in poultry isolates according to the RIRDC *P. multocida* MLST database and 13 STs had several host species. The five STs that were found in this study to be shared by both chicken and pig isolates were among the 13 STs listed with different hosts.

Discussion

The importance of Heddleston serotyping has arisen because it was believed that killed *P. multocida* vaccines provided protection only against those serovars in the vaccine (Glisson et al, 2010). However, it has been recently shown that vaccines based on killed whole cells of *P. multocida* only give protection against strains that express highly similar or identical LPS structures (Harper et al, 2016a). As there is inter- and intra-strain LPS structural heterogeneity within an LPS PCR genotype or a serovar (Harper et al, 2013), it is no longer possible to be confident that isolates of the same serovar or LPS PCR type are indeed cross-protective. In the original development of the PCR (Harper et al, 2016b), the gold standard method of a full

chemical and structural analysis showed that LPS PCR was consistently correct, while the traditional serotyping methodology was in error, when these two tests were in disagreement. Hence, the Heddleston serotyping scheme is not suitable for *P. multocida*, especially for pig isolates.

Our finding that some *P. multocida* STs were shared between the two hosts (poultry and pigs) is in contrast with observations from a study in 2011 (Hotchkiss et al, 2011) looking at bovine *P. multocida* isolates. That study examined data then available from the RIRDC MLST scheme and showed no relationship of STs between pig and poultry isolates of *P. multocida* (Hotchkiss et al, 2011). The shared STs identified in our study (ST7, ST11, ST20, ST24 and ST58) mostly consisted of Australian isolates, with only ST58 consisting of isolates from animal species from overseas. It is possible that regional influences play a role in the occurrence of STs. As an example, ovine isolates from Spain and New Zealand have been shown to belong to distinct STs (Hotchkiss et al, 2011).

It is worth noting that the RIRDC MLST database currently contains a total of 21 isolates of *P. multocida* from humans. Isolates of ST 13 and 74 have been recognised in pigs, poultry and humans. This raises the possibility of zoonotic potential of at least some STs. As well, this sharing of genotypes across pigs, poultry and humans raises biosecurity issues.

Overall, this study has shown the value of rep-PCR as a typing method. Importantly, this work has shown that for strain typing purposes, rep-PCR is a low cost, easy to use, rapid option that is particularly suited to laboratories without access to sequencing facilities. There are dis-advantages with rep-PCR. The major problem is that the technology does not allow easy comparisons across time or laboratories. Comparisons have to be performed within the same laboratory and at the same time. Nevertheless, rep-PCR does have relevance as a typing option. In the current study, we have used rep-PCR to screen a large collection and followed up with MLST on representative isolates selected from the rep-PCR results.

7.4.3 Validate the use of MALDI-TOF to serotype *P. multocida*

Results

After considerable experimentation, LPS extraction via the method reported by Pupo et al (2004) was found to be the best for generating MALDI-TOF profiles that gave the best possible separation of the reference serovar strains. (see Appendix 11.4.3 for typical profiles).

The hypothesis of this work was that each reference strain would give a unique profile of peaks and, further, that the profile of the peaks of the 16 strains would be markedly different. These two features, unique and markedly different profiles, could not be established. Despite, all efforts to optimise the methodology, only minor differences (typically only one peak) could separate the reference strains. Indeed, for the reference strains for serovars 4 and 16, the profiles were very similar except for some small differences in the height of two peaks.

Discussion

The convenience of a kit for the extraction of the LPS was our main reason for including this method into our work. It was felt that front line diagnostic

laboratories would adopt a kit easily. However, the extraction method did not yield enough LPS for the analysis via MALDI-TOF and hence proved unsuitable. The method that yielded the most LPS was the method originally reported by Pupo et al (2004). The yield of LPS was dependent on the starting concentration of the bacteria and increasing the concentration by 10 fold (using 1 mL rather than 100 µl).

Our use of MALDI-TOF analysis of the LPS was based on the fact that the 16 Heddleston reference strains have been subjected to detailed analysis of the LPS structure and all sixteen reference strains do indeed have different structures (Harper et al 2016b). The challenge is that some of the reference strains share very similar structures that differ only slightly. As an example, Heddleston serovar 2 and 5 reference strains (M 1404 and P 1702, respectively) have an identical LPS structure except that strain P 1702 has a phosphoethanolamine residue linked to the third L-glycero-D-mannoheptose that is not present in strain M 1404. These types of minor structural differences can have a marked biological significance. As an example, Heddleston serovar 2 is linked only with haemorrhagic septicaemia in Africa while Heddleston serovar 5 is associated with haemorrhagic septicaemia in Africa, Asia and occasionally other parts of the world (Petersen et al, 2014).

With considerable effort at standardisation and optimisation, the MALDI-TOF profiles did allow the separation of most of the Heddleston serovar reference strains, albeit in some cases only by one peak. Even the two very closely related serovars, serovars 2 and 5 (see paragraph above), could be held apart by a peak at 777.6 m/z. Unfortunately, it is questionable if these types of minor differences can be maintained if different field isolates were investigated. In our previous collaborative studies, we have found a marked diversity in the LPS structure, particularly in fowl cholera isolates of LPS PCR type 3 (Harper et al, 2013). With this level of known field isolate diversity, it is highly unlikely that the minor differences we have detected in the 16 reference strains will be of any value. Indeed, it is our conclusion that the minor differences that exist within the reference strains will be overwhelmed by the diversity of minor changes that are known to exist in the field isolates. Hence, at this stage, MALDI-TOF does not offer a realistic or relevant alternative when seeking to identify either serovars or immunovars.

7.4.4 Evaluate the use of bead-based PCR to serotype *Haemophilus parasuis*

Results

Validation on reference strains

The validation of the bead-based PCR on the serovar reference strains showed that both mixes amplified the correct reference serovar (see Appendix 11.4.4). For each reference strain, the correct result was obtained using the bead-based PCR assay.

Evaluation using field isolates

Based on an evaluation of the difference between the highest value and the second highest value for both mixes when used on the reference strains, the standard for the cut-off point of positive results was set as follows - the difference between the highest value and the second highest value should be more than 20% to be considered as an acceptable result.

On the basis of the above criteria, the results for the use of the bead-based PCR typing system for the 73 field isolates (along with the results of the two forms of conventional serotyping and the multiplex conventional PCR) are given in full detail in Appendix 11.4.4. To help analyse the results of this work, the multiplex PCR was defined as the gold standard. Where the two molecular assays disagreed, the conventional serotyping was used to allocate the isolate to a serovar. The summarised ability of the bead-based PCR to serotype the field isolates is given in Appendix 11.4.4. The key points to emerge from this comparison are as follows:-

A) For 55 of the 73 isolates, the multiplex PCR and the bead-based PCR were in full agreement;
B) The isolates in agreement covered serovars 1, 2, 3, 5, 6, 9, 10, 11, 13 and 15;
C) Both assays gave no reaction for 7 isolates;
D) The bead-based PCR outperformed the multiplex PCR for serovar 5, as all 10 isolates of this were correctly identified by the bead-based PCR but reacted as serovars 5 and 12 in the multiplex PCR;
E) The bead based PCR failed to recognise all nine isolates of serovar 4 and all 3 isolates of serovar 13 (all of which were serotyped by the multiplex PCR
F) The bead based PCR also had problems with the five serovar 7 isolates – one giving no reaction and four reacting with both serovars 1 and 7.

Discussion

A key finding of this work is that the recently developed multiplex serotyping PCR of Howell et al (2015) gave both non-typeable results (ie no reaction) as well as cross-reactions (ie a positive reaction with more than one serovar). The original report of this molecular serotyping PCR did not report any non-typeable isolates. However, studies in our laboratory have highlighted the genetic diversity of Australian *G. parasuis* isolates (Turni et al, 2018). It would appear that this diversity has meant that the multiplex PCR developed in Europe has failed to serotype seven of the Australian isolates of *G. parasuis.* This points to the necessarily to include all the variations of a species or serovar before developing an assay that is based on the sequence of genes. It is not clear if the non-typeable isolates detected in this study are new serovars or isolates with variations in the gene sequence of one of the targeted genes. As six of the seven isolates non-typeable in the Howell et al (2015) multiplex PCR were also nontypeable by conventional serotyping (see Appendix 11.4.4), it seems likely that the isolates do indeed represent novel serovars.

The bead-based assay worked well for 10 of the 15 serovars but gave cross-reactions for isolates that were identified as serovar 7 by the multiplex PCR. The bead-based PCR gave positive reactions to serovars 1 and 7 for these isolates. For the reference strain for serovar 7, it was possible to differentiate these two serovars. However, for the field isolates of serovar 7 this difference does not seem to exist. This ultimately means that serovar 7 is coming up with two bands (serovar 1 and 7), while the true serovar 1 isolates will only display one band (the band for serovar 1).

Three serovars were problematic in the bead-based PCR. The nine isolates of serovar 4, the sole isolate of serovar 8 and the three isolates of serovar 14 all gave no reaction in the bead-based PCR. Of particular importance is serovar 4 as this serovar is a commonly encountered serovar around the world (Nedbalcova et al, 2006) and also present in many commercial vaccines (Zhao et al, 2017). There is clearly marked differences in the serovar 4 Australian field isolates as the reference strain for serovar 4 was recognised by the bead-base PCR. In terms of practical use, all isolates that give a non-typeable reaction in the bead-based PCR would need to be examined in a reduced form of multiplex PCR of Howell et al (2015) that would be used to confirm such isolates as serovars 4, 8 or 14.

A significant advantage of the bead-based assay is that the assay can differentiate between serovars 5 and 12, both highly pathogenic serovars (Kielstein and Rapp-Gabrielson, 1992). Serovar 5 is a very common serovar in many parts of the world (Nedbalcova et al, 2006). Specifically, in Australia, serovar 5 was the most common serovar recognised in the two serotyping studies undertaken to date (Blackall et al, 1996; Rafiee and Blackall, 2000) and is commonly present in Glässer's disease vaccines around the world (Zhao et al, 2017).

The other advantage of the bead based assay is that the assay gives a good separation of all the serovars with results that show a larger than 20% difference from all other results for all isolates that were assigned to a serovar. Hence, there should be no differentiation problems and this will make the workflow easier, results being able to be created in a much shorter timeframe with confidence in the results. In contrast, the multiplex PCR of Howell et al (2015) requires very careful evaluation of band size as there are only minor differences in the sizes of some bands. As an example the band given by serovars 5 and 12 (no differentiation of these serovars is 450 bp while the band for serovar 7 is 490 bp. In routine diagnostic applications, a confident recognition of a relatively small size difference of 40 bp is a significant challenge that could result in mis-serotyping or the need for repeat testing.

It is the diversity within *G. parasuis* that explains our inability to design a bead based PCR that works effectively for serovars 4 and 14. The bead-based PCR approach requires the design of new primers as the assay requires a smaller sized target than a conventional PCR. While the bead-based PCR worked well for the reference strains of serovars 4 and 14 (which are not Australian in origin), the assay failed when applied to the Australian isolates of both serovars. This suggests that the targeted region for serovars 4 and 14 in the bead based PCR is different for the Australian isolates as compared with the reference strains. Similar problems (unrecognised diversity) may apply to isolates of these serovars from regions outside Australia.

Despite the problems with serovars 4 and 14, the bead-based PCR developed in this study offers considerable advantages. The assay can correctly serotype most of the recognised serovars, with the bead-based system overcoming the problems of cross-reactions with serovars 5 and 12. As well, the bead-based PCR provides a far clearer differentiation of serovars than is possible with the conventional multiplex PCR that requires an ability to confidently identify bands of very similar molecular weight.

7.4.5 Improve the understanding of the role of porcine circovirus-2 (PCV2) in pig disease in Australia

Results

A published qPCR method was established in the laboratory, and a plasmid containing the whole PCV2 genome was used to generate standard curves to allow for quantification of PCV2 virus load in serum samples.

A longitudinal study conducted on 24 pigs revealed that PCV2 DNA was first detected in serum at 10 weeks of age (13/24 pigs), coinciding with declining maternal antibody levels. By 19 weeks of age 18/24 pigs were PCV2 positive in serum and virus DNA was detected in lymph nodes of all 21 slaughtered pigs. No PCV2-associated disease was observed in the herd. However, there was a correlation between PCV2 antibody levels and a decline in average daily weight gain, both measures likely reflecting an effect of circulating virus in the preceding 2-3 week period.

A PCV2 vaccination trial using 100 pigs was conducted to further explore the impact of PCV2 on weight gain. The results indicated that PCV2 vaccination prevented virus DNA being detectable in the serum of pigs. PCV2 DNA was detected in the lymph nodes of 19 of 44 vaccinated pigs and 42 of 48 non-vaccinated pigs at slaughter. There was no significant difference in the average daily weight gain of PCV2 vaccinated and unvaccinated pigs.

In the phylogenetic study, 7 samples derived from 7 farms were designated PCV2b genotype, 2 samples from one farm were PCV2d and one sample from one farm was PCV2f. The other 7 samples from 4 farms were grouped as an “intermediate group” that sat as a sister clade to the PCV2d genotype on the whole PCV2 genome analysis.

For the pen-side PCV2 diagnostic test, DNA was extracted from oral fluids using a novel cellulose dipstick method, and compared to a commercial DNA extraction kit. The oral fluid was spiked with known quantities of plasmid containing PCV2 genome. The work showed that the dipstick method was effective in extracting DNA from oral fluids. A Loop-mediated isothermal amplification (LAMP) method was developed to amplify PCV2 DNA. However, non-specific and inconsistent amplifications were observed with this method. A series of experiments was conducted using different sets of primers, different concentrations of reagents and different cycling conditions. However, the results showed inconsistency in terms of repeatability and non-specific reactions and a decision was made not to pursue LAMP further. Work is now focusing on the use of a portable miniPCR machine for pen-side PCV2 DNA amplification. Initial results are very promising and work is on-going to optimise the reactions and identify the most appropriate visualisation method.

Discussion

This research has contributed to knowledge on the nature of PCV2 infection in Australia. It has demonstrated potential production impacts of PCV2 infection in a herd that showed no obvious PCV2-associated disease. The work showed the efficacy of PCV2 vaccination in reducing serum viral load to undetectable levels.

The research identified PCV2b, PCV2d, PCV2f and an intermediate clade as the predominant PCV2 genotypes in Queensland. This is the first report of PCV2d and PCV2f in Australia. The finding of PCV2d is of concern because of its virulence and association with PCV2-associated systemic disease outbreaks in many countries.

Work on the development of a rapid pen-side diagnostic test has shown promising results. Such a test will be beneficial to the pig industry in Australia as it would allow producers to assess vaccine efficacy and determine the timing of PCV2 infection in their herds.
Further research on PCV2 in Australia should focus on exploring the true pathogenicity of the virus in this country. PCV2 vaccination is widely practised, despite the lack of solid evidence on the clinical and production impacts of PCV2 in Australia.

7.5 Objective 5: Assessment of adoption and impact

7.5.1 Desktop Assessment

Results

A desktop matrix was developed for specific components of the two ACIAR projects. The various components of the matrix (field work, laboratory work, and information, extension, training and communication) are provided in Appendix 11.5.1. (Tables 11.5.1.1, 11.5.1.2, 11.5.1.3).

Discussion

This desktop exercise involved the research team providing a critical self-assessment of the adoption of the field and laboratory components of ACIAR AH-2009-022 and ACIAR AH-2012-066 as relevant in the Philippines situation. The assessment identified that in 15 areas of activity in the field work area, the research team concluded that seven areas had resulted in high adoption by relevant areas and staff of the Philippines Department of Agriculture. A similarly high level of adoption for the laboratory work in the Philippines was achieved – four of six areas being assessed as having a high level of adoption.

7.5.2 Quantitative and Qualitative assessment stage

Laboratory and Field Aspects

A full summary of the interviews with the key actors in ACIAR AH-2009-022 is provided in Appendix 11.5.2. There was strong agreement on the following significant outcomes from the Philippines based work:

- Lung Scoring Manual
- Field Sample Collection Manual
- Disease Outbreak Investigation Manual
- Biosecurity Protocol
- Bacterial isolation protocols for *Actinobacillus pleuropneumoniae* (APP), *Mycoplasma hyopneumoniae, Haemophilus parasuis, Pasteurella multocida, Streptoccocus* and *Bordetella*
- Polymerase Chain Reaction protocols for *Actinobacillus pleuropneumoniae* (APP), *Mycoplasma hyopneumoniae, Haemophilus parasuis, Pasteurella multocida, Streptoccocus* and *Bordetella*

- Water Analysis protocols.

The field manuals were valued by the research team members but there was limited on-going use (particularly of the lung scoring manuals). This limited use was connected to the fact that the government offices that held the manuals were not directly involved in on-going activities in the various slaughterhouses. The laboratory protocols now appear to be imbedded (in varying forms in the formal protocols of the veterinary laboratories of the Department of Agriculture – national and regional levels).

The international and Australian impact of the Australian-based laboratory was undertaken by independent reviewers (see Appendix 11.5.1 for full details) and provided clear evidence of the high impact of this work. At the international level, independent reviewers confirmed that the work on the recognition of an entirely novel bacterial species (*Glaesserella australis*) and the development of standard methods for performing antimicrobial sensitivity tests for *Glaesserella parasuis* were significant impacts. The reviewers confirming the relevance of this work were based in both major universities (Denmark, Germany and the UK) as well as inside a major multi-national pharmaceutical company.

In terms of the Australian and Philippines impact, Dr Peter Scott was uniquely placed to assess this impact due to his role in commercial vaccine and diagnostic companies in both countries. His independent review identified the relevance and importance of the impact of the laboratory assays flowing from both ACIAR projects and thus confirms the impact of these projects in both Australia and the Philippines.

Formal scientific publications linked to this project have already been cited 93 times in the peer review literature. As the publications only began appearing in 2014, this is futher clear evidence of the impact of this work at the international level. At the national level in Australia, the technologies reported in these publications are now in routine use in the reference services provided by UQ to guide sustainable prevention and control programs for porcine respiratory disease in the Australian pig industry.

Farmer Survey

A total of 27 of 31 members of Group A farmers, 18 of 27 members of Group B farmers and 46 of 360 members of Group C farmers were surveyed across April and May 2019. Females constituted 63%, 33% and 48% of the Groups A, B and C respectively.

There was a major adoption of the nipple drinker technology (project changed use from 13% to 70%), a positive recognition of the usefulness of the practice and a commitment to continue the use of this technology into the future (70% commitment into the future) (see Appendix 11.5.2, Tables 11.5.2.1 and 11.5.2.2 and Figure 11.5.2.1).

There was a similar marked adoption of the creep box technology (project changed use from 38% to 94% adoption), a positive recognition of the usefulness of the practice and a commitment to continue the use of this technology into the

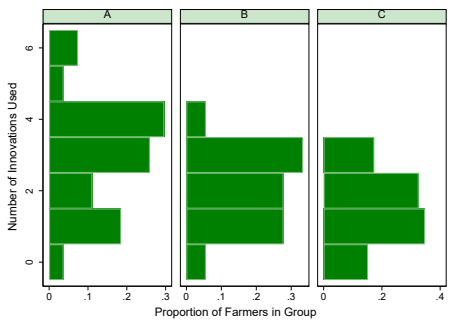
future (55% commitment into the future) (see Appendix 11.5.2, Tables 11.5.2.3 and 11.5.2.4 and Figure 11.5.2.2).

There was a more limited adoption of the project notebook to record both business and production records. Before the project, 33 of the 41 famers who responded in this area were using very informal systems (writing on the wall of the piggery and/or in a calendar) while 19 were using some form of a formal notebook for production records. After the project intervention, the wall/calendar option was used by 16 farmers while a formal notebook system was being used by 23 farmers. Thus, the change to a formal system only gained 4 farmers over the life of the project (see Appendix 11.5.2, Figures 11.5.2.3 and 11.5.2.4).

The market text price blast technology was quickly accepted by the farmers with 39/58 farmers that responded using the technology. As well, of 39 farmers who used the technology, 1 farmer found the technology to be not useful, another farmer could not decide if the technology was useful while the remaining farmers found the project innovation to be useful (20 farmers) or very useful (17 farmers).

Of the two environmental innovations introduced by the project, 14 of the 91 survey farmers had adopted the rain water harvesting technology (essentially rain water tanks) while 4 farmers had adopted the biogas generator technology. The costs associated with the installation of the biogas units (both purchase of the equipment and installation costs) on these four farms were subsidised by BAI (equipment) and the project (installation costs). who linked with the project to stimulate on-going farmer interest in this innovation. The shared interest of the project and BAI in biogas digestors suggests that future interactions on the environmental impact of small holder pig production systems should be a high priority for research projects.

In terms of an increase in income, 31 of the 91 surveyed farmers felt that they had increased their income by >25%, 44 farmers believed that their income had increased but by less than 25%, 12 farmers felt that there has been no change while 4 farmers could not say if their income had changed. An overall impression of the adoption of project interventions is provided in Figure 7.5.1.



Project Interventions:-

- Nipple drinker
- Creep box
- Project record keeping book
- Market text price blast
- Rainwater harvesting
- Biogas

Figure 7.5.2. Number of project interventions adopted by farmers in Groups A, B and C as at May 2019.

Agricultural Extension Worker Survey

A total of seven agricultural extension workers (AEWs) were surveyed. The survey revealed that prior to the project only two AEWs had received any swine-related training. As a result of the project, six AEWs gained new skills the Upskilling Workshop provided by the project and two gained new skills via a Lung Scoring Workshop provided by the project.

Barangay Co-ordinator Survey

The 14 barangay co-ordinators (12 male, 2 female) were surveyed. Survey responses were obtained from 10 of the co-ordinators. These responses showed that only 4/10 had been exposed to swine-related training prior to the project. Over the course of the project all 10 co-ordinators attended project seminars, workshops and training activities. The detailed results (Appendix 11.5.2.5) show the barangay co-ordinators were active supporters of the project and provided advisory and technical services to their fellow small-holder farmers. As an example of the impact of the project in this area, Figure 7.5.3 shows the changes in the advise and services provided by the co-ordinators from before the project to during the project for farmers raising piglets and fatteners.

Figure 7.5.3. Changes in the advice and services provided by barangay co-ordinators from before the project to during the project (data collected in May 2019).

7.5.3 Final impact assessment

Laboratory and field aspects

In the Philippines context, the project has been able to achieve adoption of project innovations or variations of those innovations in the laboratory context by input into the protocols adopted by the animal disease diagnostic laboratories (national and regional) within the Bureau of Animal Industry. The impact is clear and obvious in those laboratories directly connected to the projects (regional level RADDL III, IV and V and national level ADDRL). As an example, standardised methods using quality control strains to ensure appropriate test performance are now in routine use. The impact of the innovations is limited by the fact that the commercial and small-holder pig farmers make limited use of the government laboratory system. However, no other realistic option exists for improving animal disease diagnostic and investigation services to small-holder pig farmers. Hence, in this context, the projects appear to have had the maximum likely impact and progress along the adoption pathway given the project resources. A key point is that the main regional laboratory associated with these projects is now the centre of a major upgrade based on a joint funding scheme with the American and Philippines

governments. Most of the project innovations will therefore continue across into this entirely new facility well into the future.

The Australian based laboratory work has had significant impact at both the national and international level. The national impact has been direct and rapid, mostly due to the fact that the host laboratory is a national reference laboratory for pig bacterial respiratory pathogens. Hence, the innovations flowing from this project have been quickly and effectively moved across to the "user pays" service provided by the University of Queensland. As well, because of the cross-connections between a key user of those services (ACE Laboratories) and a Philippines-based associated company (9 Global ACE Technologies), the innovations developed in the Australian laboratory component are now available to the Philippines pig industry via 9 Global ACE Technologies, a company based in Pampanga. At the international level, the independent reviewers, based at Imperial College, University of Copenhagen and Elanco Animal Health, have confirmed significant impact and uptake for two key findings of the laboratory work – the development of a standardised protocol for antimicrobial resistance testing of *G. parasuis* and the recognition of a novel bacterium present in diseased pig lungs (*G. australis*). As confirmed by Prof Simjee, the project work will ultimately result in a standardised protocol officially approved by CLSI (one of the main accrediting agencies for laboratory protocols) for antimicrobial resistance testing for *G. parasuis*. Given the significant importance of antimicrobial resistance as a major "One Health" issue, the ability of laboratories around the world to perform standardised testing for a major pathogen is major impact of these ACIAR projects. The impact of the work recognising the occurrence of *G. australis* will continue to grow as the UQ research team has now obtained funding to develop a diagnostic assay for this organism.

Overall, the laboratory components of the two projects have moved significantly down the impact and adoption pathway with benefits flowing to the pig industries of Australia, the Philippines and the rest of the world.

Small-holder pig farmers

The general details of the adoption pathway for the work involving small-holder pig farmers is illustrated in Figure 7.5.3. As detailed in Section 7.5.2, project AH-2012-066 has clearly reached the stage of achieving impacts and adoptions of innovations at the "Next User" level. However, the innovations were not adopted in a uniform manner. Figure 7.5.4 summarises how the farmer perceptions of the project innovations have influenced the adoptions. It is clear that innovations that are perceived by the farmer as being useful and which require only a low capital and time investment (ie nipple drinkers, creep boxes, market text price blasts) are innovations that can be the focus of future activities to reach a much wider audience of small-holder farmers. While the adoption rate for record-keeping and biogas digestors was low, the farmers clearly recognised the innovations as being useful. This would suggest that factors identified by the farmers such as time requirements (record keeping) and capital requirement (biogas) are overcoming the perception of usefulness and inhibiting adoption. Future activities to assist small-holder farmers should include these innovations but need additional support mechanisms e.g. case studies from this project that show the value that can be gained from innovations (and which require good record keeping to validate those gains).

To assist in these future activities, a partial budgeting activity has been performed and full details of the economic returns are shown in Appendix 11.5.3. This analysis has shown that a net positive change of ₱2,625 ($US50) per litter. As well, a cost-benefit analysis of a biogas digestor system is provided in Appendix 11.5.3. This analysis indicates that a return on investment occurs after 1.5 years, provided that three households are connected to the unit, with the unit having a 15 year life span.

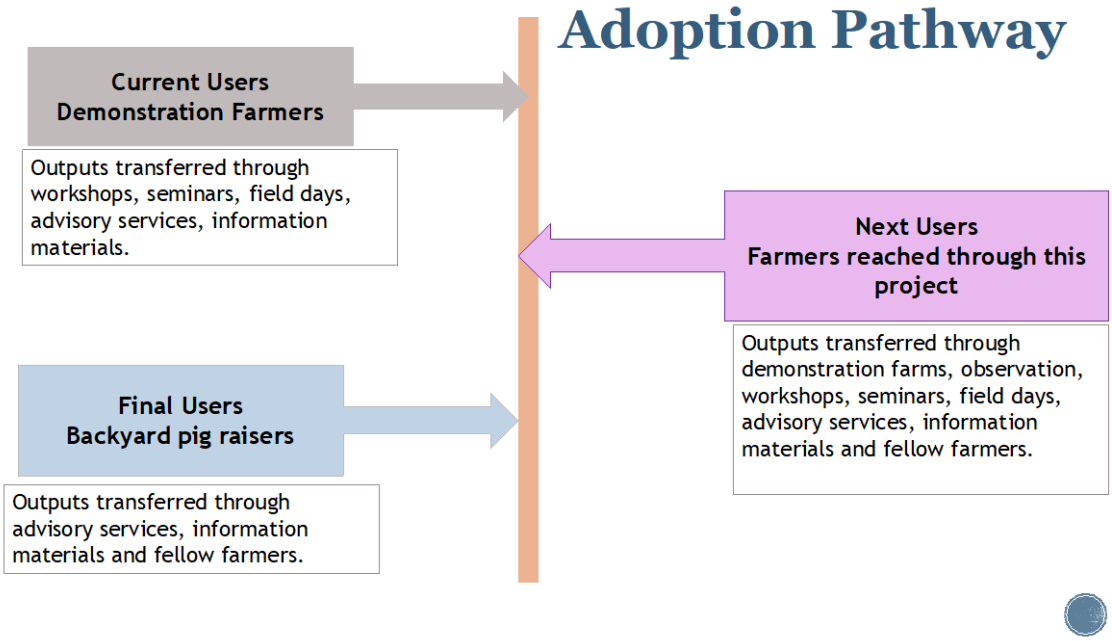


Figure 7.5.3. Generalised vision of the adoption pathway envisaged for small-holder pig farmers.

		Farmer Adoption of Techniques	
		High Adoption	**Low Adoption**
Farmer Perception	**Highly Useful**	Nipple Drinker Creep Boxes Market price text blast	Record Keeping Biogas
	Low Useful		Rainwater harvester

Figure 7.5.4. Interaction of farmer perception of the level of usefulness of the project innovations and the adoption of those techniques by the farmers.

8 Impacts

8.1 Scientific impacts – now and in 5 years

A full detailing of the current scientific impacts of both projects (AH-2009-022 and AH-2012-066) has been provided under Objective 5. A highlight is that, at the international level, independent reviewers have confirmed that the recognition of an entirely novel bacterial species (*Glaesserella australis*) (AH-2012-066) and the development of standard methods for performing antimicrobial sensitivity tests for *Glaesserella parasuis* (AH-2012-066) were significant impacts.

In 5 years' time, the two key scientific advances highlighted above are likely to have been progressed even further. Specifically, within the next five years, CLSI (one of the major international leading laboratory standard protocol authorities) will have formally approved the antimicrobial sensitivity testing protocol developed for *G. parasuis*. This is a major scientific impact. Antimicrobial resistance is major issue that links both animal and human health. Until the work undertaken in these projects (specifically AH-2009-022), one of the major pathogens that drives antimicrobial use in the pig industry (*G. parasuis*) has had no standardised protocols to test for antimicrobial resistance. This means that all treatments were essentially being undertaken without any validated, reliable and reproducible laboratory assays.

The second major international level impact has been the recognition of *G. australis* (occurred within AH-2012-066). The University of Queensland research team has continued to work on this pathogen using funds obtained from the Australian pig industry. The current activity of this industry funded research is the development of a rapid, specific and sensitive molecular assay that can be used on cultures obtained in the laboratory or directly on relevant pig tissues. The work has progressed well and this assay will be available via formal scientific publications within five years. Hence, laboratories around the world will have a diagnostic assay that can be used to identify this organism and help clarify the role of the organism in pig respiratory disease. These outcomes (a novel, previously un-recognised bacterial species and a specific and sensitive assay) are significant outcomes at the international level.

8.2 Capacity impacts – now and in 5 years

At the national level in the Philippines, developments in laboratory protocols have been imbedded into the government laboratory system, both via the national laboratory (ADDRL, Quezon City) and in the regional laboratory network provided via the Bureau of Animal Industry. Hence, the capacity of the government diagnostic systems have been significantly improved by these projects. As well, the field investigation skills of the provincial veterinary offices have been markedly improved. The capacity impact at the national laboratory level, the three involved regional diagnostic laboratories and the four provincial veterinary offices directly involved in these two projects has been convincingly demonstrated in the research activities undertaken in both projects. A diffusion of the capacity impact to other regional laboratories and provincial offices has occurred but will not have been as

solidly founded as in the laboratories and provincial offices directly involved in this project.

Due to the involvement of an Australian vaccine and diagnostic company in both the Australian pig industry (ACE Laboratories) and the Philippines pig industry (9 Global ACE Technologies), the high technology laboratory assays developed by these projects for the Australian pig industry are now also available to the broad Philippines pig industry via the diagnostic services provided by 9 Global ACE Technologies. As 9 Global ACE Technologies also provides killed autogenous vaccines that are supported by the diagnostic assays transferred by the Australian research team, the Philippines pig industry has the capacity of using locally produced autogenous vaccines for pig bacterial respiratory diseases that are supported by relevant diagnostic assays. This capacity did not exist prior to these projects and has only been possible because of the Australian based research program and the dual connection of that research team to both the Australian (ACE Laboratories) and Philippine-based (9 Global ACE Technologies) specialised diagnostic and vaccine companies.

In the Philippines context, the next five years should see the completion of a significant upgrade of the key regional veterinary diagnostic laboratory (RADDL III) involved in both AH-2009-022 and AH-2012-066. This upgrade is proceeding under a USA-Philippines funding scheme. Hence, the capacity improvements achieved by the ACIAR projects will be further enhanced by a significant investment in laboratory infra-structure.

A characteristic feature of both projects has been that the Philippines research team has mainly been based in government agencies. This has meant that Philippines staff (field and laboratory) have, in most cases, had their first opportunity for active involvement in research activities. Philippines staff in these government agencies have gained real-life research experience across many areas – e.g. laboratory work, field work, bacteriology, virology, and epidemiology. Most of the Philippines research team have been permanent government employees, meaning that these new skills remain available within the relevant government agencies (both at the national and regional level) and represent a considerably enhanced capacity to assist the animal industries of the Philippines. As well, a number of the research team members have been young scientists just beginning their working career. These young scientists have been upskilled in essential research skills such as experimental design, data analysis and oral and written communication skills (with the latter involving effective communication for a range of audiences from small-holder farmers to professional colleagues). Finally, both the young and more mature Philippines team members have been provided with extensive opportunities to attend both national and international meetings, resulting in a significantly enhanced professional network that will provided long-term and on-going support and inspiration.

In terms of the Australian research team, the project has provided significant career enhancement and improvements in capacity. Specifically, Dr Tamsin Barnes has gained experience in research leadership via her role as deputy-leader of the Australian research team. This experience has given Dr Barnes a full exposure to all of the range of activities associated with research leadership, particularly in large international projects. In five years time, it is highly likely that

Dr Barnes will be a key research leader of international projects. This progression from assistant leadership to full leadership is already underway with Dr Barnes leading ACIAR funded research activities in Timor Leste. Dr Gomathy Palaniappan is a mid-career researcher who has also gained considerable new capacities and experiences from this project. Specifically, Dr Palaniappan has an extensive background in working with small-holder vegetable farmers. Her involvement in an ACIAR project based on small-holder pig farmers has given her valuable experience and insight into an entirely different, but related, farming system. Again, this experience will significantly enhance the capacity of Dr Palanippan to contribute to international research in developing countries.

8.3 Community impacts – now and in 5 years

8.3.1 Economic impacts

As outlined in earlier parts of this report, both cost-benefit analysis and partial budgeting methods have been used to gauge the economic impact of the implementation of the innovations introduced by this project. While differing methods have been used, the consistent finding has been that small-holder farmers gain a net economic advantage from implementation of project innovations. As an example, the partial budgeting approach used in Objective 5.3 demonstrated that adoption of two innovations (nipple drinker and creep box) resulted in an increase of ₱2,625 ($50) per litter. The adoption survey demonstrated that the use of the nipple drinker rose from 13% to 70% of farmers and the use of creep box from 38% to 94%. This would suggest that a reasonable estimate of the adoption of both nipple drinker and creep box induced by the project is 32% (the prior use of the nipple drinker being 38% and the project induced adoption of the nipple drinker resulting in a 70% usage – ie a double adoption caused by the project of 32%). This means that 134 farmers adopted both innovations and were gaining ₱2,625 ($US50) per litter. This means, that assuming just one litter a year, the project has resulted in a positive impact of ₱351,120 ($US 6,754) for the San Simon small-holder pig farmer community.

8.3.2 Social impacts

Human Capital

The project's 14 barangay coordinators play two indispensable roles: one for the attainment of the project's overarching goals and the other for their respective community. For the former, they primarily support the project team in terms of disseminating project-related findings. Their community role, on the other hand, is to liaise with different key community actors such as barangay officials, *purok* leaders, and the barangay people to meet the needs of the pig farmers.

A before and after survey was conducted with the barangay coordinators. The results exhibited that majority of the barangay coordinators attended the project seminars that included Water and Nutrition Seminar series; Piglet Management and Diarrhoea Seminar series; and Record Keeping Workshops. The barangay coordinators have shifted their services from technical (castration, iron injection, and vaccination) to promoting project innovations such as the use of creep box and use of nipple drinker. Barangay coordinators read the diarrhoea leaflet, followed by water management, piglet management, and creep box material during dissemination on innovations. The barangay coordinators expressed

willingness to continue to disseminate information to the pig farmers in their community.

Social capital

As an impact of the project, social capital has been generated through establishment of a Hog raiser cooperative with around 25 to 30 members. A timeline of activities for the establishment of the cooperative is provided below:

Timeline for establishing a cooperative

Dates	Activities
February 14, 2018	Orientation of farmers on how to start forming a cooperative. Ms. Julieta Romero informed them about the requirements in creating a coop and how to sustain it.
August 30, 2018	Pre-registration Seminar of the Cooperative Development Authority (CDA) was conducted at Benigno Hall, Capitol Compound, City of San Fernando, Pampanga. Some of the interested barangay coordinators and their friends from their respective barangays attended the seminar. This is a pre-requisite before a group can start creating a formal cooperative.
February 19, 2019	Refresher Course on Pre-Registration for Cooperatives Since the Pre-Registration Seminar conducted at Benigno Hall was attended by a huge crowd from different areas of Pampanga, the farmers had a hard time grasping the seminar. Thus, Ms. Julieta invited a speaker from CDA to deliver the seminar. Now, its audience is only the San Simon farmers.
February 28, 2019	Organizational meeting was conducted for the voting of the following: • Voting for the Board of Directors (at least five, through a secret ballot). • Voting for the cooperative's official name. • Voting for their depository bank. • Voting for the official mailing address of the cooperative. • Voting for the initial membership fee.
March 2019	They have reserved a name for the cooperative at CDA: San Simon Hog Raisers' Producers Cooperative. Organization of documentary requirements
April 2019	They are working on treasurer's affidavit. They are working on completing their payments for the registration (500PHP) and shares (500PHP per share – each member with 4 shares) They have opened a bank account for their coop. Organization of documentary requirements.

Dates	Activities
June 2019	Status: Continuous collection of registration fee and solicitations were done to meet a total of 57,500 pesos (2,000 capital share and 500 membership fees per member)

8.3.3 Environmental impacts

The project sought to encourage the adoption of two technologies that have the potential for a marked positive environmental impact. As noted in the main body of this report, one of these innovations – treatment of the piggery waste in a biogas digestor – was perceived as a highly useful technology but there was little adoption of the technology. It would appear that the high capital cost of this innovation has resulted in a low level of adoption. The cost-benefit analysis undertaken within the project confirmed that a return on investment was possible after 4 years (single household use) or 1.5 years (4 household use). All future attempts to encourage use of this technology should emphasise both the environmental benefits as well as the economic benefits. The cost-benefit analysis on the biogas digestor undertaken in this project should be highlighted as evidence of the economic benefits of this technology in future attempts to promote this technology.

The second environmentally connected innovation was the rain-water harvester. However, this innovation was seen as having little usefulness by small-holder farmers. As the location of this project was a region that has traditionally suffered regular floods, the farmers clearly could not see the relevance of this technology. The concept of water conservation remains important and any future projects involving small-holder farmers should continue to consider this innovation.

8.4 Communication and dissemination activities

A number of dissemination and communication activities were undertaken over this project. Full details are provided in Appendix 11.6. A summary of the key communication and dissemination activities is provided below:

Two Eco-Health Workshops – attendees – farmers, baranagay co-ordinators, Municipal Agriculture Officers, traders, agrivet owners;
Multiple seminars on Water and Nutrition for Small-holder pig farmers – attendees – 198 farmers (102 females, 96 males);
Multiple seminars on Piglet Management and Diarrhoea – attendees – 155 farmers (85 females, 70 males).

As well, press articles directed a general audience were produced as follows
Backyard pig raising – more than a piggy bank – MARID Agribusiness Digest (December 2015, 45-46);
Facing the realities backyard swine raising – SWINE Magazine (February 2016, 23)
Facing the realities backyard swine raising – MARID Agribusiness Digest (January 2016, 20-21).

The project also maintained a Facebook page. This was a closed group and allowed quick communication. The total group members reached 39 with a number of the small-holder pig farmers of San Simon being active members of the group.

9 Conclusions and recommendations

9.1 Conclusion

9.1.1 Objective 1: To improve the diagnostic systems for respiratory disease in two selected Regions of the Philippines

The key aim of this objective – the establishment of improved and integrated diagnostic services that linked the field aspects, the slaughterhouse aspects and the laboratory aspects in two new Regions – was achieved. The successful completion of the small-scale studies, one in each of the two new regions, provided clear evidence of the achievement of this aim.

9.1.2 Objective 2: To identify, document and address constraints to the use of good disease prevention practices by smallholder pig farmers in Region 3

The use of an Eco-Health approach has underpinned the work performed in this Objective. The research team had to initially acquire a range of new skills and techniques. These new skills were then used by the research team to proceed through two full cycles of a classic eco-health project – knowledge generation, action and reflection. A key component of this project was the full involvement of the small-holder farmers. A range of interventions (seminars to address a lack of knowledge, development of a record keeping system, adoption of nipple drinkers, creep boxes, market price text blasts, biogas digestors and rainwater harvestors) were identified in a participatory process and then applied by the research team. The feedback provided from stakeholders, particularly small-holder farmers, indicated strong support for the interventions.

9.1.3 Objective 3: To estimate the incidence and economic impact of two disease syndromes of importance to smallholder farmers

Work under this objective focussed on the incidence and economic impact of disease on small holder pig farms in San Simon. While the original proposal called for work on two disease syndromes, the work undertaken as part of Objective 2 revealed that the key problems facing small holder pig farmers were very basic problems in pig management and nutrition. Indeed, work under Objective 2 identified only one disease as being of any significance – piglet diarrhoea. Hence, this objective had, with approval from ACIAR, a focus on just piglet diarrhoea. The research allowed the development of an understanding of the overall economics of small-holder pig production in San Simon. As well, some understanding of the economic implications of piglet diarrhoea was developed. As noted under Objective 2, all economic analysis work was greatly limited by the poor record keeping activities of typical small-holder farmers. One major outcome of this project is the development of a simplified record keeping notebook that will allow far better economic analysis in future studies.

9.1.4 Objective 4: To develop new diagnostic tests for, and undertake applied epidemiological research on, pig diseases important to Australia

Work under this objective has focussed on the development of technologies and diagnostic assays that are highly relevant and immediately applicable to the Australian pig industry, an industry that is supported by well funded research and diagnostic laboratories within government (State and Federal levels), Universities

and private companies. The work has had a series of important practical and relevant outcomes. In. the bacteriology work, there has been the recognition of a previously unrecognised bacterium that is linked to porcine respiratory disease and the development of novel tools for the typing of key bacterial pathogens that has provided a solid basis of knowledge for the Australian pig industry and the associated vaccine companies as they have sought to improve the efficacy and coverage provided by the current vaccines for porcine pleuropneumonia and Glässer's disease. In the virology component of this work, significant new knowledge on the nature of PCV-2 infection in Australia has been gained – particularly the understanding that high levels of the virus can occur in herds with no obvious PCV-2-associated disease, suggesting potential production impacts in the absence of frank clinical disease. Vaccination was shown to be effective, being able to reduce levels of the virus in serum to undetectable levels.

9.1.5 Objective 5: Assessment of adoption and impact

The impact adoption study has clearly demonstrated that the laboratory and field aspects of AH-2009-022 and AH-2012-066 have achieved marked progression along the adoption pathway. Indeed, there are key innovations that have clearly reached the end of the adoption pathway (e.g. the looming integration of the protocol for antimicrobial sensitivity testing as a fully approved CLSI methodology).

Project AH-2012-066 had a strong focus on assisting small-holder farmers. There has been progress in this area with most innovations developed by the project being perceived as being useful by the farmers (five of six innovations), although only three achieved a strong adoption. The results of the impact study indicate that these three innovations (nipple drinkers, creep boxes and market price text blasts) have moved from demonstration farmers to the level of "Next Users". Additional strategies to support the other innovations that were perceived as being useful by the farmers but were not widely adopted are provided to guide future activities.

9.2 Recommendations

1. Future work with small-holder pig farmers in the Philippines should continue to have a participatory focus as this approach allows a strong ownership by the farmers and other stakeholders and therefore increased likelihood of adoption by end-users.
2. Future work with small-holder farmers in the Philippines should focus on a total holistic approach. Small-holder pig farming is a key activity in the overall economy of the Philippines. However, small-holder pig farming is often only part of the activities occurring on the farm. Future projects should focus on a holistic approach that covers all the operations (animals, crops and vegetables) that are often typically part of a small-holder farming operation.
3. Future projects on small-holder farming systems in the Philippines should seek close linkages with the relevant authorities (local, regional and national) that deal with public health and environmental health. Initial contacts and linkages were made in the current project. These initial, preliminary contacts highlighted the shared overlapping interests of small-

holder farmers as well as their immediate local surrounding community and ultimately the larger community that consumes the food produced by the farmers. The small-holder farmers, their local community and ultimately broad society all operate within an eco-system that is influenced by all present in the eco-system. There is a need for projects to work broadly across the eco-system (ie adopt an Eco-Health approach) to ensure benefits from project activities flow to all within the eco-system.

4. Food safety, antimicrobial resistance and waste management are topics that have been little covered in projects with a focus on small-holder pig farming. These topics are now significant issues and there is an urgent need for research to understand the current status of these issues within this form of farming. With a basis of knowledge, it would then be possible to support actions to ensure that this form of farming is producing quality food in a sustainable, responsible manner.
5. The dual approach used in this project (specific activities to benefit the relevant, targeted Philippines agricultural industry and then specific activities to benefit the Australian pig industry) is an approach that provides considerable direct benefits to both countries and should continue to be encouraged.
6. The opportunities for shared relevant project activities between Australia and developing countries will increase as many Australian animal industries move, due to consumer demands, to organic, free range type production systems. These emerging organic, free range production systems share many of the characteristics of the small-holder operations that are a feature of developing countries. Hence, the opportunities for highly relevant, shared interest projects relevant to both Australia and our developing country partners should be the focus of attention into the future.

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10.2 List of publications produced by project

10.2.1 Formal Scientific Publications

1. Turni, C., Singh, R., Blackall, P.J. 2018. Genotypic diversity of *Pasteurella multocida* isolates from pigs and poultry in Australia. Australian Veterinary Journal 96, 390-394.

10.2.2 International Conference Presentations

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27. Turni, C., Singh, R., Blackall., P.J. 2017. Genotypic diversity of *Pasteurella multocida* from pigs in Australia. In TropAg 2017 (Brisbane, Australia).

10.2.3 National Conference Presentations

1. Allam, A.G., Palaniappan, G., Barnes, T.S., Lantican, T.L., Lapuz, E.L., Alvaran, P.J., Villar, E.C., Baluyut, A., Blackall, P.J., DeCastro, R., Domingo, R., Ignacio, C., Mananggit, M., Meneses, S.M., Meers, J., Palmieri, C., Parke, C., Turni, C. 2018. Utilizing sociology in understanding the adoption of record keeping among smallholder pig farmers in San Simon, Pampanga, Philippines. In 55th Scientific Seminar and Annual Convention, Philippines Society of Animal Science (Davo City, Philippines).
2. Alvaran, P.J., Allam, A.G., Ancog, R.C., Baluyut, A.S., Bernales, S.M., Lantican, T.L.D., Barnes, T.S., Blackall, P.J., de Castro, R., Ignacio, C., Lapuz, E.L., Lantican, T.L.D., Mananggit, M.R., Meers, J., Palaniappan, G., Palmieri, C., Parke, C.R., Turni, C., Villar, E. 2017. Using an Ecohealth approach to identify constraints to smallholder pig production in the municipality of San Simon, Pampanga, Philippines. In 54th Scientific Seminar and Annual Convention, Philippines Society of Animal Science (Cebu City, Philippines).
3. Alvaran, P.J., Barnes, T.S., Bernales, R.P., Lapuz, Jr., E.L., Villar, E.C., Allam, A., Baluyut, A., Blackall, P.J., DeCastro, R., Domingo, R., Ignacio, C., Lantican, T.L., Mananggit, M., Meneses, S.M., Meers, J., Palmieri, C., Parke, C., Palaniappan, G., Turni, C. 2018. Lung scoring as a tool to evaluate thoracic lesions in pigs from selected slaughterhouses in the province of Albay, Philippines. In 55th Scientific Seminar and Annual Convention, Philippines Society of Animal Science (Davo City, Philippines).

4. Barnes, T.S., Alvaran, P.J.J., Lantican, T.L.D., Lapuz, E.L., Baluyut, A., Parke, C.R., Palaniappan, G., Cameron, D., Meneses, S.M.A., Ancog, R.C., Domingo, R., Mananggit, M.R., Turni, C., Meers, J., Alawneh, J.I., Palmieri, C., de Castro, R., Villar, E., Blackall, P.J. 2016. Understanding the smallholder pig production system in San Simon, Pampanga, Philippines: a mixed methods approach. In Science Week Meeting - Australian and New Zealand College of Veterinary Scientists (Surfers Paradise, Australia).
5. Barnes, T.S., Alvaran, P.J., Parke, C.R., Lajarca, A., Villarba, L., Rosales, J.S., Moog, S.J., Lasay, J., Tolentino, J., Abe, F.S., Bernales, R., Adonay, F., Tapel, M., Lantican, T.L.D., Allam, A.G., Lapuz, E.L., Mananggit, M.R., de Castro, R. Baluyut, A.S., Ignacio, C., Ancog, R.C., Domingo, R., Palaniappan, G., Alawneh, J.I., Turni, C., Meers, J., Palmieri, C., Villar, E.C., Blackall, P.J. 2018. Evaluation of gross thoracic lesions in pigs from selected slaughterhouses in the provinces of Batangas and Albay in the Philippines using latent class analysis. In Science Week Meeting - Australian and New Zealand College of Veterinary Scientists (Surfers Paradise, Australia). https://drive.google.com/drive/folders/15pitglmRMHKHj1U4u-jhRLKCOEaOWBpC
6. dela Cruz, A., Meers, J., Turni, C., Azul, R., Legaspi, C., Barnes, T.S., Parke, C.R., Retes, L., Alawneh, J.I., David, J.E., Mananggit, M.R., Blackall, P.J., Villar, E.C., Lapuz, Jr., E.L., Baluyut, A.S., Basinang, V., Domingo, R., de Castro, R.O., Palmieri, C. 2018. Evaluation of PCV2 infection in pigs by histopathology, IHC and qPCR in tissue and serum samples from the Philippines. In Australian Society for Microbiology Annual Scientific Meeting (Brisbane, Australia).
7. Lantican, T.L.D., Barnes, T.S., Lapuz, E.L., Palaniappan, G., Villar, E.C., Baluyut Jr., A., Palmieri, C., Parke, C., Turni, C., Meers, J., Mananggit, M., Blackall, P.J., Alvaran, P.J.J., Ancog, R., de Castro, R., Domingo, R., Meneses, S.M. 2017. Understanding the smallholder pig marketing system in San Simon, Pampanga, Philippines. In 54th Scientific Seminar and Annual Convention, Philippines Society of Animal Science (Cebu City, Philippines).
8. Lantican, T.L., Barnes, T.S., Palaniappan, G., Allam, A.G., Lapuz, Jr., E.L., Alvaran, P.J., Villar, E.C., Allam, Baluyut, A., Blackall, P.J., DeCastro, R., Domingo, R., Ignacio, C., Mananggit, M., Meneses, S.M., Meers, J., Palmieri, C., Parke, C., Turni, C. 2018. Understanding the importance of record keeping to assess profitability and enhance financial literacy of selected smallholder pig raisers in San Simon, Pampanga, Philippines. In 55th Scientific Seminar and Annual Convention, Philippines Society of Animal Science (Davo City, Philippines).
9. Mone, K., Turni, C., Kyaw-Tanner, M., Barnes, T.S., Parke, C.R., Alawneh, J.A., Blackall, P.J., Meers, J. 2018. Molecular characterization of porcine circovirus type 2 in South East Queensland pig herds. In Australian Society for Microbiology Annual Scientific Meeting (Brisbane, Australia).
10. Padiernos, R.B.C. Alvaran, P.J., Mananggit, M.R., Parke, C.R., Barnes, T.S., Allam, A., Baluyut, A., Blackall, P.J., DeCastro, R., Domingo, R., Ignacio, C., Lantican, T.L., Lapuz, E., Meneses, S.M., Meers, J., Palmieri, C., Palaniappan, G., Turni, C., Villar, E. 2018. Evaluation of fecal samples from smallholder pig farms in San Simon, Pampanga, Philippines. In 55th Scientific Seminar and Annual Convention, Philippines Society of Animal Science (Davo City, Philippines).

11. Turni, C., Giang, N., Wu, Y., Omaleki, L., Blackall, P.J., Christensen, H. 2018. *Glaesserella australis* sp. nov., isolated from the lungs of pigs. In Australian Society for Microbiology Annual Scientific Meeting (Brisbane, Australia).
12. Turni, C., Giang, N., Wu, Y., Omaleki, L., Christensen, H., Blackall, P.J. 2017. *Actinobacillus* Taxon C - a new species not to be ignored**.** In Australasian Pig Science Association Meeting (Melbourne, Australia).
13. Turni, C., Giang, N., Wu, Y., Omaleki, L., Christensen, H., Blackall, P.J. 2017. *Actinobacillus* Taxon C - a new species not to be ignored. In Australian Pig Veterinarians Conference 2017 (Perth, Australia).
14. Turni, C., Giang, N., Wu, Y., Omaleki, L., Christensen, H., Blackall, P.J. 2017. *Actinobacillus* Taxon C - a new species not to be ignored. In Australian Association Veterinary Laboratory Diagnosticians Conference 2017 (Sydney, Australia).