Australian Government

Australian Centre for International Agricultural Research

Final report

Small research and development activity

project	**A one health approach to establish surveillance strategies for Japanese encephalitis and zoonotic arboviruses in Papua New Guinea**
project number	LS/2018/213
date published	November 2022
prepared by	David Williams
co-authors/ contributors/ collaborators	Mr Orlando Mercado, Dr Ilagi Puana, Ms Bridgit Kavana, Ms Rose Yalwan, Dr Stephan Karl, Ms Joelyn Goi, Dr William Pomat, Dr Paul Horwood, Dr Leanne Robinson, Dr Melanie Koinari
approved by	Dr Anna Okello
final report number	FR2022-025
ISBN	978-1-922787-45-3
published by	ACIAR GPO Box 1571 Canberra ACT 2601 Australia

Contents

1 Acknowledgments

The project leader and collaborating lead scientists wish to express their thanks and gratitude for the valuable contributions of the staff involved in this project.

In particular, from PNG National Animal Health & Quarantine Authority (NAQIA), we thank and acknowledge:

- Ms Bridgit Kavana (Project Officer) for her tireless laboratory work and commitment to the project, as well as Ms Rose Lisania, Ms Elaine Hevoho, Mr Fova Naoka and the staff of the National Animal Health and Food Testing Laboratory for their support, dedication and contributions to laboratory testing associated with the project.
- Mr Pius Clement, Mr Martin Pain, Mr Fova Naoka, Ms Elaine Kagena, Mr Daniel Kelly, Mr, Ligani Libama, Dr Estelo Quimbo, Dr Ian Enriquez, Mr Heni Nigani and NAQIA Animal Services staff involved in organising and sampling sentinel animals in Central, Morobe and Western provinces. We also thank the North Fly Department of Agriculture and Livestock for their assistance with mosquito surveillance in Kiunga.
- Mr. David Tenakanai (General Manager Technical Services), Plant Health Officers (Mr Noah Saruwa and Ms Marilyn Apa and cadets Mr Felicitas Bureng, Ms Fiona Timea and Mr Steve Tamuki), Animal Health Officers (Mr Pius Clement, Ms Elaine Kagena, Dr Ian Enriquez, Mr Heni Nigani and Mr Raymond Niaka) for facilitating access to farms for mosquito trapping and providing workspace for mosquito sorting.
- Dr Nime Kapo (PHAMA Plus) and NAQIA Animal Health Officers Mr Wilson Guhe, Mr Paskalis Ominipi, Ms Valentina Yamenea and Dr Tania Areori, for their contributions to the field evaluation of African swine fever rapid tests.

From PNG Institute of Medical Research, we thank and acknowledge:

- Ms Joelyn Goi (Project Officer) for leading and organising the mosquito trapping trips in Port Moresby, Lae and Kiunga, as well as Ms Rebecca Vinit and Muker Sakur for the technical support of these trips.
- Ms Tamarah Koleala, Ms Rosemary Simbil and staff at the Partnership in Health Laboratory, Port Moresby for providing support and training in PCR testing for Ms Bridgit Kavana (NAQIA).

We thank Dr Melanie Koinari (formerly of Divine Word University, now at James Cook University), for her support of studentships and analysis of the mosquito trap evaluation. We also thank Dr Scott Ritchie and Dr Dagmar Meyer Steiger (James Cook University) for their expert advice on mosquito traps.

We also wish to acknowledge the generosity and support of the farms and private owners of pigs for the use of their valuable animals as sentinels and allowing location access for mosquito trapping. In particular, we thank farm managers Mr. David Duran and Dr Anchie Cruz of Rumion Ltd (Lae), Mr Craig Bradfield of Boroma Piggery (Hiri District), Mr Steven Qiu of Eden Green farm (Hiri District), and Mr Peter Moody of City Mission Farm (Hiri District). We also thank Mr Nunu Vaguia (Papa village), Mr Ranu Mase Ori (Lea Lea Kampa village), Mr Nao Nao (Papa village), Mr Rei Roni (Roku village), as well as farm owners in North Fly District for hosting mosquito trapping activities.

At the Australian Centre for Disease Preparedness, the commitment and dedication of the following staff involved in laboratory testing and training of NAQIA staff is gratefully acknowledged: Mr Antonio DiRubbo, Ms Julie Cook, Dr Tim Bowden, Mrs Shaylie Latimore, Dr Jianning Wang, Mrs Som Walker, Mrs Donna Gillies and Dr Matthew Neave. Thanks also go to Dr Peter Durr and Mrs Kerryne Graham for epidemiology support and analysis, and to Dr John Allen for his early encouragement and ongoing support.

The support of the Australian Department of Agriculture via Dr Jenny Baird for funding the supply of African swine fever PCR reagents to NAQIA is gratefully acknowledged.

The project leader wishes to specifically thank the numerous contributions of his more experienced collaborating scientists, and for sharing their advice, expertise, knowledge, contacts, enthusiasm and good will; especially Dr Ilagi Puana, Mr Orlando Mercado, Mr Andy Yombo. Mr David Tenakanai (NAQIA), Prof. Willie Pomat and Dr Moses Laman (IMR), Dr Stephan Karl and Prof Paul Horwood (James Cook University) and Prof. Leanne Robinson (Burnet Institute).

Finally, special thanks and gratitude go to the ACIAR program managers Dr Francette Dusan (now DFAT) and Dr Anna Okello for their constant support, advice and encouragement.

2 Executive summary

Zoonotic arthropod-borne (arbo)viruses of public and veterinary health importance are known to circulate in Papua New Guinea (PNG), including Japanese encephalitis (JE) virus, the major cause of arboviral brain disease in humans in Southeast Asia. Despite their disease potential, little is known about the epidemiology of these arboviruses in PNG. The overall aim of this project was to establish pilot surveillance activities for JE and other zoonotic arboviruses at selected sites in PNG, using a combination of sentinel animal and mosquito trapping methods. To achieve this, a One Health strategy was adopted, underpinned by laboratory and field capacity building, involving the National Agriculture Quarantine & Inspection Authority (NAQIA) and the PNG Institute of Medical Research (IMR) as major partners, along with Australian partners at James Cook University and the Burnet Institute.

Laboratory capacity building primarily focussed on the NAQIA National Animal Health and Food Testing Laboratory (NAHFTL) and was achieved via a combination of laboratory training placements at ACDP, equipment upgrades, in-country technical backstopping visits, and online meetings. ELISA format antibody testing was operationalised at NAHFTL for use in arbovirus testing as well as other priority diseases such as African swine fever (ASF), which emerged in PNG in 2020. Inter-laboratory comparison testing undertaken between ACDP and NAQIA demonstrated the competency of NAHFTL staff to perform these tests. Two laboratory assessments of animal disease testing were undertaken in 2019 and 2021, which identified specific gaps in laboratory infrastructure and performance, and facilitated awareness of NAHFTL staff and NAQIA management of the requirements to meet national laboratory quality assurance and biosafety standards.

A second major capacity building outcome was the implementation of molecular (PCR) testing for NAHFTL, which was achieved through collaboration and support from ACDP and PNG IMR. This was undertaken at the PNG IMR Partners in Health (PiH) PCR laboratory. This capability has the potential to be applied to PCR testing for other priority animal diseases. A fundamental gap that remains is laboratory infrastructure for NAHFTL to accommodate a fit-for-purpose PCR suite. Towards fulfilling this objective, ACDP worked with NAHFTL on the scope and design of PCR suite on the NAHFTL site.

The major scientific finding of this project was evidence of continued circulation of JE and other zoonotic flaviviruses in PNG. Serological evidence of JE virus infection was found in sentinel pigs in National Capital District (NCD), Central and Morobe provinces in 2019-2020, as well as in pig samples collected from 2016-2020 in NCD, Sandaun, New Britain, Enga and Western Province for disease surveillance programs. Antibodies to Kunjin and Murray Valley encephalitis viruses were also detected in small numbers of pigs and chickens from Sandaun, NCD and Morobe, indicating co-circulation of these viruses.

Mosquito surveillance was performed in NCD, Central, Morobe, Madang and Western provinces in 2019-2020. A pilot comparison of three mosquito-trapping devices to identify a suitable and cost-effective surveillance tool for the major vectors of zoonotic arboviruses (*Culex* species) showed that CDC traps with ultraviolet light were were more effective than CDC traps with incandescent light and BG sentinel traps. A total of 22,735 female mosquitoes were caught, of which the majority (~84%) were *Culex*. From PCR testing of 253 pools of mosquitoes, two were positive for JE virus (Morobe) and five positive for Kokobera virus (NCD/Central, Western Province, Madang), which is related to JE virus but not known to be a significant cause of human disease.

Taken together, these findings are significant for public health in PNG in relation to potentially unrecognised clinical cases of flavivirus disease and strengthen the justification for ongoing surveillance and investment in zoonotic arbovirus surveillance to better understand the epidemiology and disease burden of JE in PNG.

3 Background

Japanese encephalitis (JE) virus is the most important cause of human viral encephalitis in Southeast Asia. Even though an effective vaccine is available to prevent JE, approximately 67,000 human cases occur annually worldwide (Campbell et al., 2011). The JE virus (genus *Flavivirus*, family *Flaviviridae*) is mosquito-borne, and pigs and waterbirds act as reservoir (amplifying) hosts. JE disease primarily affects rural communities, with highest rates of disease in children. Most infections are asymptomatic, but when disease occurs, it is associated with high rates of morbidity (~50%), mortality (~30%) and long-lasting side effects (~25%).

In Papua New Guinea, there is strong epidemiological evidence that JE is endemic (reviewed in Mackenzie et al. 2007 and Jonduo et al., 2012). Human sera collected between 1989-1995 from the Western, Gulf and Southern Highlands provinces were found to be antibody positive to JE virus. Virus isolations were made from *Culex* species mosquitoes collected in Western province in 1997-98, as well as from neighbouring Torres Strait islands where an outbreak occurred in 1995 and was followed by frequent seasonal activity in subsequent years. Unconfirmed human outbreaks have also been reported in Milne Bay province and confirmed human cases have been reported from Port Moresby.

JE is also an economically important disease of animals, causing potentially fatal encephalitis in horses and reproductive failure in pigs. In horses, JE can result in similar outcomes to human disease. Infection of pregnant sows or gilts can lead to abortions and stillbirths, while infection of boars can cause aspermia. Encephalitis has also been observed in piglets following experimental infection (Yamada et al., 2004).

There is currently no JE vaccination program in PNG. However, because of the complexity of the JE virus ecology, even if routine vaccination were implemented, it would not prevent virus circulation and potential for disease in the unvaccinated. Thus, surveillance is critical to monitor virus activity, and to initiate preventive measures before peak periods of circulation. Currently, there are no routine surveillance programs in PNG for vectors and hosts of JE virus. Existing knowledge of JE epidemiology in PNG has largely been derived from the occurrence of human cases and from limited serological surveys or vector studies (Anga et al., 2010; Hanson et al., 2004; Johansen et al., 1997; 2000). Typically, the detection of JE virus in vectors or reservoirs early in an epidemic cycle precedes human infection by several weeks, providing an opportunity for disease preparedness and response.

Several other zoonotic arthropod-borne (arbo)viruses of medical importance have also been found in PNG, including Murray Valley encephalitis (MVE) and West Nile-Kunjin (KUN) viruses, close relatives of JE virus, and Ross River (RR) virus (genus *Alphavirus*, family *Togaviridae*). MVE virus is the major cause of arboviral neurological disease in Australia, affecting humans and horses. In PNG, MVE virus has been isolated from *Culex* mosquitoes in the East Sepik and Western provinces (Johansen et al., 2000), and despite serological evidence of widespread activity in lowland areas, only two human cases have been documented (in 1956 and 1960) (French et al., 1957; Essed et al., 1965). KUN virus can cause a febrile illness and mild encephalitis in humans and has also been associated with outbreaks of equine encephalitis in Australia. No human cases of KUN have been identified in PNG; however serological evidence in children in the Sepik district has been found (R. Hawkes, cited in Marshall, 1988).

RR virus can cause a crippling arthritic disease in humans and is the most common cause of arboviral disease in Australia, causing an average of ~4,700 cases each year (Claflin and Webb, 2015). Equine RR disease is also common, causing poor performance, lethargy and muscle stiffness in affected horses (Barton and Bielefeldt-Ohmann, 2017). RR virus has been isolated from over 40 species of mosquitoes including *Aedes* and *Culex* species. Marsupials are a major reservoir host and humans and horses have also been implicated in the absence of macropods (Claflin and Webb, 2015). Human cases of

RR disease have also been reported in PNG, and there is serological evidence of human infection across several provinces (Tesh et al. 1975; Hii et al., 1997). RR virus has also been isolated from mosquitoes collected in the Western province (Johansen et al., 2000).

Despite the pathogenic potential of these arboviruses for humans and livestock, little is known about their epidemiology in PNG, including accurate information of distribution, prevalence and seasonality. There is also a poor understanding of the impact of these viruses on human health, due primarily to a lack of surveillance and clinical reporting. The latter is confounded by high rates of other diseases with similar clinical presentations (e.g. fever and neurological signs), such as dengue and malaria (Anga et al., 2010). Even less is known about the contribution of these viruses to animal health. Shortages of veterinary services, remote locations of many villages and reluctance of many farmers to seek veterinary assistance for sick animals are likely contributors to this situation (Brioudes et al 2017).

The overall aim of this project was to establish pilot surveillance activities for JE virus and other zoonotic arboviruses that may affect rural and urban communities of PNG. To achieve this, a One Health strategy was adopted, involving human and veterinary/animal health organisations within PNG and Australia. Underlying this approach is closer cooperation between human and animal health sectors, and recognition of the link between human disease, animals and the environment. This approach is acknowledged as being optimal for leveraging capabilities in resource-poor countries and is appropriate for controlling zoonotic diseases that are influenced by human agricultural practices.

African swine fever in Papua New Guinea

In March 2020, the deadly swine disease African swine fever (ASF) emerged in the Highlands provinces of PNG (OIE, 2020). ASF is a highly lethal and contagious disease affecting wild and domestic pigs that has spread rapidly throughout China and Southeast Asia since 2018. It's emergence in PNG poses a serious threat to village production systems, and the incomes and livelihoods of rural communities, where pigs are highly valued as a cultural and economic livestock animal. To assist ASF control activities within PNG, led by NAQIA, the project aims were extended to include laboratory diagnostic capacity building and support for ASF surveillance. The ASF diagnostic activities complemented and extended those of the ACIAR SRA on 'Point of Care Diagnostics for Animal Disease Surveillance' (LS/2018/203), which had a focus on ASF field testing.

Justification

This project aimed to address a major gap in environmental surveillance of vertebrate hosts and mosquito vectors of zoonotic arboviruses of public health concern. The project team was assembled to represent relevant areas of animal and human health, with expertise in arbovirus and animal disease surveillance. The CSIRO Australian Centre for Disease Preparedness (ACDP; formerly Australian Animal Health Laboratory) was the lead organisation of this project. ACDP is an internationally-recognised animal health laboratory with a long history and strong track-record in diagnosis of and research on arboviruses. It is a national reference laboratory for arbovirus diagnosis and participates in national arbovirus surveillance programs. ACDP is a World Organisation for Animal Health (OIE) reference laboratory for several diseases of livestock, including ASF, and operates under an accredited quality assurance system. It is also an OIE Collaborating Centre for Veterinary Laboratory Capacity Building, with an active overseas training program providing capacity building throughout South-East Asia.

The project partners engaged in PNG represent key institutions involved in research, diagnosis, response and investigation of animal and mosquito-borne diseases. The two major project partners in PNG were the National Agriculture and Quarantine Inspection Authority (NAQIA) and the Institute for Medical Research (PNGIMR). In addition, the Central Public Health Laboratory in Port Moresby and Divine Word University in Madang

were partner organisations representing public health stakeholder and higher education, respectively.

Collaborating Australian researchers involved in the project were from James Cook University and the Burnet Institute. These researchers contributed relevant expertise and experience in mosquito-borne diseases and associated field work in PNG, and facilitated coordination and engagement with the complementary project 'Stronger Surveillance and Systems Support for Rapid Identification and Containment of Resurgent or Resistant Vector Borne Pathogens in Papua New Guinea' (STRIVE PNG), funded by DFAT. Several members of the project team are also investigators on the STRIVE PNG project, which focusses on mosquito-borne human febrile diseases such as malaria and dengue.

The key livestock species the project focussed on was pigs, the most important livestock animal for the predominantly rural population of PNG. Pigs are raised to cater for cultural exchange, to provide cash income, and as a dietary source of protein (Amben et al. 2017). In many parts of PNG, the number of pigs owned, along with their size and condition, is a measure of the social ranking of the owner. Pig husbandry also reflects gender relationships whereby women in some communities look after the pig but do not own them (Dwyer, 2006). Thus, pigs – an important amplifying host of JE virus – are an intrinsic part of the socio-cultural fabric of PNG.

It has been estimated that there are at least 1.8 million pigs in the smallholder sector, raised by over 360,000 smallholders. Annual production was estimated to be ~27,000 tonnes, which supplies over 93% of the pork consumed in PNG (Ayalew 2011, 2013; Bourke and Harwood 2009). In comparison, the commercial sector supplies ~3,200 tonnes of pork per annum (Ayalew, 2013).As the population of PNG continues to grow, pork production is expected to increase. Furthermore, as smallholders are encouraged towards commercial and semi-commercial production systems, the commercial sector has been predicted to grow (Ayalew 2011).

This project addressed Australia's goals to work in partnership with PNG on regional issues affecting the poor, including sustainability and resilience of production systems, including livestock health and production. In the 2017-18 ACIAR Operational Plan, it was recognised that capacity building and close collaboration with relevant Australian and PNG agencies plays an important role in strengthening biosecurity arrangements. The project also aligns with the ACIAR Animal Health program's focus on diseases that are of regional significance, transboundary, zoonotic, and affecting production. In addition, it aligns with the ACIAR 10-year (2018-27) strategy objective of building scientific and policy capability within partner countries.

The project addressed PNG's Vision 2050 strategy through addressing the pillar of human capital development, gender, youth and people empowerment. Specifically, it aimed to address the 'need to increase, improve and support current research-based institutions and universities to produce top quality research and development outcomes that will provide solutions to challenges in areas such as medicine, climate change and disease patterns'.

4 Objectives

The aim of the project was to establish pilot surveillance activities for JE virus and other zoonotic arboviruses of public health importance that may affect the predominantly rural population of PNG. To achieve this, the major objectives were to:

1. Evaluate current methods to detect zoonotic arboviruses (and ASF) in the field and in the laboratory, and build capacity where gaps were identified

2. Establish pilot surveillance activities at selected sites using one or a combination of sentinel animal (pigs, chickens) and mosquito trapping methods

3. Establish and develop linkages and coordination between human and animal health agencies

The primary outputs of this project were field- and laboratory-based capacity to develop longer term surveillance activities that can contribute to an early warning system for public health and provide a better understanding of the ecological drivers of viral and disease activity in PNG. The project also aimed to foster sharing of knowledge and capability between PNG and Australia.

5 Methodology

5.1 Objective 1. Evaluate current methods to detect zoonotic arboviruses and African swine fever in the field and in the laboratory, and build capacity where gaps are identified

5.1.1 Building flavivirus ELISA testing capacity at the National Animal Health and Food Testing Laboratory (NAHFTL)

Laboratory capacity-building activities for NAQIA scientists were undertaken for serological detection of antibodies to JE, MVE and KUN virus infections. A competitive enzyme-linked immunosorbent assay (C-ELISA) in use at ACDP was employed (Williams et al., 2001) for this purpose using tests reagents produced and supplied by ACDP for the detection of flavivirus-specific antibodies in both chickens and pigs. In total, three separate shipments of ELISA reagents were sent to NAQIA over the course of the project to address testing needs.

Implementation of ELISA testing began with an initial scoping visit to the NAHFTL at Kila Kila in May 2019 to meet NAHFTL-based project members and to evaluate existing laboratory equipment and infrastructure. A workplan was established to address equipment requirements, involving new filters for the ELISA plate reader and spare parts, and later replacement of the ELISA plate washer. Progress and troubleshooting were addressed as part of subsequent visits to PNG by ACDP staff and at project meetings.

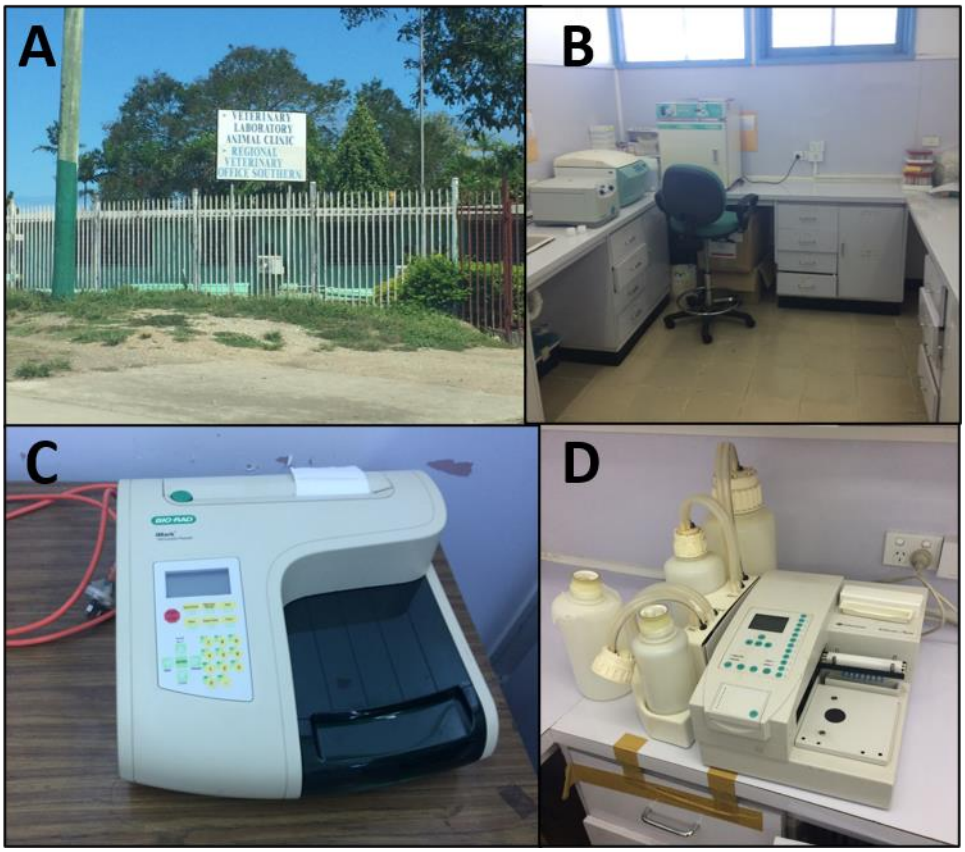


Figure 1. The entrance to the NAQIA National Animal Health and Food Testing Laboratory, Kila Kila (A). A work bay within the Serology laboratory (B); key equipment used for ELISA testing: ELISA plate reader (C) and plate washer in disrepair (D).

Staff training was undertaken via a placement of two NAHFTL staff members (Ms. Bridgit Kavana and Ms. Elaine Hevoho) at ACDP in September 2019 (Figure 2), which focussed on practical training in diagnostic ELISA methods and accompanying quality assurance procedures. Test documentation (e.g. worksheets, SOPs and progressive monitoring forms) were provided to NAHFTL to enable them to set-up testing. Editorial and technical support was also provided by ACDP for adapting SOPs to the NAHFTL format. Once testing was underway at NAHFTL, regular technical support and troubleshooting was undertaken via online meetings and email. Due to COVID-19 restrictions, further laboratory placements and in-country backstopping visits could not be undertaken, as originally planned.

Figure 2. Training placement at ACDP for NAQIA staff. From left to right: Mr Antonio DiRubbo (ACDP), Dr David Williams (ACDP), Ms Bridgit Kavana (NAQIA), Ms Elaine Hevoho (NAQIA), Dr Tim Bowden (ACDP).

5.1.2 Inter-laboratory Comparison Testing

An external quality assurance (EQA) inter-laboratory comparison testing (ILCT) assessment was undertaken to provide an objective measure of the ability of NAHFTL staff who had received ELISA training at ACDP to test serological samples from chickens and pigs for flavivirus infection using the flavivirus ELISA assays established at NAHFTL, and to support NAQIA in their capacity and capability to adopt and implement new test methods. The samples were prepared under ISO17025 and to ISO17043 principles at ACDP and the assessment was coordinated by the ACDP Proficiency Testing team. ACDP is a NATA-accredited provider of proficiency testing panels for a variety of diseases and tests. The flavivirus serology ILCT comprised a panel of 15 coded and well characterised positive and negative sera from pigs and chickens experimentally infected with either JE, MVE or KUN viruses at ACDP. A planned laboratory backstopping visit to support the inert-laboratory comparison testing could not be undertaken due to COVID-19-related travel restrictions.

Instructions were provided to NAHFTL for sample testing using the three C-ELISA methods for detecting antibodies to JE, MVE or KUN viruses. For samples that were positive in two or more C-ELISAs, further testing with samples titrated (diluted) from 1/10, in a four-fold dilution series to 1/640 was carried out to determine endpoint antibody values. If a sample was reported negative in the screening test, then there was no requirement to repeat the testing and titrate the sample. Endpoint titre values were compared for each test to determine the flavivirus species involved, using a document-

controlled Titre Estimation Spreadsheet. Differences in endpoint titre values of ≥4-fold was considered diagnostically significant.

5.1.3 Supporting African swine fever diagnostic testing at NAQIA

Laboratory and field diagnostic capacity building for ASF extended assessments previously undertaken via involvement of Dr Williams in the FAO Emergency Preparedness Mission to PNG in 2019, along with subsequent engagement with NAQIA for ASF diagnostic testing as part of ACDP's OIE ASF Reference laboratory role. For supporting existing laboratory diagnostics at NAHFTL, this activity focussed on ASF antigen ELISA testing, which is the main laboratory test performed at NAHFTL to detect the ASF virus in blood samples. Technical problems experienced in ELISA testing over the course of the project were addressed through troubleshooting with ACDP staff and a review of equipment levels at NAHFTL.

ASF field testing support focussed on the development of SOPs and worksheets for the use of the commercial (Ingenasa) rapid antigen test kits, which was identified as a priority be NAQIA staff to ensure that samples are fit-for-purpose and that testing is performed accurately. An evaluation of alternative commercial rapid test kits was also undertaken to provide a basis for back-up options should resourcing challenges be experienced with the Ingenasa kits. An assessment of commercially available field rapid antigen tests was performed to identify the best performing option(s) in terms of sensitivity and practical considerations (e.g. costs, delivery times etc). This involved a review of peer-reviewed literature on rapid antigen test validation data, supported by a side-by-side evaluation at ACDP of the Ingenasa kit with two other commercial rapid tests (Anigen Bionote Rapid ASFV Ag kit and the Pencheck Rapid ASFV screen kit). For this evaluation, blood samples collected from pigs experimentally infected with the pandemic strain of ASF virus were tested using each of the rapid antigen tests and real-time PCR (Zsak et al., 2005).

From this assessment, the Anigen Bionote ASFV Ag rapid test was selected to progress to a side-by-side field evaluation with the incumbent Ingenasa antigen test. An SOP was developed for the Bionote test and online training provided by ACDP to NAQIA field officers involved in this evaluation, which was performed as part of ongoing ASF disease investigation activities in affected PNG highlands provinces.

5.1.4 Building diagnostic PCR Testing Capacity at NAQIA

This objective focussed on ASF PCR to support NAQIA's ASF laboratory diagnostic capacity, which was identified as a priority diagnostic gap following the emergence of ASF in PNG. The pandemic strain of ASF virus that emerged in PNG typically causes acute disease and most pigs succumb before producing antibodies. Therefore, virus detection methods are the frontline diagnostic tests for ASF. Of these, the PCR test is considered the most sensitive and specific. Prior to the commencement of this project, NAQIA did not have PCR testing capability. ASF laboratory diagnosis was primarily undertaken using antigen detection tests (ELISAs), which lack sensitivity compared to PCR, resulting in false-negative results for a proportion (~20-30%) of samples tested.

To improve ASF laboratory diagnostic capacity at NAQIA, the ACDP supplied reagents for 500 ASF PCR tests for the OIE-recommended King assay (King et al., 2003). Primers and probe mixes were supplies as freeze-dried reagents, and positive and negative controls were shipped frozen, together with commercial PCR mastermix (AgPath-ID™ One-Step RT-PCR) and DNA extraction kits (Qiagen DNeasy Blood and Tissue). Funding for the PCR kits and reagents was supported by the DAWE.

ASF PCR capacity building was provided through a series of activities facilitated by ACDP, including presentations at NAHFTL on basic concepts of PCR diagnostics (June 2019) and PCR workflow and laboratory design (October 2019). A planned 4-week laboratory placement at ACDP for Ms. Bridgit Kavana (NAQIA) for PCR training and laboratory biosafety (scheduled for 22nd March – 18th April 2020) could not take place

due to COVID-19 travel restrictions. Alternative PCR training was provided to Ms. Kavana at the PNG Institute of Medical Research Partnership in Health Laboratory (PiH) located at University of PNG, Port Moresby, by IMR staff (Figure 3). This was facilitated through Dr Williams' involvement in the STRIVE PNG project, which also involved malaria and arbovirus PCR testing activities using human samples at the IMR PCR laboratory.

Figure 3. NAQIA project officer Ms. Bridgit Kavana performing PCR testing at the PNG IMR Partnership In Health PCR laboratory at University of PNG, May 2020. Credit: Tamarah Koleala

Initial training involved the use of samples collected for the STRIVE PNG project. Establishment of ASF testing was supported by SOPs and test worksheets for DNA extraction and ASF PCR provided by ACDP, which were then adapted to the NAHFTL Quality Assurance Manual format. Once ASF PCR testing was established at PNG IMR, testing was undertaken on serum and whole blood samples collected from pigs as part of disease investigation and surveillance activities by NAQIA from different highlands provinces. Where parallel testing had been performed at ACDP for confirmatory testing, results were compared to determine proficiency.

Planning activities for NAHFTL-based PCR testing were also undertaken, including advice on design and refurbishment of existing laboratory space, workflow and equipment needs. While the further development of this lab suite was beyond the funding of this project, this is expected to be achieved by future capacity-building projects. Although alternative temporary PCR workflow options at NAHFTL were considered, none of these were found to be suitable.

5.1.5 Laboratory audits

Two laboratory audits were performed using the FAO Laboratory Mapping Tool, a standardized spreadsheet-based assessment tool developed to help support national, regional, and global efforts to maintain an effective network of animal health and veterinary public health laboratories (Mouille et al., 2018). The FAO LMT format allows data to be captured either by external evaluators or through self-assessment. The tool is designed to facilitate the assessment of laboratory functionality in a systematic and semi-quantitative manner, to evaluate strengths and weaknesses. Results served to measure general progress of laboratories and targeted interventions, such as improvement of Quality Assurance (QA) and biosafety, over successive assessments.

The FAO LMT is based on a questionnaire that generates a laboratory profile or 'map' by scoring performance in different aspects of laboratory management, infrastructure, and technical performance. The primary FAO LMT Core module (LMT-C) was designed to

identify strengths and gaps in five broad categories: a general laboratory profile; infrastructure, equipment and supplies; technical performance; quality assurance, biosafety and biosecurity; and collaborations and networking. A second LMT-Safety Module (LMT-S) was also designed for a more detailed evaluation of the current and ongoing status of laboratory safety, including chemical and radiation safety, and with a strong focus on biosafety and biosecurity in veterinary laboratories (Mouille et al., 2018). Both tools are used to determine gaps in laboratory functionality and safety and define mechanisms and targets for capacity building to fill these gaps.

The LMT-C assessment was undertaken at NAHFTL by Dr David Williams in October 2019 during a project trip (Figure 4). A second assessment, including both the LMT-C and LMT-S was undertaken virtually by Mr Chris Morrissy (FAO) in May 2021. The assessments were performed with the approval of the FAO (Dr. Filip Claes, personal communication with D. Williams).

Figure 4. Laboratory assessment at NAHFTL, 22nd October 2019.

5.1.6 Database Management

To facilitate public health responses to results from surveillance activities and to enable epidemiological interpretation of arbovirus surveillance data, a database management activity was planned for data integration, developing data analyses procedures, and data management strategies. A review of database software used by the major project partners NAHFTL and PNG IMR was undertaken by ACDP project staff during the first two project trips in May and June 2019. Options for database management and integration of relevant project partners involved in arbovirus surveillance were explored at the first project workshop in June 2019.

A project database to capture sentinel animal surveillance data was also developed using Microsoft Excel software. Data included sentinel sites, locations and GPS coordinates, animal species, numbers of animals per site/bled, animal ages at sampling, and sampling dates.

An additional aim within this activity was to explore the integration of the vector and animal surveillance database with the Tupaia Db used for the STRIVE PNG project, in collaboration with relevant STRIVE project staff.

5.2 Objective 2. Establish pilot surveillance activities over consecutive transmission seasons

5.2.1 Mosquito Surveillance

Pilot mosquito surveillance activities were undertaken involving PNG IMR staff and targeted *Culex* species. The study was conducted at 14 agricultural farming sites in National Capital District (NCD), Central, Morobe and Western provinces, from November to December 2019 (NCD, Central), September to October 2020 (Morobe) and July to August 2021 (Western; Table 1). The high levels of rainfall and temperature at these sites are ideal for the development and reproduction of vector mosquitoes. The farms were selected based on consultation with relevant provincial NAQIA Animal Services teams, who undertake routine surveillance for livestock diseases but not mosquito surveillance. In Central and Morobe provinces, four farms (two piggeries and two chicken farms) were selected, and in Western province, four pig farms and two chicken farms were selected. Traps were placed adjacent to livestock pens at each of the farms. A provincial map of PNG and satellite locations of the sentinel sites is shown in Appendix 1.

In addition to mosquitoes collected from these surveillance sites, Culex mosquitoes collected as by-catch near Maprik in East Sepik for the International Center of Excellence in Malaria Research, and from Bogia, Morobe province for the Death to Onchocerciasis and Lymphatic Filariasis project were included in this study. Mosquitoes were collected using the human landing catch method in Bogia in August to September 2019 and in East Sepik in March to April 2019.

Table 1. Details of mosquito surveillance sites used in this project.

Province	Local level government	Collection dates	Site location details (animals*)	Average rainfall (mm)	Average temperature (°C)**
Central	Hiri Rural	26/11/2019-20/12/2019	City Mission farm (P,C) Papa-Lealea village (P) Roku village (P)	1,150	30
National Capital District	National Capital District	26/11/2019-20/12/2019	Boroma Piggery (P) Eden Green farm (P,C)	1152	27.0
Morobe	Lae Urban Wampar Rural	20/9/2020-16/10/2020	EBC Farm (P,C) Rumion Ltd Farm, (P,C)	4677	26.5
Western	Kiunga Urban	19/7/2021-20/8/2021	Kilo 1 (P) Kilo 6 (P) Kilo 8 (P) Peter's Pig farm (P) Broken Hill (C) Mepu Village (C)	4521	26.7

*Animal lures: P, pigs; C: chickens

**Climate data from Climate-Data.org and ClimateCharts.net

Figure 5. Different mosquito traps types employed for this project: BG Sentinel trap (A), CDC light trap with incandescent light (B) and UV light (C). Traps being hung and changed at EBC farm (D), Boroma Piggery (E) and Kiunga (F).

Three types of traps were evaluated to capture mosquito species responsible for transmission of zoonotic arboviruses. Trap types selected were those that did not use carbon dioxide, a commonly used mosquito attractant in the form of dry ice or gas from cylinders, which was considered impractical for use in remote parts of PNG. The three traps evaluated were: BG sentinel trap (BioGents, Regensbourg, Germany), which uses visual and olfactory cues to lure mosquitoes, and the miniature Centers for Disease Control (CDC) light trap with incandescent light (CDC_I) or ultraviolet light (CDC_UV; BioQuip, California, USA), which use light as the primary attractant (Figure 5).

Traps were placed for two periods of 5 days per farm. Specifically, for the first five-day period, five CDC_I traps and one BG trap were placed in one animal shelter while, while five CDC_UV traps and one BG trap were placed in another animal shelter. In the second five-day period, CDC_I and CDC_UV traps were rotated. CDC light traps were hung on animal housing 2 meters above the ground, while BGS traps were placed on the ground. Every 24 hours, mosquitoes were collected and transported to the laboratory for species identification. Mosquitoes were placed on white filter paper in a Petri dish, and the species identified morphologically under a stereo microscope (Zeiss, Jena, Germany) using taxonomic keys.

Culex mosquitoes, the main target vector species of this project, were pooled (up to n=10) according to species and feeding status and placed into the stabilisation and storage reagent RNAlater (ThermoFischer Scientific), using 100µl per mosquito. Pooled mosquitoes in RNAlater were stored at -4^{o}C overnight and then frozen at -20^{o}C.

Mosquitoes were sent to the ACDP for molecular testing by real-time reverse-transcriptase polymerase chain reaction (RT-PCR) to detect viral genomic RNA. Only unfed mosquito pools were tested to allow association of vector status to the

Table 2. Details of real-time RT-PCR assays used for testing pools of Culex mosquitoes.

Virus	Gene target	Source
Japanese encephalitis	NS1	Shao et al. (2018)
Murray Valley encephalitis	Env	Niazi et al. (2013)
West Nile-Kunjin	NS5-3'UTR	Pyke et al (2004)
Ross River/Barmah Forest	E1	Based on an in-house PCR from PathWest Laboratory Medicine WA
Kokobera	3'UTR	ACDP in-house test
Sindbis	nsP1	Sane et al. (2012)
Sepik	NS5	ACDP in-house test

corresponding species (blood-fed mosquitoes may carry viruses in their bloodmeals that do not reflect vector potential). Individual pools of mosquitoes were further combined based on trap location, date of collection and species in pools of up to 100 mosquitoes. The pools were then homogenised prior to nucleic acid extraction. The details of the RT-PCR tests used are shown in Table 2. In addition to JE, MVE, KUN and RR viruses, other arboviruses known to circulate in PNG and cause (or suspected of causing) human disease were also tested for; namely, Kokobera (KOK), Sepik (SEP) and Sindbis (SIN) viruses. A RT-PCR test targeting 18S ribosomal RNA (ThermoFischer Scientific) was also performed for each mosquito pool to ensure that extraction was successful and that no PCR inhibitors were present.

Minimum infection rate (MIR) was calculated as described previously (Bernard et al., 2001). MIRs were determined for single mosquito species from a defined location and trapping period and only if a minimum of 1,000 mosquitoes were tested.

Figure 6 shows images of the different sorting and processing stages following collection in the field and prior to RT-PCR testing at ACDP.

Figure 6. Sorting and processing mosquitoes for molecular testing. A NAQIA cadet officer removing insects from catch bags (A). Total insect catch placed into Petri dishes for sorting (B). Morphological identification and speciation of sorted mosquitoes using a dissecting microscope (C, D). Mosquitoes pooled by species and placed in labelled tubes containing RNAlater then stored in freezer boxes (F). Further processing of mosquitoes at ACDP by removing RNAlater and combining pools (G) followed by homogenisation in lysis buffer using carbide shards.

5.2.2 Animal Surveillance

Sentinel Animals

Sentinel animal surveillance for this project involved pigs and chickens located at commercial farms or villages. Sentinel animals are so-called because they can act as sensitive hosts for the detection of important viruses circulating in the environment and can potentially be employed to provide advanced warning of circulating mosquito-borne viruses. When infected mosquitoes feed on animals, they can pass the virus into the blood stream, which can lead to infection and antibody response against the virus. Blood collected at frequent intervals from sentinel animals is then tested for these antibodies in the laboratory. This project focussed on the detection of antibodies to JE, MVE and KUN viruses using established laboratory test methods.

Animal sentinel sites were selected based on existing sites used by NAQIA for livestock disease surveillance (Table 3). This work was approved by the ACDP Animal Ethics Committee (Approval no. AEC1978), in accordance with the Australian Government National Health and Medical Research Council *Australian code for the care and use of animals for scientific purposes*. A condition of this approval was that private owners of animals used for this project sign a CSIRO Animal Welfare Statement Use of Privately Owned Animals form, which outlined the scope of the project, potential risks to their animals and their responsibilities (Appendix 2). As incentive, pig owners were provided with sampling fees and veterinary services and advice by NAQIA officers, if required. Where possible, sentinel pigs were selected at sites that were nearby or adjacent to chicken flocks to enable a comparison of these two sentinel animal strategies. Examples of sentinel sites are shown in Figure 7.

Animals were tested at intervals for an approximately three-month period from the end of October 2019 to the end of February 2020. Blood sampling was undertaken by trained NAQIA Animal Services officers. For chickens, blood was collected from the brachial wing vein using a syringe and needle. For pigs, blood was collected from the jugular vein or anterior vena cava, depending on the size of the pig. For most sites, numbers of animals bled varied between collection dates, due to sale or slaughter or animal, or problems obtaining sufficient volumes of blood due to difficulties encountered when sampling. The ages of pigs at the commencement of sample collections varied depending on whether they were located at a village/backyard setting or commercial farm. Weaner piglets at 4 weeks of age were selected at commercial farms (i.e., Rumion Ltd, Boroma Piggery, City Mission and Eden Green farm), while in villages weaned, younger pigs of indeterminate age were selected. For chickens, mixed age birds were sampled from free-range layer flocks.

Testing of stored surveillance samples

In addition to blood samples collected from sentinel animals for this project, serum samples previously collected in PNG and submitted to ACDP for antibody testing for a range of porcine infections by NAQIA or by the Australian Department of Agriculture and Environment (DAWE) were tested to determine the seroprevalence of target zoonotic arboviruses. The details of these samples, collected between 2016 and 2018, are shown in Tables 4. Testing of these samples was undertaken with the permission of the Chief Veterinary Officer (CVO) of NAQIA and the DAWE. Samples collected by NAQIA for ASF surveillance in the highlands provinces in 2020-21 were also tested at NAHFTL using the flavivirus ELISAs, with the permission of the NAQIA CVO (Table 5).

A graphical representation of the locations and total number of serum samples collected from sentinel animals and surveillance programs is shown in Figure 8.

Figure 7. Animal sentinel sites used in this project. Sentinel pigs on commercial farms (A, B) and villages (C-F); sentinel chickens (F); NAQIA and Rumion Ltd staff involved in sample collection, Lae; engagement with village pig owners (H).

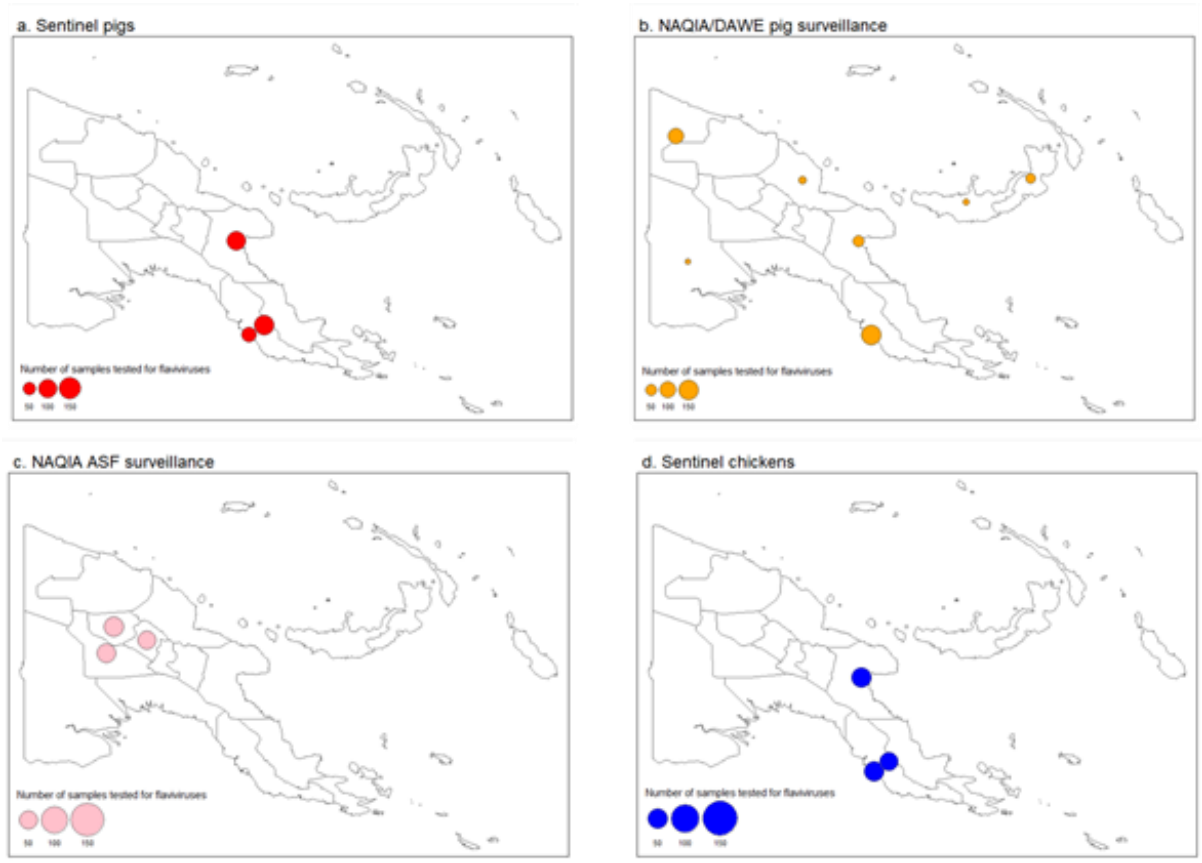


Figure 8. Number of serum samples tested for antibodies to the three flaviviruses of interest (JE, KUN and MVE viruses), according to the source and province from which the samples were collected.

Table 3. Details of sentinel animal surveillance sites and sample collections.

Province	Local level government	Site location details	Collection dates	No. pigs sampled	Chickens sampled
Central	Hiri Rural	City Mission farm	31/10/- 4/11/2019 13/12/2019 16/1/2020	16 15 10	16 13 15
		Papa-Lealea village	13/11/2019 11/12/2019 17-18/1/2020	15 12 14	NA
		Roku village	11/12/2019 17/1/2020	6 5	NA
		Hiri LLG Office	22/11/2019 16/1/2020	6 2	NA
		Sibore village	4/11/2019 13/12/2019 16/1/2019	5 8 4	NA
National Capital District	National Capital District	Boroma Piggery	4/11/2019 13/1/2020	20 19	NA
		Eden Green farm	2/12/2019 16/12/2019 15/1/2020 10/2/2020	10 10 10 ND	16 12 16 5
Morobe	Markham Valley*	Rumion Ltd Piggery	10/9/2019 25/9/2019 10/12/2019 15/1/2020 29/1/2020 12/2/2020 26/2/2020	20 20 20 20 10 10 NA	ND ND ND ND ND ND 10
		Ubix village	10/12/2019 15/1/2020 29/1/2020 26/2/2020	NA	9 10 9 10

*LLG not specified

Table 4. Details of pig samples collected as part of the Australian Department of Agriculture, Water and the Environment and NAQIA porcine disease surveillance in PNG, 2016-18.

Province	Location (LLG, District)	Collection dates	No. pig samples tested
East New Britain	Rabaul, Rabaul	2-9/9/2016	21
East and West New Britain	Various locations*	16-25/10/2017	36
National Capital District	National Capital District	4/2018-6/2018	147
Madang	Karkar, Sumkar	4-5/1/2018	4
	Karkar, Sumkar	2-6/4/2018	19
Morobe	Markham Valley*	17-26/9/2018	53
Sandaun	Vanimo, Vanimo/Green River	7-13/9/2016	80
		14-18/2/2017	17
Western	Daru, South Fly	5/3/2018	1
	South Fly*	19-28/2/2018	5
	Various locations*	6-18/3/2017	9

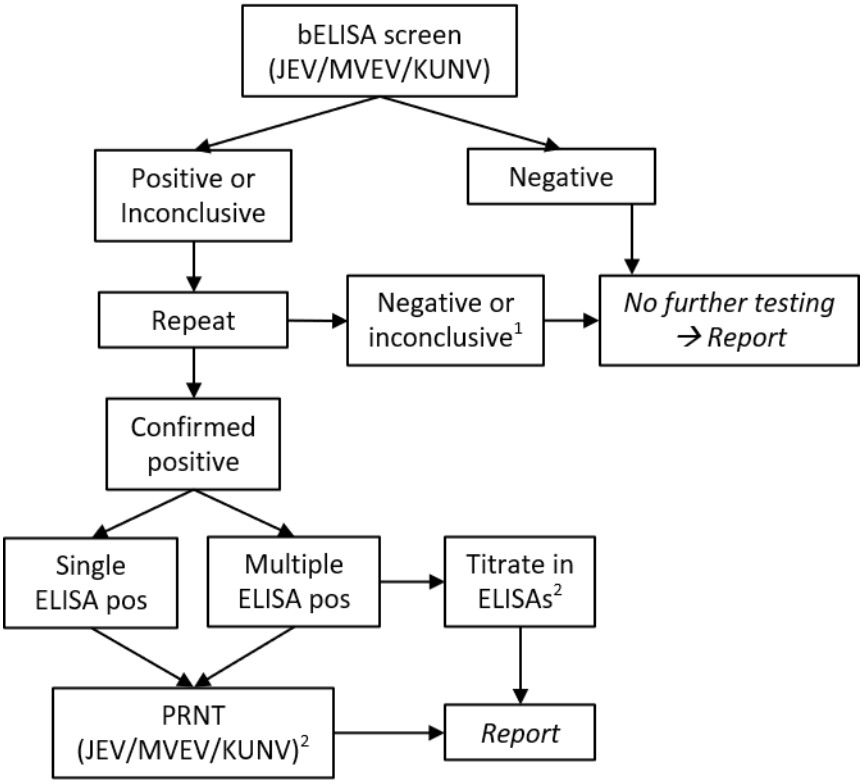
*LLG or district not specified

Table 5. Details of NAQIA pig sample collections for African swine fever surveillance in PNG.

Province	Location (LLG, District)	Collection dates	No. pig samples tested
Enga	Various locations	17/5-24/11/2020	50
Jiwaka	South Waghi, Anglimp/South Waghi	12/10/2020	2
Southern Highlands	Various locations	12/2/2020-2/2/2021	51
Western Highlands	Various locations	10/7/2020-2/2/2021	50

Laboratory Testing

Following sample collection, samples were sent to the NAHFTL for processing and frozen storage, and accession into the LIMS. Due to delays experienced at NAHFTL in establishing ELISA testing, chicken samples collected prior to the 15th January 2019 and pig samples collected prior to the 29th January were sent to ACDP to expedite testing. Remaining samples were tested at NAHFTL once the ELISA was established and verified in 2021. The testing algorithm at both ACDP and NAHFTL is shown in Figure 9. The gold standard plaque reduction neutralisation test (PRNT) was performed on selected samples at ACDP to confirm ELISA titration results. This test was not in scope for capacity building at NAHFTL due to its complexity.



[1]Confirmed inconclusive results may be tested further by PRNT at discretion of ACDP team
[2]If diagnostically significant difference in titre to any one virus (i.e., ≥4-fold), then report for that virus. If no significant difference (<4-fold), report as evidence of flavivirus infection.

Figure 9. Serological testing algorithm for samples collected from chicken and pig sentinel animals.

Ecological analysis of flaviviruses positivity in pigs and chickens

A digital reference map of PNG was used to display the number of serum samples from each province that were tested for antibodies to the three flaviviruses of interest, i.e. JE, KUN and MVE viruses. These samples were collected from four surveillance activities, *viz*. sentinel pigs for this study (2019-2020), sentinel chickens for this study (2019-2020), DAWE/NAQIA porcine disease surveillance (2016-2019), and NAQIA ASF surveillance (2020-2021).

To explore the effect of the timing and sampling location on seropositivity for the three flaviviruses for the four surveillance groups, we undertook a contingency table analysis. Each serum sample was classified as being overall flavivirus positive if one or more of the results for each virus ELISA was positive. To assess equality of proportions across the tabulated cells, we applied Pearson Chi-square test with a p-value <0.05 considered significant.

To enable an interpretation of the contingency table analysis of the effect of province on the percentage of samples that were flavivirus positive, we produced maps of long term average temperature and annual precipitation across PNG using the WorldClim 2.1 dataset (Fick and Hijmans, 2017). The global 5 minute BioClim gridded datasets for Annual Mean Temperature (“Bio1”) and total Annual Precipitation (“Bio12”) for the period 1970-2000 were downloaded from the WorldClim 2.0 website (https://www.worldclim.org/data/worldclim21.html#) and clipped to the digital vector map of PNG.

To enable a visual assessment of the impact of the seasonality of temperature and precipitation on the flavivirus seropositivity at the sentinel sites we constructed climatographs for the long term temperature and rainfall data collected at Port Moresby and Lae, using the ClimateCharts.net website (Zepner et al., 2021).

All mapping and statistical analyses were undertaken using the *R* statistical software environment (https://www.r-project.org/) version *4.02*. For the production of the maps we used the *tmap* package (Tennekes, 2018) with the vector spatial data read into *R* using the *sf* package (https://r-spatial.github.io/sf/articles/sf1.html) and the raster spatial data using the *stars* package (https://r-spatial.github.io/stars/articles/). For the contingency table analysis, we used the *chisq.test* functions of the *stats* package.

5.3 Objective 3. Establish and develop linkages and coordination between human and animal health agencies

Initial efforts to develop a framework for formal linkage and coordination were made through the first project workshop (Figure 10), which involved partners from the animal health (ACDP, NAQIA), human/public health (PNG IMR, CPHL and PNG Department of Health). Existing or potential cross-sector linkages were identified and explored, along with opportunities and options for reporting frameworks between the laboratories involved in arbovirus surveillance and response and other stakeholders. Successful models employed elsewhere (e.g. Australian state systems) were reviewed for potential adoption. It was anticipated that linkages between the relevant human and animal health partners will strengthen and develop as the project progresses through regular communication between partners and stakeholders (email updates, reports, project meetings) and through the planned inter-laboratory visits.

Figure 10. The first project partners workshop at NAQIA Headquarters, Port Moresby, June 2019.

5.4 Communication and travel

Multiple means of communication were employed between ACDP and the PNG country project partners, including email as the primary means, and complemented by the use of WhatsApp for both text-based and audio-visual calls. Following the emergence of COVID-19 and the availability of online software tools (e.g. Zoom, Teams, Webex), these were also used, particularly for larger group meetings involving partners from multiple locations. Once the project officer at NAQIA (Ms. Bridgit Kavana) was appointed, weekly meetings were scheduled via WhatsApp to review objectives and progress of NAHFTL-based activities. Prior to COVID-19, five trips were undertaken to PNG by ACDP staff for project activities from May 2019 to January 2020 (Appendix 3). All trips involved Port Moresby-based activities involving NAQIA and partners located there. Two trips each were also made to the Entomology laboratory at PNG IMR in Madang and to NAQIA at Lae.

Only one of the two project workshops originally planned was able to be completed on 26th-27th June 2019 at NAQIA Headquarters in Port Moresby (Appendix 4). This was followed by a technical workshop on Animal Surveillance at NAHFTL on the 27th June. The second and final face-to-face project meeting originally planned was not able to be undertaken due to COVID-19 travel restrictions. A virtual final project meeting is planned in early 2022.

Advanced planning for a NAQIA ASF Diagnostic Workshop was undertaken throughout 2021 as part of the ASF extension to the project. This involved regular online planning meetings organised by NAQIA with relevant groups involved, including ACDP and DAWE.

6 Achievement against activities and outputs/milestones

6.1 Objective 1: To Evaluate current methods to detect zoonotic arboviruses in the field and in the laboratory, and build capacity where gaps are identified

No.	Activity	Outputs/ milestones	Completion date	Comments
1.1	Project Meetings	First Project meeting – organisation of and delivery of presentations	27th June 2019	The first workshop of partners of the ACIAR project 'A one health approach to establish surveillance strategies for Japanese encephalitis and zoonotic arboviruses in Papua New Guinea', held over two days at NAQIA HQ. This meeting incorporated the zoonotic arbovirus workshop outlined in the SRA proposal (section 3.2).
		Technical workshop on flavivirus diagnostics – organisation of and delivery of presentations	28th June 2019	Held at NAQIA National Animal Health and Food testing Laboratory (NAHFTL), Kila Kila.
		Final project meeting - organisation of and delivery of presentations	To be confirmed	Due to delays in the final delivery of laboratory-based testing, the meeting was not held as originally planned. A virtual meeting is planned in early 2022.
		ASF Diagnostic Workshop – advanced planning and preparations	To be confirmed	Advanced stages of planning and preparation undertaken (Appendix 4), including programme, budget, staff nominations, and the majority of presentations written. Due to ongoing restrictions and lockdowns in PNG, it has not been possible to hold this meeting. Now planned for early 2022.
1.2	Laboratory Capacity building	Laboratory Training at ACDP (ELISA testing) - delivery	4th October 2019	One-week training visit at ACDP involving Ms. Bridgit Kavana and Ms. Elaine Hevoho from NAQIA, on performance and interpretation of flavivirus ELISAs; 30th Sept-4th Oct.
		Laboratory Training at ACDP (PCR testing) - delivery	June 2021	Second 4-week laboratory placement for Ms. Bridgit Kavana (NAQIA) and Ms. Joelyn Goi (IMR) in PCR training and laboratory biosafety was scheduled for the 22nd March – 18th April, 2020; partly funded by the DFAT Indo-Pacific Centre for Health Security. This was not completed due to COVID-19 travel restrictions. Alternative PCR training was facilitated at the PNG IMR PCR laboratory at University of PNG, accompanied by remote technical support from ACDP.

		ASF PCR testing capability	September 2021	PCR and DNA extraction kits and PCR controls sent to PNG IMR PiH PCR lab for NAQIA use. SOPs and worksheets written in collaboration with ACDP/NAQIA. Training facilitated for Ms Bridgit Kavana (NAQIA) by Ms Tamarah Koleala (PNGIMR). Pig samples collected as part of ASF investigations tested at IMR and compared to ACDP test results, with 100% result concordance. Ms Kavana considered proficient in PCR testing by Ms Koleala and ACDP.
1.3	Laboratory audits	Laboratory audit of NAHFTL – completed laboratory mapping tool	December 2020 October 2021	The FAO LMT-Core module assessment was undertaken for at the NAHFTL, 21st-25th Oct. Informal assessments were also undertaken during each of the visits to NAHFTL in 2019. A second LMT-Core audit was performed in 2021, along with the LMT-safety module.
1.4	Interlaboratory comparison testing (ILCT) and Laboratory Backstopping	ILCT panel produced and delivered to NAQIA, tested and report from ACDP	November 2021	ELISA test kits and ILCT panels sent to NAQIA, along with laboratory SOPs and benchnotes, which were also adopted to NAHFTL format. ILCT completed and final results submitted to ACDP July 2021. Final report issued Nov 2021.
		Backstopping visit and report	Cancelled	The scheduled laboratory backstopping was cancelled due to COVID-19 travel restrictions. Technical troubleshooting was performed remotely via a combination of web meetings, calls and emails.
1.5	Database Management	Review of PC databases	September 2019	A review of the databases in use by the major partners NAQIA and IMR was undertaken in the first and second project trips in May and June by ACDP staff. Development of a new LIMS for NAQIA is underway through the Fleming Fund Country Grant. It was therefore decided that the originally proposed enhancements to the NAHFTL MS Access LIMS database would be redundant and was not carried out. No changes were needed for the IMR EpiCollect Db.
		Development of sentinel animal surveillance Db	February 2020	A project Db for animal surveillance data was established and is in current use.
		Integrated databases for epidemiological analyses	Not completed	The originally planned integration of vector and animal surveillance Db with the Tupaia Db used for the STRIVE PNG project was not completed due to the late delivery of animal surveillance and mosquito testing.
1.6	Supporting ASF testing	Technical advice and support for lab and field diagnostics	May 2021	Reviewed ASF Antigen ELISA testing procedures at NAHFTL and advice provided on troubleshooting technical problems experienced. Advice also provided for ASF surveillance strategy.

		Evaluation of ASF rapid antigen tests	Partially completed	Lab-based assessment of three different available commercial tests completed at ACDP in April 2021. SOPs and training in use of Ingenasa and Bionote kits provided to NAQIA animal services officers; Bionote kits provided. Only limited testing in the field has so far been undertaken due to limited opportunities. This activity will continue beyond the project into 2022 until sufficient numbers of field tests have been performed.

6.2 Objective 2: Establish pilot surveillance activities at selected sites using one or a combination of sentinel animal (pigs, chickens) and mosquito trapping methods

No.	Activity	Outputs/ milestones	Completion date	Comments
2.1	Mosquito surveillance	Pools of mosquitoes collected from three trapping trips in Western, Morobe and NCD provinces in PNG.	October 2021	Mosquito trapping trips for this project were completed in four provinces (Table 1). In addition, Culex mosquitoes caught as by-catch for other projects at Bogia and East Sepik were contributed for testing. A total of 22,735 mosquitoes were collected, of which 19240 were Culex.
		Trap evaluation	February 2020	Three mosquito traps were evaluated during for the collection of Culex species mosquitoes during each of the trapping trips: Biogents Sentinel, CDC Light Trap Normal and CDC Light Trap UV. The latter was found to be optimal for this purpose. A manuscript reporting these findings has been accepted for publication.
		PCR testing of mosquito pools	November 2021	A total of 15,617 unfed female Culex species mosquitoes from 253 pools were tested by PCR for JE, MVE, KUN, KOK, SEP, SIN and RR/BF viruses at ACDP.
2.2	Sentinel Animal Surveillance	Serological test results for sentinel animal samples Assessment of seroprevalence for target viruses at sentinel locations	October 2021	Nine sentinel sites were established in three provinces and 2 to 7 rounds of sampling were undertaken during 2019 and 2020. ELISA testing of 438 samples was performed at ACDP and NAHFTL An additional 393 pig serum samples collected by DAWE in various provinces of PNG as part surveillance activities was tested at ACDP. Another 153 pig serum samples collected for ASF surveillance from the highlands provinces of PNG were tested at NAHFTL.
2.3	Preliminary epidemiological study of zoonotic arboviruses in PNG	Report on combined vector and animal surveillance activities and findings	November 2021	Analysis of the results of laboratory testing of mosquito pools and pig and chicken serum samples is reported below. A high level of seroprevalence to JE virus was found in pigs, which were found to be a more sensitive sentinel animal than chickens. JE virus RNA was detected in 2 pools from Morobe province. Several KOK viruses were also detected from multiple provinces.

6.3 Objective 3: To Establish and develop linkages and coordination between human and animal health agencies

No.	Activity	Outputs/ milestones	Completion date	Comments
3.1	Support cross-sector linkages and reporting	Awareness between partners of roles and activities	Partially completed	First project meeting brought together relevant public health, animal health, entomology and vector surveillance partners and stakeholders. Cross-sector linkages identified at this meeting and via the FAO LMT-C assessments. Further scoping required for a formal reporting framework for arbovirus surveillance results, and to confirm key public health stakeholders/contacts. This will be followed-up as part of post-project activities to disseminate the project findings.
		NAQIA assist and support to PNGIMR for vector surveillance	October 2021	NAQIA provided advice, staff and facilities for mosquito trapping trips to NCD, Central, Morobe and Western provinces (Table 1) for ZAPPA and PNG STRIVE projects.
		PNG IMR assisted and supported NAQIA for PCR training and capability	December 2020	Refer to above output on ASF PCR testing capability. A MoU to promote and maintain a sustainable collaborative One Health Approach between NAQIA and PNG IMR has been drafted to underpin future one health collaborations.
		Historical human JE case data	Partially completed	Contacts at CPHL made for provision of unpublished human JE data to complement animal and mosquito surveillance results, to provide a public health context for the findings on JEV surveillance work completed in this project. Data not yet obtained and will be completed as part of subsequent manuscript write-up post project.

7 Key results and discussion

7.1 Diagnostic test capacity building at the National Animal Health and Food testing laboratory (NAHFTL)

7.1.1 Flavivirus ELISA testing capacity

At the commencement of this project in 2019, general ELISA testing capacity was not operational at NAHFTL. Through the assistance provided by the project, general ELISA testing capacity was re-established at the NAHFTL, underpinning the implementation of specific C-ELISA tests required to test for antibodies to JE, MVE and KUN viruses in pig and chicken serum samples. C-ELISA test capability was enabled through the provision of staff training, test materials and reagents, and test documentation (e.g., SOPs and worksheets), electronic progressive monitoring documents and an electronic calculator to determine test results and endpoint titres.

Key items of equipment were also upgraded, repaired, or purchased to support general ELISA testing capacity at NAHFTL. This included the servicing and addition of new spectral filters for the existing NAHFTL plate reader in Brisbane, and repair of the existing automated plate washer in 2019. The subsequent failure of the plate washer prompted the purchase of a new machine in 2021, providing an automated, efficient, and reproducible means of washing ELISA plates, which is a critical element of this test method. Through the provision of spare parts and repairs funded and coordinated by this project, ELISA testing capacity was re-instated at the NAHFTL early in 2020. This was significant because African swine fever emerged in PNG in March 2020 and the frontline laboratory diagnostic test for ASF virus detection available to NAQIA at that time was ELISA-based.

7.1.2 Inter-laboratory comparison testing

Following establishment of the flavivirus C-ELISA at NAHFTL and prior to the commencement of diagnostic testing of sentinel samples, NAHFTL participated in an ILCT activity coordinated and resourced by ACDP under the principles of ISO 17043 for general requirements for proficiency testing. ACDP is an ISO 17043-accredited proficiency testing provider. The final interpretation of test results provided by NAHFTL were consistent with those from ACDP and the final qualitative interpretation was considered as correct and as expected.

These results demonstrated competence in flaviviruses C-ELISA testing at NAHFTL and provided the basis to undertake subsequent testing of sentinel samples with confidence.

7.1.3 FAO Laboratory Mapping Tool assessments

Two laboratory assessment audits were performed using the FAO LMT-C module to identify strengths and gaps in five major areas of laboratory performance and operations. The first assessment was performed over the 21st-23rd October 2019 and the second performed over several months in 2021 (finalised 2nd May). The results for each of the major areas of assessment in 2019 and 2021 are shown in Table 5. Similar overall scores were obtained from both LMT-C assessments; however, the 2021 LMT-C total score (28.7%) was slightly lower than that in 2019 (33.7%), reflecting the inclusion of histopathology, virology and molecular testing in 2021. These disciplines were not assessed in 2019 due to the lack of functional capacity in these areas.

Both LMT-C assessments identified strengths in general laboratory profile (location, organisation and supply of electricity and water), stable & experienced staff, a functioning QA system for the food testing area (but not animal health), international collaborations/projects, and expertise in the use of disease-related web resources and e-platforms for epidemiology.

Table 5. Results of the FAO Laboratory Mapping Tool Core module for NAHFTL in 2019 and 2021.

LMT Area	Score (%) 2019	Score (%) 2021
General laboratory profile	46.7	46.7
Infrastructure, equipment, supplies	38.6	26.9
Laboratory performance	21.7	19.2
Quality Assurance, biosafety/biosecurity	32.2	29.9
Lab collaboration and networking	37.5	33.3
Total score %	**33.7**	**28.7**

Gaps were identified in infrastructure, equipment and supplies, especially for the animal health testing area. The laboratory budget was considered to be insufficient for NAHFTL to implement activities under a QA system (ISO 17025) to ensure accuracy of testing and standard levels of laboratory biosafety/safety and biosecurity. A major gap recognised in both assessments was the absence of molecular diagnostics capacity on site at NAHFTL, which has become a standard tool for disease diagnosis in national laboratories. The establishment of a dedicated PCR laboratory for testing relevant agents (virus, bacteria & parasites, including aquaculture) was a key recommendation of both assessments. The recent capacity building for ASF PCR testing for NAQIA at the PNG IMR laboratory was noted for the second assessment; however, for the purposes of the assessment, this was not considered a capacity available on site at NAHFTL.

Other gaps identified included the lack of virology, pathology and histopathology capability; improvements to QA are required, especially for SOPs and internal quality control. Updated animal facilities and procedures for using animals for reagent production, and including animal ethics approval procedures, were also recommended. Further active participation in national and international laboratory networks was also considered to be beneficial for NAHFTL.

The LMT Safety module assessment identified gaps in policies, guidelines, procedures, and documentation for laboratory safety/biosafety. Notably, there was no safety/biosafety manual and agent-specific risk assessments, and limited staff medical surveillance. Operational gaps in staff safety training, handling chemical hazards, waste disposal management, equipment maintenance, cleaning & housekeeping, disinfection, and signage were found, and improvements to sample reception were also noted.

For laboratory infrastructure, deficiencies in electrical supply to some areas, chemical security, building condition and furniture, pathology facilities, fire safety & emergency procedures and preventative maintenance for building and equipment were identified by the LMT-S. Improvements in supply, use, maintenance and disposal of Personal Protective Equipment (PPE) are also needed.

A summary of the major recommendations made from these assessments were:

- NAHFTL should be given support and budget to build laboratory capacity, and upgrade and maintain the laboratory to international standards to meet ongoing requirements for disease control in PNG
- Priority should be given to establishing diagnostic PCR capacity at NAHFTL, including laboratory infrastructure and equipment

- NAHFTL should be provided support to implement best practice QA following ISO 17025 guidelines, and international standards of laboratory biosafety/safety to allow the safe and secure handling and storage of infectious materials
- A Quality Management (QM) system should be implemented, and a QA manager and Biosafety Officer appointed, together with the formation of a QA/Biosafety committee to implement and manage QA/Biosafety at NAHFTL
- The LMT-C and LMT-S are undertaken on a regular basis (e.g., annually) and national LMT experts and laboratory focal points are established

The FAO LMT has led to the identification specific gaps in laboratory infrastructure and performance and facilitated awareness of the requirements to meet QA and biosafety standards for a national laboratory. This information will enable targeted capacity building by NAHFTL, and also provides a reference for collaborating partners for the purpose of planning and resourcing laboratory capacity building activities. A further benefit of undertaking regular LMT assessments is that this can provide justification for increased funding and support from PNG government and donor organisations.

Although the LMT scores were relatively low for NAHFTL, they were comparable to other regional animal health laboratories that have experienced similar challenges in receiving adequate budgetary and technical support. Over the course of the project, there were notable improvements in laboratory performance and capacity, as evidenced by the flavivirus ELISA ILCT results and the successful implementation of ASF PCR testing (see below). *The dedication, knowledge, and enthusiasm of NAHFTL staff was also well noted and appreciated by external project team members who were directly involved in project activities.*

7.1.4 Capacity building for African swine fever diagnostic testing

Support for ASF antigen ELISA testing

Technical issues experienced at NAHFTL using the commercially available Ingenasa antigen ELISA were addressed by ACDP OIE ASF Reference laboratory staff, following a request from NAHFTL. A short report was provided to NAHFTL management on the 14/1/2021 with recommendations on sample type and quality, sample processing prior to testing, the need for an SOP, and advice on the use of laboratory plastics for diagnostic testing.

Support for ELISA testing capability was also provided through the provision of two ASF antigen ELISA test kits in June 2020 to meet a shortfall in kit supply and ensure continuity of testing for delimiting surveillance activities underway at that time.

Support for ASF field testing

Field test documentation (SOPs and worksheets) were developed, with assistance from the ACDP OIE ASF Reference laboratory, for use by NAQIA veterinary services staff during field testing and for QA purposes. A draft of the NAQIA Veterinary Sample Advice document was also reviewed for NAHFTL by the ACDP Veterinary Investigation team members, and feedback provided.

The major activity under this objective was an evaluation of ASF rapid antigen tests (RAT). This involved a preliminary laboratory evaluation at ACDP of three commercially available test kits using blood samples derived from pigs experimentally infected with the pandemic strain of ASF virus (Georgia/07). To simulate field testing conditions, blood samples were tested on the day of collection. These samples were also tested by ASF PCR, as a reference or ‘gold standard’ test. Details of these kits and a summary of the results is shown in Table 6.

Table 6. Results of laboratory evaluation of ASF rapid antigen tests using whole blood from pigs experimentally infected at ACDP.

Manufacturer	Kit name (catalogue no.)	Format	% PCR positive samples detected	No. false positive samples detected
Ingenasa	INgezim ASF CROM Ag (11.ASFV.K.42)	Lateral flow	80.9	0
Bionote	Anigen ASFV Ag rapid test (RG1407DD)	Lateral flow	70.2	4
PenCheck™	Rapid Screening Test for ASFV (PC-888)	Dipstick	27.7	3

In total, 47 PCR-positive samples and 40 PCR-negative samples were tested. Of the RATs evaluated, the Ingenasa test performed with the highest levels of specificity and sensitivity, detecting 38 of the 47 PCR-positive samples (80.9%), with no false positives. The levels of specificity observed for the Ingenasa test kit was comparable to those published previously (Sastre et al., 2016); however, the level of sensitivity was higher, with only 67.8% of PCR-positive samples testing positive by RAT in the evaluation reported by Sastre et al.. This may reflect a relatively higher proportion of positive blood samples containing high levels of ASF virus antigen used in our study.

The Bionote test performed with slightly lower sensitivity than the Ingenasa RAT (33 PCR-positive samples detected; 70.2%) but the lowest specificity of the kits tested (4 false positives). The PenCheck test had the lowest levels of sensitivity (13 PCR-positive samples detected; 27.7%) and similar level of specificity (3 false positives) compared to the Bionote RAT.

Given the low levels of sensitivity observed for the PenCheck RAT, only the Bionote test was progressed to a field evaluation alongside the Ingenasa RAT, already employed by NAQIA for field testing. Due to limited opportunities by the field teams involved in this work to investigate suspect ASF cases, only two comparison tests have been performed (both negative). The field study will continue beyond the end of the project to complete the evaluation.

The availability of a second field test for NAQIA staff provides an alternative option in the event of supply constraints for the Ingenasa test kit. Should the Bionote test prove to have comparable performance in the field, this may be a suitable back-up. In this regard, NAQIA staff have received training in the use of this test and test documentation is also already in place.

Other field (point-of-care [PoC]) testing options recommended for future consideration are molecular PoC tests, which offer higher levels of sensitivity, comparable to laboratory-based real-time PCR assays. There are several molecular platforms now available that allow very sensitive ASF virus DNA detection in infected pigs, even at the early stages of disease. These tests can also be used for detection of contaminated carcases, pork and environmental samples at the point-of-need. However, they are technically more complex than rapid antigen tests and require a much higher level of staff training and competency for accurate testing. Molecular field tests also require expensive equipment and maintenance, and ongoing reagents for amplification. For most PCR-based platforms, additional equipment or test kits are also required for extracting viral DNA prior to testing.

Ultimately, the choice of PoC method to use is influenced by many factors including cost, continuity of reagent supply, ease of use and training requirements. Simple rapid tests

may be an appropriate test for certain situations (e.g. low training requirements) and can be readily provided to multiple field teams. Whereas the higher cost and complexity of molecular platforms may limit their use to a small number of regional settings such as regional animal health centres or laboratories.

Diagnostic PCR testing

ASF PCR testing was established for NAQIA in collaboration with the PNG IMR at the PiH PCR laboratory. This was achieved through provision of ASF PCR kits and reagents from ACDP and supported by the Australian DAWE. NAQIA-formatted test SOPs for DNA extraction and PCR testing were written by NAQIA staff in collaboration with ACDP, based on manufacturer's instructions, and ACDP work instructions and bench notes. These documents will be incorporated into the NAHFTL QA manual.

Once the ASF PCR was operational at the PiH laboratory and staff training requirements and proficiency expectations had been met, ASFV PCR testing was undertaken using whole blood and serum samples collected from pigs as part of disease investigation and surveillance activities by NAQIA. In total, 29 samples were selected for testing from pigs for which confirmatory testing had been performed at ACDP and results previously reported to NAQIA. In some cases, the same sample was tested in both locations, while for most pigs only the whole blood was tested at ACDP, whereas serum was tested at IMR since duplicate whole blood samples were unavailable or exhausted.

Comparison of the ASF PCR results from ACDP and PNG IMR/NAQIA demonstrated almost complete agreement. There was only one discrepancy, with one sample testing as weak positive in one of two ACDP PCRs used, which was at the threshold of detection. The second ACDP test was negative for this sample, in concordance with the NAQIA result.

There was however a notable difference in Ct values between ACDP and PNG tests, ranging from ~Ct 4-10. This may be explained by different samples types from individual animals being tested in each laboratory. Where serum was tested by NAQIA and whole blood tested by ACDP, from the same animal, a larger difference in Ct values was observed. Since ASF virus is haemagglutinating and attaches to red blood cells, whole blood typically has higher levels of virus present than serum (cell-free), which can lead to lower levels of ASF virus detected in the latter. This can result in differences in Ct values obtained from serum compared to whole blood.

However, of the eleven samples tested by ACDP and NAQIA that were aliquots of the same sample, differences were still observed. A likely contributing factor was the use of different extraction methods and specific PCR tests at each laboratory. ACDP uses an automated MagMax^{TM} extraction system and different PCR tests (Zsak et al., 2005; Haines et al. 2013), while the King assay is now used as a back-up test and for reference purposes. The extraction kit selected for use by NAQIA is an established column-based method compatible with the equipment available at the PiH laboratory. Although the Zsak and Haines assays are not OIE-recommended, they have been found to be more sensitive than the OIE-recommended King assay at ACDP. The King assay was selected for use at NAQIA because of its OIE-recommendation, and for consistency with the test provided to other regional animal health laboratories by ACDP on behalf of FAO. Future PCR capacity building at NAQIA is expected to provide the opportunity to introduce more sensitive ASF PCR tests.

The successful implementation of ASF PCR testing for NAHFTL represents the first molecular diagnostic test capability for infectious animal disease diagnosis at NAQIA. The training provided and format of testing can potentially be applied to PCR testing for other priority animal diseases. A fundamental gap that remains to be addressed is laboratory infrastructure at NAHFTL to accommodate a fit-for-purpose PCR suite. Over the course of the project, scoping and design of a PCR suite was undertaken in collaboration with NAHFTL staff, which provided the basis for architectural and engineering designs

commissioned by NAQIA, along with cost estimates for the refurbishment of an existing building on the Kila Kila site. While full laboratory refurbishment was beyond the scope and resources of the current project, a recent announcement of funding support from the Australian Department of Foreign Affairs and Trade (DFAT) for laboratory infrastructure, including PCR capability, provides the means for a major step forward for sustainable diagnostic PCR capacity at NAHFTL.

Other training activities to support animal health

With support from the ACIAR Capacity Building program and the PNG Country Manager, this project facilitated the enrolment of two NAQIA Animal Health Officers (Mr Erik Makui and Ms Dorothy Pagru) in the University of Natural Resources and Environment (UNRE) Certificate in Fundamentals of Para Veterinary Technology. This course was run at the UNRE Rabaul campus over 14 weeks from November 2020 to February 2021 and covered modules in:

- Animal Anatomy and Physiology
- Veterinary Epidemiology and Ecology
- Clinical Examination of Animals
- Necropsy Techniques for Disease Investigation
- Diseases of Ruminants
- Diseases of Poultry
- Diseases of Pigs
- Veterinary Drugs and Laboratory Disease Testing Techniques

Seventeen students were enrolled in the course, from the livestock or animal health sector. The course was delivered face-to-face and included lectures, tutorials and practical sessions on the University Farm Operations Division to address clinical diseases investigation, necropsy techniques and simulated ASF outbreak investigations.

This project also played a major role in developing the NAQIA ASF Diagnostic Workshop, led by NAQIA and in collaboration with the DAWE, and with in-principal support from the ACIAR Capacity Building program. Approximately 30 NAQIA Animal Health and Laboratory Officers from 8 provinces have been nominated to participate. Advanced planning involving development of the program (Appendix 5) and presentation materials, as well as logistical planning and budgeting has been undertaken. The workshop will focus on sample collection, field testing, post-mortem and sample packaging and shipping. The workshop is planned to be held as a combined online and face-to-face format, involving two ‘hubs’ in PNG (Port Morseby and Lae) in order to minimise COVID risks associated with face-to-face meetings. Unfortunately, due to ongoing COVID-19 restrictions in PNG, it has not been possible to hold the workshop, and it is now expected that this meeting will take place in 2022 or once restrictions in PNG ease.

Short- and long-term training opportunities such as these provide a valuable avenue for building staff expertise and capacity for NAQIA in field-based disease investigation and surveillance, and in support of backyard and village-based livestock systems.

7.2 Mosquito surveillance for Japanese encephalitis and zoonotic arboviruses

7.2.1 Comparison of Different Mosquito Traps for Zoonotic Arbovirus Vectors in Papua New Guinea

The following section has been adapted from Goi et al. (2022), which reports a major component of this objective of the project.

Numerous trapping devices are available for capturing mosquitoes but trapping success may vary depending on species-specific cues (e.g., visual, olfactory, carbon dioxide; Silver et al., 2008) and sampling conditions (Gorsich et al., 2019). One of the simplest and most widely used trap types for capturing mosquitoes is the CDC light trap (Sudia et al., 1962). CDC light traps can be used with only light as attractant but can also be supplemented with other mosquito attractants (such as CO_2 or octenol) to enhance mosquito collection (Kline, 2006; Newhouse et al., 1966). Previous studies conducted elsewhere have evaluated trapping devices in many environments (Gorsich et al., 2019; Farajollahi et al., 2009; Li et al., 2016; Luhken et al., 2014; Mboera et al., 2019; Ponlawat et al., 2017; Roiz et al., 2012). Some studies showed that CDC light traps are more effective in capturing *Anopheles* and *Culex* than *Aedes* mosquitoes (Gorsich et al., 2019; Ponlawat et al., 2017; Sriwichai et al., 2015). Biogents-sentinel trap (BG), which uses both visual and olfactory cues, was designed to capture *Aedes albopictus* (Johnson et al., 2020). Previous reports comparing BG to light traps have shown that BG traps with or without lures are more effective at capturing *Aedes* than the light traps (Farajollahi et al., 2009; Li et al., 2016; Luhken et al., 2014). Light emitting diodes (LEDs) with ultraviolet light (Johnson et al., 2020) are also increasingly being assessed for capturing mosquitoes and previous studies showed that CDC light traps with UV light have higher trapping efficacies than those with incandescent light (Mwanga et al., 2019; Chaiphongpachara et al., 2019; Moore et al., 2001). Different trap types may yield higher capture rates for different mosquito species based on their visual attractant. So far, there has been no published work on the evaluation of different traps for improved capture of field populations of mosquitoes in PNG.

For this project, the effectiveness of three mosquito-trapping devices were compared to identify a suitable and cost-effective surveillance tool for major vectors of neglected zoonotic arboviral diseases in PNG. The traps evaluated were the light trap with incandescent light (CDC_I), the CDC light trap with ultraviolet light (CDC_UV) and the Biogents Sentinel trap (BG),

The evaluation was conducted at eight agricultural farming sites in NCD, Central and Morobe provinces, from November to December 2019 and September to October 2020, respectively (Figure 10), as described in section 5.2.1. Traps were placed for two periods of 5 days per farm. Trapping periods were calculated as the number of traps multiplied by the number of 24h-trap day for each trap. Altogether, there were 480 trap periods (80 for BG, 2 traps operated over 5 days x 8 farms; 200 for CDC_I: 5 traps operated over 5 days x 8 farms; and 200 for CDC_UV).

Due to the lack of normality of the data and large standard deviations, non-parametric tests were used to analyze mosquito densities. Trapping rates (number of mosquitoes collected per trap per day) were calculated. Differences in mosquito trapping rates among the three trap types were assessed using Independent Samples Median Tests, followed by Bonferroni correction for multiple tests. Statistical analysis was performed using IBS SPSS Statistics Software v27 (IBM, USA).

Four hundred and eighty 24-h trapping periods yielded 13,788 female mosquitoes of which 13,041 (95.6%) were identified to species level. Mosquito species captured included *Aedes albopictus*, *Ae. aegypti*, *Anopheles bancrofti, An. farauti, An. koliensis,*

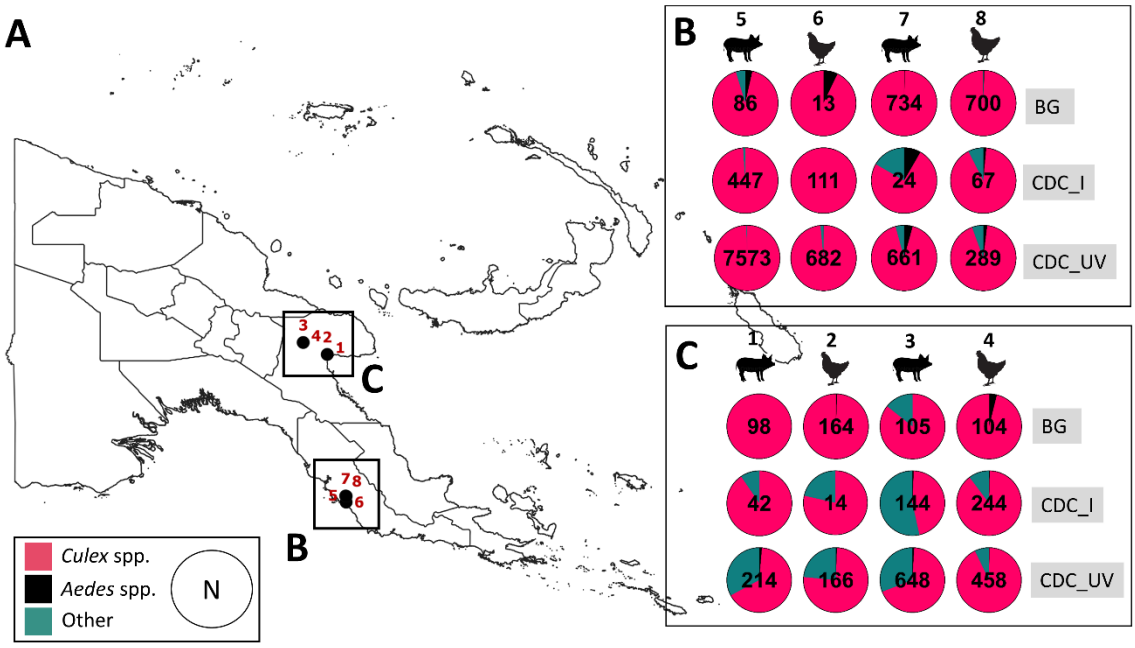


Figure 11. Summary of mosquito collections. Panel A shows the collection locations in the two areas of PNG, NCD/Central Province (inset B) and Morobe Province (inset C). In each location, traps were set up in 2 pig farms and 2 chicken farms. Panels B and C show mosquito distribution and abundance per collection site (farms labelled 1 to 8) and trap type. Each pie chart represents the mosquito distribution in one collection site and for one trap type. The numbers inscribed within the pie charts denote the overall number of mosquitoes collected.

An. longirostris, An. punctulatus s.s., Culex annulirostris, Cx. gelidus, Cx. quinquefasciatus and *Cx. sitiens*. Two genera, *Armigeres* and *Mansonia,* were also captured. Mosquitoes belonging to the *Culex* genus were the most abundant caught in all traps and at all study sites, comprising 95.4% of the total catch (Figure 11). The predominant species captured by BG traps was *Cx. quinquefasciatus* (96.7%), while for the other two traps, it was *Cx. gelidus* (CDC_I: 54.0%; CDC_UV: 80.4%). CDC_UV traps caught all of the eleven species identified in the present study, followed by CDC_I (8 species, 72.7%) and BG trap (7 species, 63.6%). All trap types caught the four *Culex* species identified (Figure 12). BG traps captured both *Aedes* species and only one *Anopheles* species, while CDC_I traps caught one *Aedes* and three *Anopheles* species (Figure 12).

Independent samples median tests showed that there were statistically significant differences in median trapping rates among the three trapping devices (all sites combined) for the following four species: *Cx. annulirostris:* $X^2(2) = 14.434$, $p = <0.001$; *Cx. gelidus:* $X^2(2) = 9.399$, $p= 0.009$; *Cx. quinquefasciatus:* $X^2(2) =9.000$, $p=0.011$; and *Cx. sitiens:* $X^2(2) = 7.000$, $p= 0.030$. There was no evidence of a significant difference for the other species, which may also be a result of the low number of mosquitoes captured for some species. In addition, the low number of anthrophilic mosquitoes observed could be due to the sampling location, which is animal houses, where human exposure is minimal.

Further pairwise comparisons of the median test and post hoc test results based on Bonferroni correction are presented in Figure 13. Among the traps compared, CDC_UV exhibited the highest efficacy for trapping *Cx. annulirostris, Cx. gelidus and Cx. sitiens,* with median trapping rates of 1.12, 4.62 and 0.54 per trap day, respectively. The BG trap showed the lowest overall median trapping rates, which was zero for the same three species ($X^2 = 9.000$, p = 0.008, in all cases). However, BG traps were significantly more effective than CDC_I at capturing *Cx. quinquefasciatus* ($X^2 = 9.000$, p = 0.008), with a median trapping rate of 8.5 per trap day compared to 0.4 per trap per day for the CDC_I

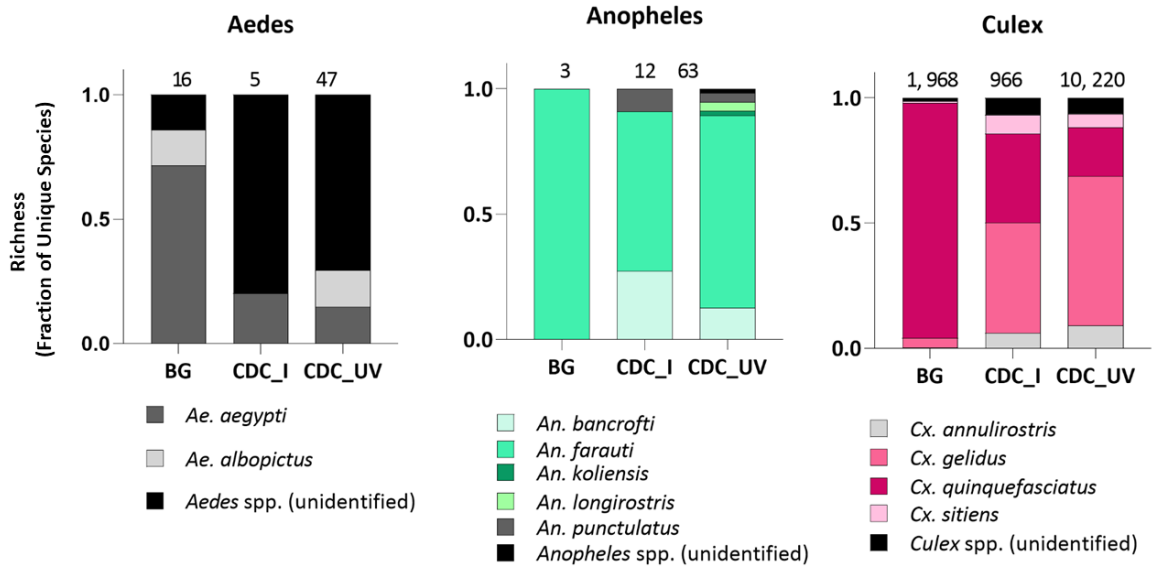


Figure 12. Species of mosquitoes caught among the three trapping devices. Numbers atop each column represent total number of mosquitoes caught by that trap.

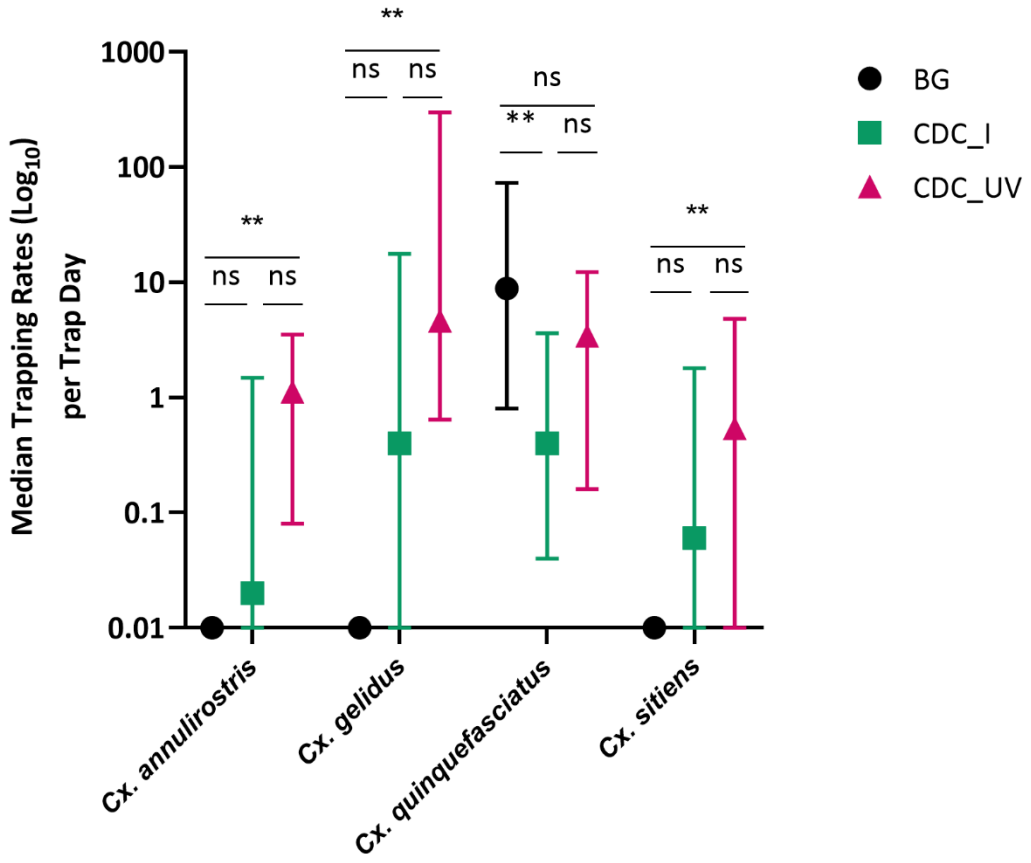


Figure 13. Median log_{10} Trapping Rates (number of mosquitoes caught per trapping day) of the three trapping devices. BG = Biogents Sentinel trap, CDC_I = CDC light trap with incandescent light and CDC_UV = CDC light trap with UV Light. Error bars represent 95% CIs. Capture rates were compared using Independent Samples Median Test with Bonferroni correction. Statistical significance is indicated by * symbols: with ** denoting a p-value of ≤0.01 and ns = non-significant.

trap. These univariate analyses were well supported by a multivariate negative binomial model that accounted for province and farm type as covariates (results not shown).

Various commercial trapping devices are available for mosquito monitoring or surveillance. This study compared traps for capturing vectors relevant for the transmission of zoonotic arboviruses in commercial farming environments in PNG. We showed that the CDC light trap with UV light captured the highest number of mosquitoes and the most species as compared to BG traps and CDC light traps with incandescent light. These differences are based on comparisons in 480 trap nights across eight farms. Similar results between UV and incandescent light traps have also been reported from studies conducted in Thailand, China and Tanzania, in which traps were placed inside homes (Mwanga et al., 2019; Chaiphongpachara et al., 2019; Moore et al., 2001). There are fewer published studies comparing the efficacies of BG traps and CDC_UV traps. For instance, a recent study in Thailand has shown that CDC light trap augmented with UV performed better than BG trap for capturing *Anopheles* species (Ponlawat et al., 2017).

While some previous studies have used BG and CDC traps in farming environments (Hou et al., 2021; Johnson et al., 2020; Möhlmann et al., 2017), we could not identify published studies that compared capture rates using the same traps as used in this study. For example, in China, Hou et al. (2021) compared CDC light trap with UV light (CDC_UV) and BG-Mosquitaire trap baited with lures in five different biotopes including a pig shelter. The pig shelter was the only place where CDC_UV outperformed BG-Mosquitaire in capturing the highest number of mosquitoes. Species wise, CDC_UV caught significantly more *Cx. tritaeniorhynchus* while BG-Mosquitaire trap caught more *Ae. albopictus* (Hou et al., 2021). In South Africa, Johnson et al., (2020) used CO2- baited traps (CDC and Mosquito tent trap) in horse farms but they did not specifically compare the two trap types. *Cx. theileri*, *Cx. univittatus*, and *Cx. pipiens sensu lato* were the most dominant species caught (Johnson et al., 2020). In Europe, Möhlmann et al. (2017) used two CO_2-baited traps (BG and Mosquito Magnet Liberty Plus (MMLP)) to capture mosquitoes in various habitats including dairy farms. MMLP trapped the largest numbers of mosquitoes in Sweden and Italy, while the BG trapped most mosquitoes in the Netherlands and the most abundant species caught *An. maculipennis* for Sweden and *Cx. pipiens* for the Netherlands and Italy. Other studies used only one trap type in animal farms; CO_2- baited CDC light traps in dairy farms in Nigeria where *Cx. quinquefasciatus* was the most abundant species caught (Victor et al. 2017) and CO_2-baited MMLP trap in horse farms in Belgium, where the most abundant adult species collected were *Cq. richiardii* (Boukraa et al. 2016). Although the traps used in other studies were different to ours, our findings are similar in that the abundant genus caught was *Culex* species in all the studied animal farms.

Limitations of this study include the small number of farms and provinces. While a balanced trapping study design was used for CDC_UV and CDC_I, the BG traps were used as ‘controls’ with one BG trap in each location run concurrently with the CDC_I and CDC_UV traps. This may have affected the capture rates and species richness observed, especially in the BG traps as it could be that mosquitoes were drawn away by nearby CDC_I or CDC_UV traps. However, our results are well aligned with recent BG trapping observations from Madang province in PNG (Staunton et al., 2021).

Based on these findings, we recommend CDC_UV traps for future research and programmatic monitoring studies of *Culex* species relevant for zoonotic arbovirus transmission in PNG.

Mosquito Collections from Kiunga, Western Province, 2021

Subsequent to the above study, a final mosquito trapping trip was undertaken in Kiunga and nearby locations in the North Fly district of the Western Province from July to August 2021 (Table 1). This trip was originally postponed following COVID-19 restrictions; however, when the project was extended, the IMR Entomology team took the opportunity to undertake this trip. Due to the timing, the results from the Kiunga collection were not

included in the above analyses for NCD/Central and Morobe, which had already been completed and submitted for publication.

As for the previous trips described above, the three different trap types were again evaluated (CDC_I, CDC_UV and BG). All three traps were placed together at each of two chicken farms, while only one trap type was placed at backyard piggeries due to their small size (5-6 pigs) and traps were rotated weekly across each of the four pig farms. The total number of female mosquitoes caught over 5 weeks (approximately 160 trap nights) was 8947, comprising 12 different species and a further 8 groups identified at the genus level. The different mosquito species collected, and their relative proportions for each trap type is shown in Figure 14.

Mosquitoes belonging to the *Culex* genus were again the most abundant caught in all traps and at all study sites, comprising 68.0% of the total catch. As for the earlier study, the predominant species captured by BG traps was *Cx. quinquefasciatus* (41.6%) and *Cx. gelidus* for the other two trap types (CDC_I: 47.9%; CDC_UV: 41.8%). Notably, for *Culex* mosquitoes, a much higher proportion of the vector species *Cx. annulirostris*, was collected in Kiunga (BG: 20.2%; CDC_I: 36.5%; CDC_UV: 35.9%) compared to other locations, indicating a relatively higher abundance of this vector in the North Fly district at the time of collection. *Cx. pullus* mosquitoes were also identified at Kiunga for the first time in the study. *Cx. pullus* is commonly found in northern Australia and isolates of MVE virus are frequently obtained from this species (Johansen et al., 2009).

The CDC_I traps caught all of the twenty species identified, closely followed by the CDC_UV trap (19 species), while the BG trap accounted for 14 of the species identified. All trap types caught the four *Culex* species and the two *Aedes* species identified (Figure 14). BG traps captured three of the six *Anopheles* species identified, while CDC_I traps caught all six and CDC_UV traps captured five. Other species identified included *Coquillettidia Xanthogaster*, and unidentified members of the genera *Armigeres*, *Hodgesa*, *Mansonia*, *Tripteroides*, and *Uranotaenia*. Both CDC traps caught all of these mosquito groups, while the BG trap caught only four.

The evaluation of the different trap types for the Kiunga mosquito collections was consistent with those from the previous study sites in NCD, Central and Morobe provinces. The CDC_UV trap caught the highest numbers of mosquitoes, including the highest numbers of each of the *Culex* species identified, and the highest level of species richness was observed for the CDC traps. These findings support our earlier conclusions that the CDC_UV trap is recommended for future vector monitoring and surveillance programs in PNG.

7.2.2 Arbovirus detection using real-time RT-PCR

The focus of arbovirus testing was *Culex* species mosquitoes, which are the main genera implicated as vectors for JE, MVE and KUN viruses, and are also important vectors of RR virus. A total of 18,951 unfed female *Culex* mosquitoes in 253 pools were processed for real-time RT-PCR testing for target arboviruses (Table 7).

Two detections of JE virus were made from *Cx. gelidus* mosquitoes collected from EBC piggery in Lae in October 2019. Five detections of KOK viruses were also made from four different species at various locations in three provinces: two positive pools of *Cx. quinquefasciatus* were detected from collections in Madang city in October 2019; one positive KOK virus pool of *Cx. annulirostris* was detected from mosquitoes caught in Boroma piggery in November and December 2019; one positive pool of *Cx. quinquefasciatus* was detected from City Mission farm collections in December 2019; and one detection of KOK virus was found in a *Cx pullus* pool collected from Kiunga in July and August 2021.

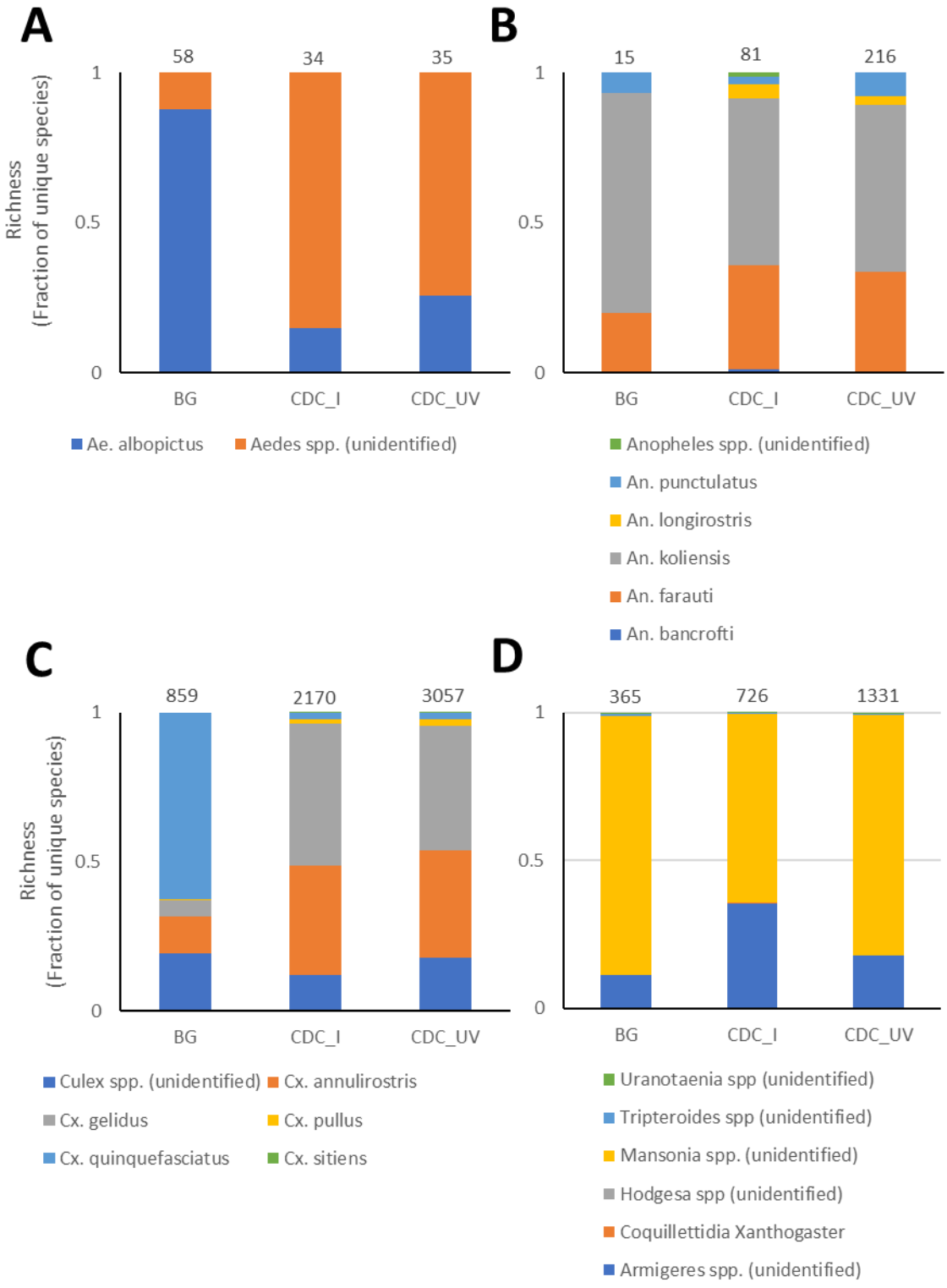


Figure 14. Species of mosquitoes caught among the three trapping devices: *Aedes* (A), *Anopheles* (B), *Culex* (C), other species (D). Numbers atop each column represent total number of mosquitoes caught by that trap.

Table 7. Numbers of mosquito species and corresponding pools processed for arbovirus real-time RT-PCR testing from collections at study sites located in Morobe (September-October 2019), National Capital District/Central (November-December 2019), and Western (July-August 2020) provinces.

Province	Species	No. processed	No. pools	Virus detection
Morobe	*Culex annulirostris*	598	11	
	Culex gelidus	6703	75	JE(2)
	Culex quinquefasciatus	4471	50	
	Culex sitiens	21	2	
	Culex spp. (unidentified)	28	5	
NCD/Central	*Culex annulirostris*	135	4	KOK(1)
	Culex gelidus	198	5	
	Culex quinquefasciatus	612	8	KOK(1)
	Culex sitiens	145	5	
	Culex spp. (unidentified)	112	5	
Western	*Culex annulirostris*	1327	17	
	Culex gelidus	2144	27	
	Culex pullus	40	3	KOK(1)
	Culex quinquefasciatus	519	9	
	Culex sitiens	490	7	
	Culex spp. (unidentified)	7	4	
Madang	*Culex annulirostris*	539	7	
	Culex gelidus	13	2	
	Culex quinquefasciatus	2791	30	KOK(2)
	Culex spp. (unidentified)	26	3	
East Sepik	*Culex annulirostris*	667	7	
	Culex quinquefasciatus	439	5	
	Culex sitiens	140	2	
	Culex spp. (unidentified)	155	2	
Total		**18,951**	**253**	**JE(2), KOK(5)**

The minimum infection rate (MIR) calculated for JE virus detections from *Cx. gelidus* at EBC piggery for the trapping period of the 6th to the 16th October was 0.34. The MIR for the two KOK virus detections from *Cx. quinquefasciatus* in Madang between the 27th July and 31st October 2019 was higher, at 1.16. Since each of the other KOK virus detections were made from collections that were under 1,000 mosquitoes, MIRs could not be accurately determined, precluding a comparison of vector species for KOK virus.

The detection of JE virus is highly significant and is the first known detection in mosquitoes collected in PNG since 1997/98 at Lake Murray, Abam and Balimo in the Western Province (Johansen et al., 2000). Viruses were isolated from pools of *Cx. sitiens*

subgroup mosquitoes (*Cx. annulirostris* and *Cx. palpalis*) collected from these locations, with MIRs ranging from 0.02 to of 0.09, considerably lower than MIRs found in the present study in Madang province. This may reflect the cell-culture-based method of virus isolation used by Johansen et al. (2000), which is recognised as being less sensitive than molecular detection methods such real-time RT-PCR. Higher levels of circulating virus in Madang during the October 2019 trapping period may also have contributed to the higher MIRs observed.

To the authors' knowledge, the detection of JE virus in Lae is also the first time this arbovirus has been detected in mosquitoes in this area, and it is the first detection in *Cx. gelidus* mosquitoes in PNG. The latter finding is not altogether surprising since *Cx. gelidus* is an important vector of JE virus in many Southeast Asian countries, and JE virus has also been isolated from this species in Torres Strait islands, neighbouring PNG (van den Hurk et al., 2001; Johansen et al., 2004).

Of interest was the multiple detections of KOK virus in 2019 and 2021. Human infections with KOK virus have rarely been associated with acute polyarthritis in Australia (Mackenzie and Williams, 2009). This virus was detected from three different species of *Culex* mosquitoes and from several different trapping locations. Three detections were made from *Cx. quinquefasciatus* pools – two from Madang in October 2019 and one from City Mission farm (Central) in December 2019; one pool of *Cx. annulirostris* collected at Boroma piggery (NCD) in late November to early December was positive; and one pool of *Cx. pullus* collected during July-August 2021 in Kiunga (Western) was also positive for KOK virus. Only one isolate of KOK virus has previously been reported from PNG from a mixed pool of *Aedes* mosquitoes collected at East Sepik (Sandaun) (Mackenzie et al. 1994). This virus was first isolated from *Cx. annulirostris* in Australia and has since been isolated from several species of *Aedes*. The finding of KOK virus in *Cx. quinquefasciatus* and *Cx. pullus* potentially broadens the range of vectors associated with this virus.

The levels of viral RNA detected by RT-PCR were relatively low, with Ct values ranging between 32 for JE virus to 36-39 for KOK virus. Conventional sequencing using a flavivirus generic PCR was attempted for JE virus but was unsuccessful, and therefore genotyping and molecular epidemiological analyses were unfortunately not able to be undertaken. Further sequencing will be attempted using next generation (deep) sequencing approaches to attempt to sequence the viruses detected.

7.3 Animal surveillance for Japanese encephalitis and related flaviviruses

7.3.1 Sentinel Animal Surveillance

Groups of sentinel pigs and chickens were established at various sites in Central, Morobe and National Capital District (Table 3, Figure 8). The initial aim of this pilot program was to sample young animals that had not been exposed to flavivirus infection.

Sentinel Pigs

Weaner pigs of known age were selected and reliably sampled at commercial piggeries, where established production systems were in operation. The remaining pig sentinel sites comprised villages where the ages of pigs varied, with many pigs being >8 months of age, or were uncertain. Despite the possibility that village pigs had been exposed to natural flavivirus infection at the time of first blood collection, they were included in the study with the intention to subsequently exclude any that were found to be antibody positive. Results from the sentinel animal surveillance also contributed to the seroprevalence analysis (section 7.3.2).

Due to delays experienced in establishing ELISA testing at NAHFTL, coupled with logistical and resourcing challenges associated with regular sampling from each sentinel group (chickens and pigs), the original plan of regular blood collections followed by immediate testing was not achieved. Instead, each group was sampled between 2 and 7 times between September 2019 to February 2020 (Table 3) and testing was performed retrospectively at ACDP and NAHFTL over 2020 and 2021.

The ELISA testing revealed that all sentinel pig herds had one or more animals that tested positive for JE virus; however specific antibodies to MVE or KUN viruses were not detected. A small number of unresolved cross-reactions with JE, KUN and/or MVE viruses were found in pigs at five sites, suggesting secondary flavivirus infections in these pigs (Table 8; Figure 15).

A complication associated with using young pigs as sentinel animals is the presence of maternal antibody. In sows that have previously been infected with JEV, maternal antibody is transmitted passively to piglets in colostrum from birth until weaning. Maternal antibody to JE virus has been shown to wane between 1-3 months after weaning (Scherer et al., 1959) and, in recent studies in Cambodia, waning maternal antibody was found to correspond to 3-4 months of age (Capelle et al., 2016; di Francesco et al., 2018). In the sentinel pigs sampled in our study, the ages of weaner piglets on commercial farms at first sampling ranged from 4 weeks (Rumion Ltd), 11 weeks (City Mission), 12 weeks (Boroma) and 16 weeks (Eden Green; Table 8).

At Rumion farm in Morobe province, all piglets sampled were positive to JE virus at 4 weeks of age, consistent with the presence of maternal antibodies (Table 8). By 4 months, this level of seropositivity had dropped to 11/20, before rising again to 16/20 and 10/10 by 5-6 months. Taken together, these results indicate that during this time period maternal antibodies levels had waned, and this was followed by natural infection. These results are consistent with the detection of JE virus in mosquitoes collected from Lae, located approximately 60km from Rumion Ltd farm, and provides evidence that JE virus was circulating in the Markham Valley in late 2019.

At Boroma and Eden Green farms, both located at nearby locations in NCD, a similar pattern of seropositivity was observed (Table 8; Figure 15). A high proportion of pigs (17/20 and 9/10, respectively) were positive to JE virus in November and December 2019, when pigs were 3-4 months of age, but by January only three pigs remained positive at Boroma and no pigs were positive at Eden Green. These results indicated that maternal antibodies had waned during this time and limited, or no natural infection occurred prior to the final round of sampling.

At City Mission farm, the numbers of pigs sampled varied and inconsistencies in animal identification were also found between collections; it was therefore not possible to reliably determine patterns of seropositivity. However, from samples collected in the final sampling round in January 2020, when pig were approximately 5.5 months old and maternal antibodies would be expected to no longer be present, one pig tested positive for JE virus and cross-reactive antibodies were found in a second pig considered to have had an unresolved flavivirus infection (Table 8).

Village pigs included in this study were located in coastal locations in Central province. Challenges were experienced with sampling the same cohort of pigs at each village and accurately identifying individual pigs over sequential collections. Obtaining a reliable estimate of animal ages was also not always possible. Despite this, high levels of seropositivity to JE virus and flavivirus were identified, ranging from 18.2% to 94.1% of total samples tested (Table 8). Highest numbers of positive pigs were found in Sibore village and the Hiri LLG Office sites, where all but one sample collected at each location

Table 8. Summary of results from flavivirus sentinel surveillance in pigs in PNG from September 2019 to February 2020.

Location (Province)	Age at 1st bleed (if known)	Sep		Oct		Nov		Dec		Jan		Feb		Totals	
		n	+ve	n	+ve	n	+ve	n	+ve	n	+ve	n	+ve	Test-ed	+ve (%)
City Mission farm (Central)	~11 weeks			6	3	10	2	15	4	10	2			41	11 (26.8)
				3JE		2JE		4JE		1JE, 1Flav					
Papa-Lealea village (Central)	Mixed (4 weeks to 2 years)					15	4	12	5	14	7			41	16 (39.0)
						1JE, 3Flav		4JE, 1Flav		7JE					
Roku village (Central)	>8 months							6	2	5	0			11	2 (18.2)
								2JE							
Hiri LLG Office (Central)	>1 year					6	5			2	2			8	7 (87.5)
						3JE, 2Flav				2Flav					
Sibore village (Central)	Not known (adults and weaners)					5	4	8	8	4	4			17	16 (94.1)
						3JE, 1Flav		6JE, 2Flav		4JE					
Boroma Piggery (National Capital District)	~12 weeks					20	17	19	3					39	20 (51.3)
						17JE		3JE							
Eden Green farm (National Capital District)	~12 weeks							10/10*	9/10	10	0			30	10 (33.3)
								9JE/ 1JE							
Rumion Ltd Piggery (Morobe)	~4 weeks	20/20	20/20					20	11	20/10*	20/10	10	10	100	91 (91)
		20JE/ 16JE,4Flav						11JE		16JE/ 9JE, 1Flav		10 JE			

Notes: No sample and test information indicate that sampling was not performed in that month; *samples were collected twice at Eden Green in December 2019 and at Rumion farm in December 2019 and January and February 2020, as indicated and separated by the '/' symbol. Sampling was split into 2x10 pigs at Rumion over Jan-Feb; Flav, sample cross-reactive in two or more ELISAs.

Table 9. Summary of results from flavivirus sentinel animal surveillance in chickens PNG from September 2019 to February 2020.

Location	Age at 1st bleed (if known)	Sep		Oct		Nov		Dec		Jan		Feb		Totals	
		n	+ve	n	+ve	n	+ve	n	+ve	n	+ve	n	+ve	Tested	+ve
City Mission farm (Central)	Adults of unknown age			16	5			13	3	15	4			44	12 (27.3)
				1KUN,2MVE,2Flav				2JE, 1 MVE		1JE, 3 MVE					
Eden Green farm (National Capital District)	Adults of unknown age							16/12*	1/2	15	1	5	0	48	4 (8.3)
								1KUN/ 1KUN,1JE		1JE					
Rumion Ltd Piggery (Morobe)	Adults of unknown age											10	0	10	0 (0)
Ubix village (Morobe)	Adults of unknown age							9	4	10/9*	2/1	10	3	38	10 (26.3)
								2KUN, 1JE, 1Flav		1KUN, 1Flav/ 1Flav		1JE, 2Flav			

Notes: No sample and test information indicate that sampling was not performed that month; *Chickens at Eden Green farm were sampled twice in December 2019, as indicated and separated by the '/' symbol.; Chickens at Ubix village were bled twice in January; Flav, sample cross-reactive in two or more ELISAs.

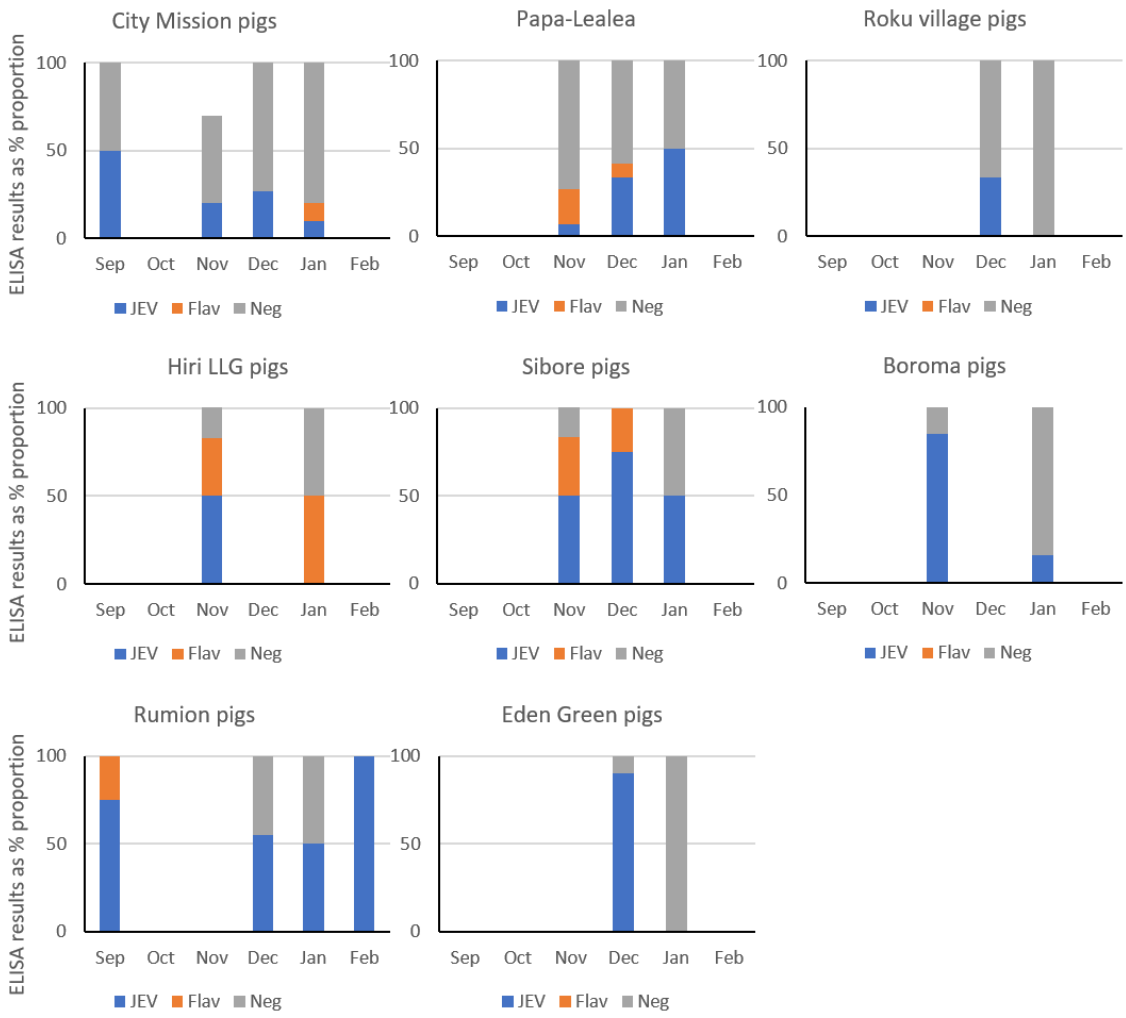


Figure 15. Proportion as percentage of pigs at each sentinel site positive for antibodies to Japanese encephalitis (JEV, blue) or non-specific flavivirus (Flav, orange).

over a three-month period tested positive for either JE virus or cross-reacting flavivirus antibodies (Table 8; Figure 15). The pigs at these villages were mostly adults and the results indicate they have had one or more infections with JE virus and/or a related flavivirus.

Sentinel Chickens

Due to various practical challenges associated with establishing dedicated sentinel chicken flocks, including housing, agistment and meeting animal ethics requirements, established adult layer chickens located at commercial farms were instead selected for this purpose. Flocks were located at City Mission (Central), Eden Green (NCD) and Rumion Ltd farms (Morobe) and one to three rounds of sampling were undertaken. ELISA test results are summarised in Table 9.

In contrast to the pig sentinel herds, the relative proportion of seropositive chickens was considerably lower, and ranged from 0 to 27.3% of total samples tested per site (Table 9, Figure 16). Furthermore, a comparable proportion of JE, MVE or KUN virus antibody positive samples were found (Figure 16). Two of the chicken sentinel sites were co-located at pig sentinel sites (Eden Green and City Mission farms) and it is therefore somewhat surprising that specific MVE- or KUN virus antibody positive pigs were also not detected on these farms. It may be speculated that the mosquito vector species for JE varied to those of MVE and KUN viruses, and that each had host preferences for pigs or

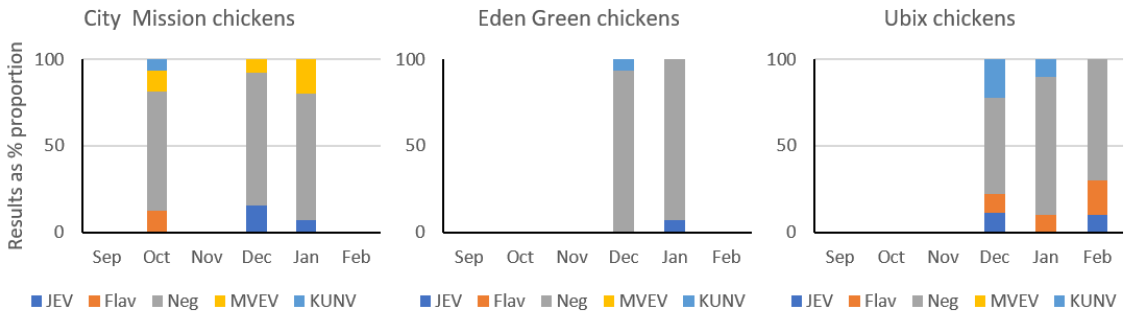


Figure 16. Proportion (%) of chickens at each sentinel site positive for antibodies to Japanese encephalitis (JEV, blue), Murray Valley encephalitis (MVEV, yellow), Kunjin (KUNV, light blue) or non-specific flavivirus (Flav, orange).

chickens, respectively. In this regard, of the mosquito species identified at each of these farms during this study (section 7.2), JE virus has been detected in PNG from *Cx. gelidus* (this study), and both JE and MVE viruses have been isolated from pools containing *Cx. annulirostris* (Johansen et al., 2000). In Australia, *Cx. annulirostris* is the major vector of MVE and KUN viruses (Mackenzie and Williams, 2009). Thus, the vector for MVE and KUN viruses, *Cx. annulirostris*, may have had a preference for chicken blood meals at Eden Green and City Mission farms, while *Cx. gelidus* (JE virus) preferred pigs. Further investigation involving bloodmeal analysis of these mosquito species collected at these sites is required to address this question.

When comparing the two sentinel animals used in this study, pigs were found to be a more sensitive indicator of JE virus infection than chickens, with a total of 57% vs 18.5% samples tested as positive, respectively. If only final round collection samples for pigs are considered, to remove bias from the presence of maternal antibodies in earlier sampling rounds, then seropositivity drops to 36.5%, which is still at a much higher level than for chickens.

Although the presence of maternal antibody can complicate interpretation of seroconversion, the findings from this study allow a more focussed approach to using sentinel pigs, whereby sampling of pigs commencing from around 2-3 months of age will enable the tracking of waning maternal antibody and subsequent seroconversion from natural infection. A further advantage of using pigs as sentinels is the potential to leverage existing collaborations between NAQIA and commercial production farms located near major population centres, such as Lae and Port Moresby.

Future pig sentinel surveillance activities may also be complimented by PCR testing of oro-nasal swabs taken from weaner pigs. It has been shown experimentally that pigs shed virus in oro-nasal secretions and are highly susceptible to oro-nasal infection (Ricklin et al., 2015; Lyons et al., 2018). The use of pen-based rope chewing methods for regular sampling could potentially be employed for this purpose.

A significant finding of this study was that chickens were more sensitive than pigs for detecting antibodies to MVE and KUN viruses, albeit in low numbers of samples. While cross-reactive antibodies between JE and MVE or KUN viruses were detected in pigs, single reactor MVE or KUN virus-specific antibodies were not found. Thus, a combination of chicken and pig sentinels may offer the most sensitive approach for detecting the circulation of all three viruses.

7.3.2 Porcine seroprevalence of zoonotic flaviviruses in PNG from 2016-2020

In addition to testing sentinel animals for this project, serum samples previously collected in PNG for surveillance of a range of priority porcine disease by NAQIA or DAWE were tested for antibodies to JE, MVE or KUN viruses. These samples were collected from eleven provinces on mainland PNG and the New Britain island, including both highlands and lowland locations, and spanning 5 years (2016-2020). Refer to Tables 4 and 5 and Figure 8 for details.

Samples collected as part of DAWE and NAQIA porcine disease surveillance in PNG were sourced from New Britain, NCD (Boroma piggery), Madang, Morobe, Sandaun and Western provinces between 2016 and 2018 (Table 4). Seropositive pigs were found in NCD (83.7% of samples tested), Western province (50%), Sandaun (28.9%), New Britain (10.5%), and Morobe (9.4%). No positive samples were detected from 23 pigs in Madang. Of the locations where seropositive pigs were detected, NCD had the highest numbers and proportions, with 48% of pigs (n=71) positive for JE virus, 32.6% (n= 48) with flavivirus cross-reactive antibodies and 2.7% (n=4) positive for KUN virus (Figure 18). High levels of seropositivity were also found in Sandaun (Vanimo), with detections of JE (n=19), MVE (n=1), KUN (n=1) and cross-reactive flavivirus antibodies (n=7). For the remaining locations where seropositivity was detected, these were only modest numbers, and included predominantly cross-reactive flavivirus antibodies. Notably, however, JE virus specific antibodies were detected in New Britain and Western provinces (3 pigs each).

The results from NCD samples are interesting since these were collected from one of the locations employed for sentinel surveillance (Boroma piggery). Samples were collected between April and June 2018, as compared to November 2019 to January 2020 for the sentinel pigs. The ages of the pigs sampled in 2018 are not known and since single samples only were tested, determination of pre- or post-weaning antibody status cannot be made. However, these results may reflect seasonal arbovirus activity leading to infection and seroconversion. Moreover, the high proportion of pigs with cross-reactive antibodies is suggestive of multiple or sequential infections, supporting the notion of arbovirus activity occurring prior to or during the time of sample collection in April-June. Average rainfall is highest in Port Moresby from January to March from the West Pacific monsoon, and this is expected to be accompanied by increases in mosquito numbers and, in turn, arbovirus activity. Thus, it may also be expected that pigs in NCD are likely to exhibit high levels of exposure to flaviviruses and subsequent seroconversion over this monsoon period. Future sentinel surveillance activities for zoonotic flaviviruses may therefore be most productive during this wet season period in NCD.

Serum samples collected from pigs for ASF surveillance purposes in the highland provinces were also tested. However, of the 153 samples tested, only 9 (5.9%) tested positive, in contrast to the findings from lowland provinces reported above. Despite this, one sample positive for JE virus antibodies was detected from Pogera, Enga province in May 2021, and two KUN virus positive samples were detected in pigs from Enga (Lagaip-Pogera) in November 2020. Three KUN virus positive samples and one flavivirus cross-reactive sample from Hagen Central in the Western Highlands province were also detected (December 2020). The remaining positive results were from a single sample from Imbongu, Southern Highlands (KUN virus), collected in January 2021, and a cross-reactive sample from Jiwaka collected in October 2020.

The contrasting results likely reflect geographic and climatic factors such as altitude and temperature, which can affect vector populations. For instance, Hii et al. (1997) found that the prevalence of malaria decreased with increasing altitude in the Southern highlands. Our results may also reflect differences in vector populations in the highlands regions compared to lowland provinces. Despite this, there is strong evidence that arboviruses

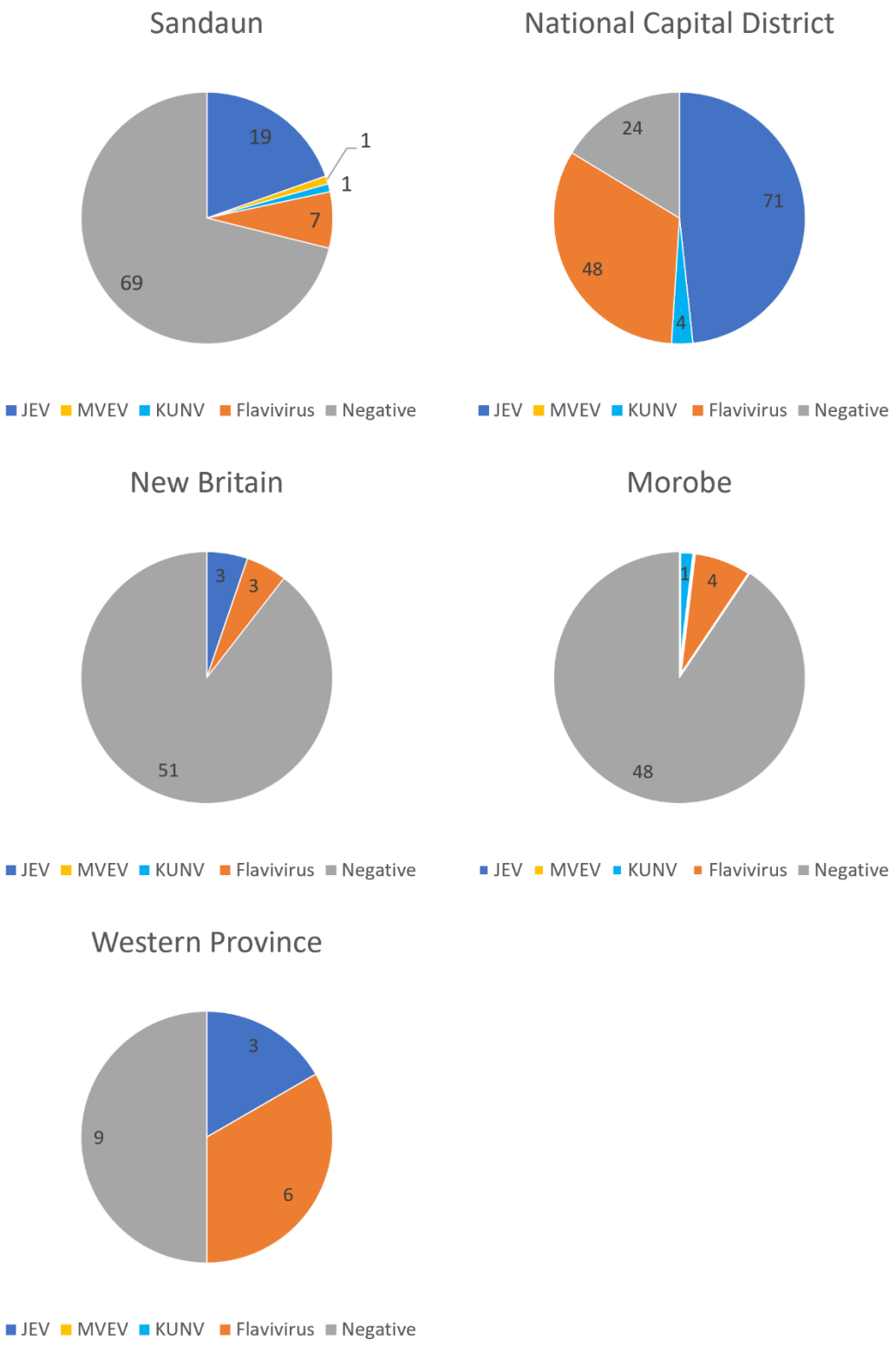


Figure 17. Seroprevalence of antibodies to zoonotic flaviviruses in pig sera collected from different provinces of PNG between 2016 and 2018 as part of NAQIA/DAWE surveillance activities.

can circulate and cause disease in highlands regions. Serological studies have reported the presence of antibodies to JE and RR viruses in human sampled in the Southern highlands (Hii et al., 1997; Johansen et al., 1997). Our findings of seropositive pigs to JE and KUN viruses in three highlands provinces support these studies and underline the potential of these viruses to cause disease in highland areas. Further investigation is warranted to fully understand the factors underlying arbovirus activity in the highland provinces of PNG.

7.3.3 Ecological analysis of flaviviruses positivity in pigs and chickens

The effect of the timing and location of sampling on seropositivity for the three flaviviruses was analysed for the four surveillance groups (viz., sentinel chickens, sentinel pigs, NAQIA ASF surveillance and NAQIA/DAWE pig disease surveillance), as described in section 5.2.2. For the purpose of this analysis, seropositivity for any of JE, MVE or KUN viruses was combined to assess overall levels of flavivirus seroprevalence.

A significant effect was observed for the region of sampling on the seropositivity for flaviviruses in pigs, however the patterns of association were complex and not entirely consistent between the studies. Thus, although the sentinel pig study showed that the sampled animals in the northern Morobe province were more likely to be seropositive as compared to the Central and National Capital District (Table 10a), this result was not shown with the porcine disease surveillance sampling where positivity was more likely to occur in pigs sampled from the Southern Region (Table 11a).

Part of this discrepancy was due to different provinces being sampled between the different surveillance programs and the current study, but also reflects different times of sampling. Thus, while the sentinel pigs were sampled between September and February, the pigs sampled as part of NAQIA/DAWE pig disease surveillance program occurred over a longer part of the year, between September and May, and over a 4-year period (2016-2019). As there were significant effects of the period of the year on seropositivity for all surveillance data (Tables 10b and 11b), there is likely to be an interaction between the location and the time of sampling. This in turn is highly likely to be due to differences in mosquito activity between the two regions, itself driven by the underlying climatology in the two regions (Figures 18 and 19). These differences apply most strongly to the rainfall pattern, where the total annual rainfall is much higher in Lae than in Port Moresby (4,677 vs. 1152 mm), and with the latter having a distinct dry season during July through to October (Figure 19a). By contrast there is little difference in the mean annual temperature of the two regions (26.5^{o}C vs. 27^{o}C) which contrasts with the considerably lower mean temperature in the highlands (Figure 18a).

Given the apparent complexity of flavivirus infection dynamics throughout PNG, the need for detailed longitudinal studies in areas of high zoonotic risk so as to prioritise sampling and testing resource is strongly suggested. In this respect our study has been successful in identifying areas of higher zoonotic risk – particularly around the population centres of Port Moresby and Lae. By contrast, the highlands are an area of low risk, as indicated by the NAQIA ASF surveillance study (Table 12a), which is consistent with the low average temperature encountered there being less hospitable for vector mosquitoes of zoonotic arboviruses (Figure 18a). The Islands region is also suggested to be an area of low risk, but as this has a comparable climate to the northern Momase region, then this might best be treated a provisional assessment.

Table 10. Numbers and proportion (in brackets) of **pig samples** which tested positive for one or more of the three flaviviruses (JE, KUN and MVE viruses) for the **sentinel pig sampling** summarized by (a) the sampling province; (b) the sampling period between September 2019 and February 2020; and (c) the production system of the pigs.

(a) Flavivirus seropositivity aggregated by the two sampling provinces (n=297).

Flavivirus serology	Central Province	Morobe Province	TOTALS
Positive	**82 (0.44)**	**96 (0.87)**	178 (0.60)
Negative	**105 (0.56)**	**14 (0.13)**	119 (0.40)
TOTALS	187 (0.63)	110 (0.37)	297 (1.0)

χ^2 test: $p < 0.001$

(b) Flavivirus seropositivity aggregated by the aggregated sampling periods (n=297).

Flavivirus serology	Sep-Oct 2019	Nov-Dec 2019	Jan-Feb 2020	TOTALS
Positive	**43 (0.93)**	**72 (0.53)**	**63 (0.55)**	178 (0.60)
Negative	**3 (0.07)**	**65 (0.47)**	**51 (0.45)**	119 (0.40)
TOTALS	46 (0.15)	137 (0.46)	114 (0.38)	297 (1.0)

χ^2 test: $p < 0.001$

(c) Flavivirus seropositivity aggregated by the two animal production systems (n=297).

Flavivirus serology	Commercial production	Village production	TOTALS
Positive	**137 (0.62)**	**41 (0.53)**	178 (0.60)
Negative	**83 (0.38)**	**36 (0.47)**	119 (0.40)
TOTALS	220 (0.74)	77 (0.26)	297 (1.0)

χ^2 test: $p = 0.2091$

Table 11. Numbers and proportion (in brackets) of **pig samples** which tested positive for one or more of the three flaviviruses (JE, KUN and MVE viruses) for the **porcine disease surveillance sampling** summarized by (a) the sampling provinces aggregated to the regional level; (b) the sampling period between September 2016 and April 2019 aggregated into bi and trimonthly periods. Note that the sampling did not occur consistently over this period.

(a) Flavivirus seropositivity aggregated by the three sampling regions (n=393).

Flavivirus serology	Islands Region	Momase Region	Southern Region	TOTALS
Positive	**6 (0.11)**	**33 (0.19)**	**132 (0.81)**	171 (0.44)
Negative	**51 (0.89)**	**141 (0.81)**	**30 (0.19)**	222 (0.56)
TOTALS	57 (0.15)	174 (0.44)	162 (0.41)	393 (1.0)

χ^2 test: $p < 0.001$

(b) Flavivirus seropositivity aggregated by the aggregated sampling periods (n=393).

Flavivirus serology	Apr-May	Jan-Feb-Mar	Sep-Oct	TOTALS
Positive	**123 (0.74)**	**11 (0.31)**	**37 (0.19)**	171 (0.44)
Negative	**44 (0.26)**	**25 (0.69)**	**153 (0.81)**	222 (0.56)
TOTALS	167 (0.42)	36 (0.09)	190 (0.48)	393 (1.0)

χ^2 test: $p < 0.001$

Table 12. Numbers and proportion (in brackets) of **pig samples** which tested positive for one or more of the three flaviviruses (JE, KUN and MVE viruses) for the **NAQIA ASF surveillance** sampling summarized by (a) the sampling provinces, all within the Highlands Region; and (b) the sampling period between January 2020 and February 2021 aggregated bi- and trimonthly periods. Note that 2 samples from Jiwaka were excluded from the province analysis due to low numbers and for 48 samples (all from Enga Province) no date of collection data was provided.

(a) Flavivirus seropositivity aggregated by the three sampling Provinces (n=156).

Flavivirus serology	Enga	Southern Highlands	Western Highlands	TOTALS
Positive	**3 (0.05)**	**1 (0.02)**	**4 (0.08)**	8 (0.05)
Negative	**52 (0.95)**	**50 (0.98)**	**46 (0.92)**	148 (0.95)
TOTALS	55 (0.35)	51 (0.33)	50 (0.32)	156 (1.0)

χ^2 test: p = 0.3846

(b) Flavivirus seropositivity aggregated by the aggregated sampling periods.

Flavivirus serology	Dec-Jan-Feb	Apr-May	Jun-Jul-Aug	Sep-Oct	TOTALS
Positive	**5 (0.10)**	**0 (0.00)**	**0 (0.00)**	**1 (0.05)**	6 (0.05)
Negative	**46 (0.90)**	**20 (1.00)**	**17 (1.00)**	**21 (0.95)**	104 (0.95)
TOTALS	51 (0.46)	20 (0.18)	17 (0.15)	22 (0.20)	110 (1.0)

χ^2 test: p = 0.2571

Table 13. Numbers and proportion (in brackets) of **chicken samples** which tested positive for one or more of the three flaviviruses (JEV, KUNV and MVEV) for the **sentinel chicken sampling** summarized by (a) the sampling province; (b) the sampling period between October 2019 and February 2020; and (c) the production system of the poultry.

(a) Flavivirus seropositivity aggregated by the two sampling provinces (n=140).

Flavivirus serology	Central Province	Morobe Province	TOTALS
Positive	**16 (0.17)**	**10 (0.21)**	26 (0.19)
Negative	**76 (0.83)**	**38 (0.79)**	114 (0.81)
TOTALS	92 (0.66)	48 (0.34)	140 (1.0)

χ^2 test: p = 0.7886

(b) Flavivirus seropositivity aggregated by the sampling months (n=140).

Flavivirus serology	Oct-19	Dec-19	Jan-20	Feb-20	TOTALS
Positive	**5 (0.31)**	**10 (0.20)**	**8 (0.16)**	**3 (0.12)**	26 (0.19)
Negative	**11 (0.69)**	**40 (0.80)**	**41 (0.84)**	**22 (0.88)**	114 (0.81)
TOTALS	16 (0.11)	50 (0.36)	49 (0.35)	25 (0.18)	140 (1.0)

χ^2 test: p = 0.4496

(c) Flavivirus seropositivity aggregated by the poultry production systems (n=140).

Flavivirus serology	Commercial production	Semi-commercial	Village production	TOTALS
Positive	16 (0.17)	0 (0.0)	10 (0.26)	26 (0.19)
Negative	76 (0.83)	10 (1.0)	28 (0.74)	114 (0.81)
TOTALS	92 (0.66)	10 (0.07)	38 (0.27)	140 (1.0)

χ^2 test: p = 0.1442

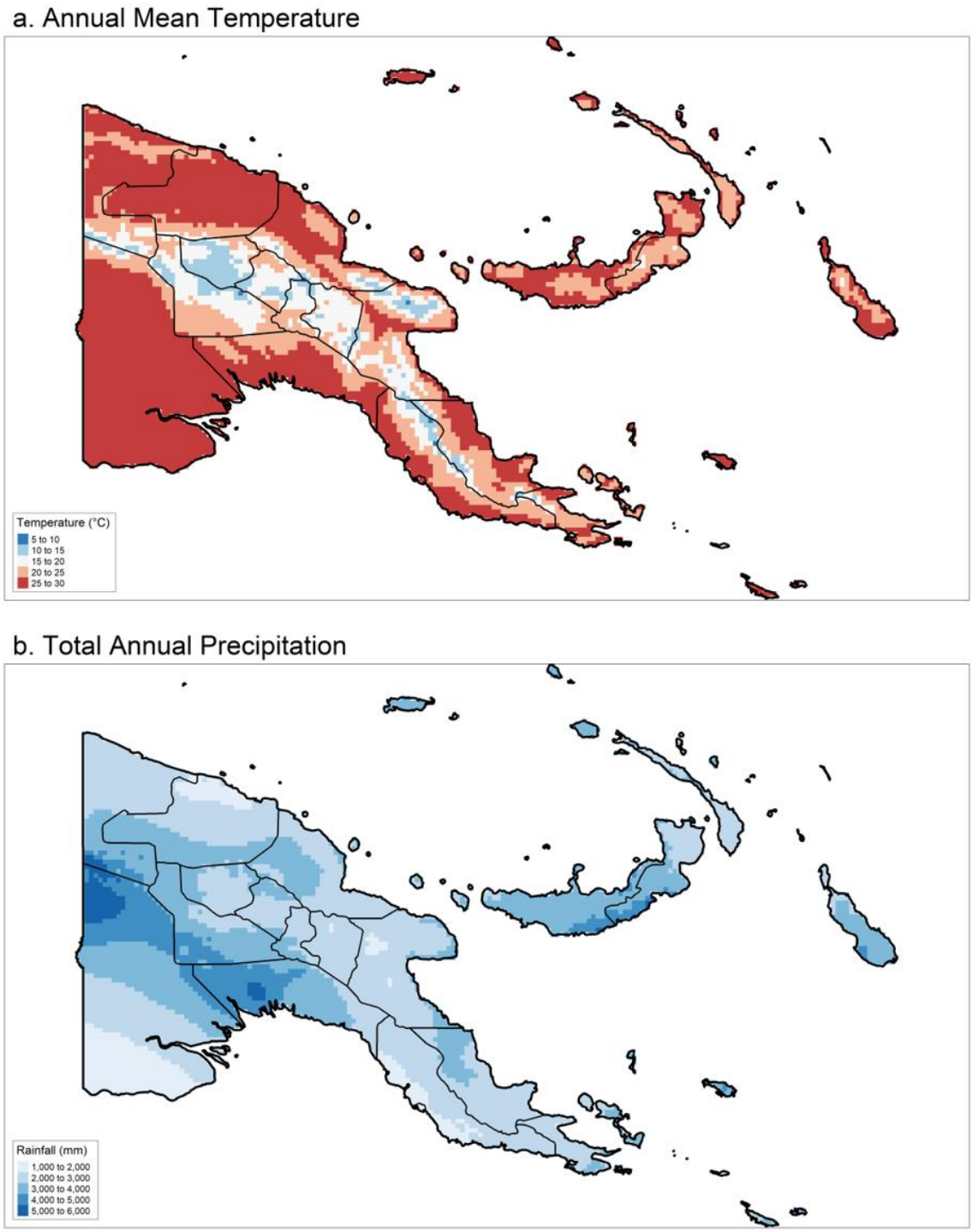
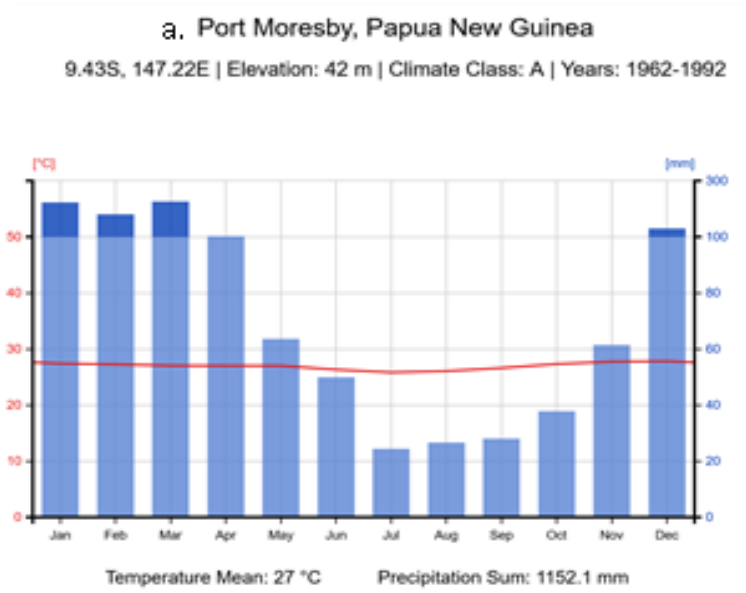


Figure 18. Interpolated long term average temperature (a) and annual rainfall (b) for PNG for the period 1970-2000 with province boundaries superimposed.



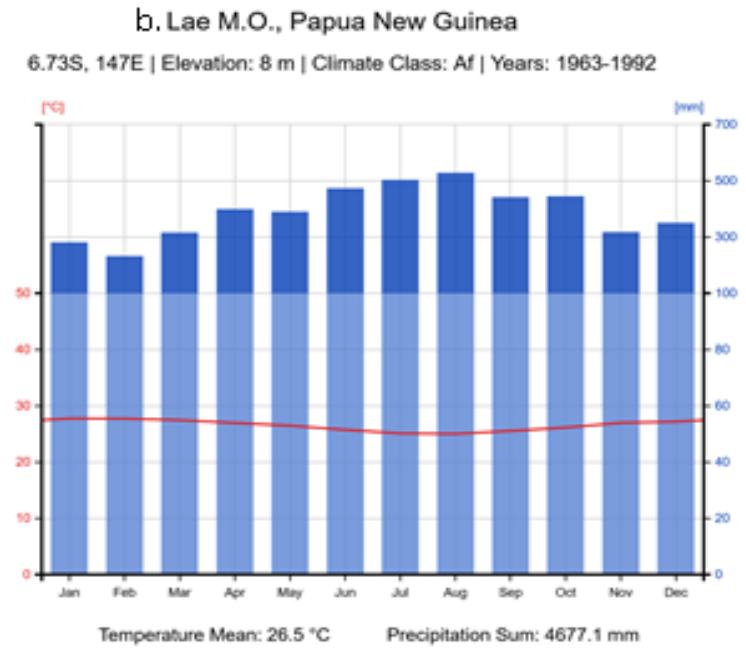


Figure 19. Long term monthly mean air temperatures and total rainfall for (a) Port Moresby, Central Province; and (b) Lae, Morobe Province. Climographs were compiled using ClimateCharts.net.

8 Impacts

8.1 Scientific Impacts now and in 5 years

This project has led to several important scientific findings that are expected to have both immediate and longer-term impacts for the control of zoonotic arboviruses and African swine fever in PNG.

The major scientific finding of this project was evidence of continued circulation of JE and other zoonotic flaviviruses in PNG. Serological evidence of JE virus infection was found in sentinel pigs in NCD, Central and Morobe provinces, as well as in pig samples collected over multiple years in Sandaun and New Britain for disease surveillance programs. Antibodies to KUN and MVE viruses were also detected in relatively smaller numbers of pig and chicken samples from Sandaun, NCD and Morobe provinces, indicating the co-circulation of these viruses together with JE virus. However, it is the high proportions of seropositivity found to JE virus in pig samples and the detection of JE virus positive mosquito pools from Morobe that are of most interest and concern, given the potential of JE virus to cause severe disease and outbreaks. These findings are significant for public health in PNG in relation to potentially unrecognised clinical cases of flavivirus disease and strengthen the justification for ongoing surveillance and investment in zoonotic arbovirus surveillance to better understand the epidemiology and disease burden of JE in PNG.

The recent case of JE in the Tiwi Islands underscores the potential threat of JE from neighbouring countries to the population centres of northern Australia. The findings from this project provide a timely update to knowledge on JE in PNG; prior to this project, the most recent published JE surveillance studies in PNG were conducted over 20 years ago (Johansen et al., 1997, 2000). Our findings are also expected to provide a reference point for future studies on zoonotic flaviviruses in PNG and will be of interest to regional countries and the scientific community.

A second scientific outcome that is likely to have impacts beyond this project is the finding that CDC_UV traps are more effective in attracting and trapping mosquitoes than the other traps tested (section 7.2.1). This finding will be relevant to future mosquito surveillance activities targeting Culex species where non-light attractants (e.g., carbon dioxide) cannot be used or are difficult to resource, such as in resource-poor countries and settings. A research article describing this work has been published; however, it is too early (at the time of writing) to gauge its wider impact, e.g., based on downloads or citation metrics.

The evaluation of commercially available rapid antigen test for the detection of ASF virus infections had immediate practical impacts through demonstrating that the Ingenasa test has the highest levels of sensitivity and specificity of the tests evaluated. This finding provided confidence for NAQIA in the continued use of this test for field testing in PNG. The results of the laboratory-based study using experimentally infected pig samples have been shared with the OIE ASF laboratory network and have influenced advice provided by the ACDP OIE ASF Reference laboratory for field testing options for the Pacific Island countries. As a result, Ingenasa test kits have recently been sent to thirteen PICs through support from Pacific Horicultural & Agricultural Market Access Plus Program (PHAMA Plus) and the Pacific Community (SPC) with accompanying online training. The test documentation provided to the PICs involved was based on those written for NAQIA as part of this project.

8.2 Capacity impacts now and in 5 years

Laboratory capacity building primarily focussed on the NAHFTL and represented a major outcome of this project, with both immediate and long-term impacts. In terms of diagnostic platform capacity building, the re-establishment and further development of ELISA testing capability at NAHFTL had a notable and immediate impact. At the start of the project, there was no operational ELISA testing capability at NAHFTL due to equipment in disrepair or not functioning. Through the provision of spare parts, repairs, upgraded and new equipment, funded and coordinated by this project, ELISA testing capacity was re-instated early in 2020. The timing of this was fortunate in that it allowed NAHFTL staff to undertake ASF testing following the emergence of this disease in March 2020 using commercial ELISA test kits. In the absence of rapid PCR testing, the NAHFTL ELISA testing played a major role in providing critical information to the CVO and response teams on the extent of spread of the outbreak in the early stages. The ELISA has also been used for the subsequent ASF delimiting surveys performed in the highlands provinces to determine the spread of ASF and effectiveness of control measures. Technical advice provided to NAHFTL by ACDP, as part of this project, to troubleshoot problematic diagnostic ELISA results have also resulted in methodological improvements and the consistency and accuracy of testing.

As part of establishing antibody testing capability for zoonotic arboviruses at NAHFTL, ELISA test kits were produced by ACDP and shipped to NAHFTL, along with interlaboratory comparison testing (ILCT) panels. To support laboratory quality assurance practices, test SOPs and worksheets, and progressive monitoring spreadsheets were also written with and for NAHFTL staff for these tests. It is expected that that this documentation will be added to the NAHFTL Quality Assurance Manual. This activity also represented a major objective of the project, which has now established serological diagnostic capability for zoonotic arboviruses at NAHFTL, which can be employed for future disease investigations and surveillance activities. By working together with ACDP on QA documentation, NAHFTL staff gained experience and insights into ACDP's QA processes and standards for QA documentation. The training and expertise gained by NAHFTL staff in developing QA documents and protocols for the flavivirus ELISAs are expected to provide a basis for revising existing documents or developing documentation for new tests in the future.

The ILCT for the flavivirus ELISA undertaken by NAHFTL demonstrated that the training provided by the project enabled accurate and proficient diagnostic testing by NAHFTL staff involved. ILCT or proficiency testing is a valuable activity for diagnostic laboratories that follow ISO/IEC 17025 standards and is a requirement for accreditation at this standard. This flavivirus ELISA ILCT round was the only ILCT/PT undertaken for the animal health laboratories during the project. This activity provided staff and management valuable experience with this form of laboratory assessment, and it is expected to lead to further involvement in future PT rounds for this and other diagnostic tests. Continued involvement in ILCT will reflect positively in future LMT assessments and is expected to continue to raise the overall technical proficiency of NAHFTL staff. Furthermore, continued involvement in proficiency testing will be an important factor for accreditation to the ISO 17025 standard, which represents a longer-term goal.

The FAO laboratory Management Tool assessments undertaken in 2019 and 2021 led to the identification specific gaps in laboratory infrastructure and performance and facilitated awareness of NAHFTL staff and NAQIA management of the requirements to meet QA and biosafety standards for a national laboratory. This information will also enable targeted capacity building by NAHFTL and provides a reference for collaborating partners involved in planning and resourcing future laboratory capacity building activities. A further benefit is that the results and findings of the LMTs can provide justification for further funding and investment from PNG government and donor organisations in the future.

A major capacity building impact of the project was implementation of ASF PCR testing for NAHFTL, representing the first molecular diagnostic testing capability for NAHFTL. Moreover, this was achieved using a One Health approach, through collaboration and support from the PNG IMR Partners in Health laboratory. Since PCR is a generic capability, there is now an opportunity to extend molecular testing to other priority animal diseases identified by NAQIA. This will be an objective of future projects, such as the recently funded DFAT LabCap PNG project. The project also facilitated an in-depth understanding of the requirements of PCR laboratory infrastructure and equipment by NAHFTL staff, which has informed subsequent planning for a PCR suite at NAHFTL, linked to the LabCap PNG project. Continued ASF PCR testing and implementation of other priority tests, accompanied by training of additional NAHFTL staff, is expected to provide a panel of rapid, sensitive and accurate laboratory diagnostic testing for animal diseases for NAQIA.

The project also facilitated valuable capacity building at PNG IMR for the surveillance of mosquito vectors of zoonotic diseases. The identification of CDC_UV traps as a sensitive and low-cost tool for *Culex* mosquito surveillance will directly benefit future surveillance activities targeting these vectors. These findings are also expected to influence trap selection for researchers and surveillance programs in other countries where resourcing CO_2 for baited traps is not feasible. The project also facilitated and fostered new linkages between PNG IMR and NAQIA, enabling access to NAQIA animal surveillance sites and advice and assistance for the mosquito trapping trips. This led to further capacity impacts outside this project through assistance provided by NAQIA to the PNG IMR Entomology department for mosquito trapping trips undertaken for other projects, such as PNG STRIVE.

This project also supported the completion of the Certificate in Fundamentals of Para Veterinary Technology at the University of Natural Resources and Environment by two NAQIA Animal Health officers (Appendix 6). The feedback from these officers was highly positive and both felt confident of transferring the skills they had learnt into the workplace (e.g., minute from Mr Erik Makui in Appendix 6). The course provided a valuable avenue for NAQIA animal health staff to consolidate and improve their levels of expertise, thereby building capacity for NAQIA and PNG in field-based disease investigation and surveillance, and for animal health in support of backyard and village-based livestock systems.

8.3 Community impacts now and in 5 years

8.3.1 Economic Impacts

In principle, economic benefits of zoonotic arbovirus surveillance may be achieved if surveillance is coupled with response. These benefits are manifested by reduced incidence of disease and associated illness and deaths in affected communities. Achieving quantifiable economic benefits was beyond the scope of this project. However, in the future, risk communications may be considered via engagement with PNG Department of Health for communities at locations where significant arbovirus activity was detected; for example, in Port Moresby and Lae.

The extension of the project to ASF diagnostic capacity building led to significant improvements in laboratory testing and enhancements to field testing. The benefit of this to NAQIA is timely and accurate diagnostics to inform targeted and effective ASF surveillance and control activities in affected highlands provinces. Pigs are the most important livestock animal for the predominantly rural population of PNG, where the smallholder sector supplies over 93% of the pork consumed in PNG (Ayalew 2011, 2013; Bourke and Harwood 2009). The small holder sector will benefit from an effective ASF response, of which accurate and rapid diagnostics is a cornerstone.

8.3.2 Social impacts

Social impacts are related to the health of affected communities and their livestock. For zoonotic arbovirus surveillance, potential impacts of this project will be based on raising awareness among villages and communities that are potentially affected by the arbovirus activity detected in this project. Such risk communications will be most effective when targeted to high-risk periods such as the wet season when mosquito numbers, and therefore arbovirus activity, are highest.

For ASF diagnostic capacity building, potential impacts to pig health will be achieved through improved diagnosis in the field and laboratory to support outbreak investigation, surveillance and response activities.

8.3.3 Environmental impacts

The arbovirus surveillance activities and outcomes of this project are not expected to lead to environmental impacts.

Improved ASF diagnostic capacity will contribute to effective control measures to prevent the spread of ASF and associated livestock losses (death or culling), which can be largescale and have environmental impacts because of carcass disposal and chemical decontamination of affected properties. Preventing stock losses are expected to reduce such environmental impacts.

8.4 Communication and dissemination activities

Communication and dissemination concerning project activities and outputs took place via several different means, including presentations at scientific conferences or workshops and government level meetings, scientific publications (see section 10.2), online blog articles and video.

Presentations on the project were presented at several forums, including:

PNG IMR Madang, Introduction to the project by David Williams, 9th May 2019;

DFAT Indo-Pacific Centre for Health Security Partners Forum on Vector Surveillance and Control: 'Project Update from ZAPPA' by David Williams, 24th February 2021;

The National Arbovirus and Malaria Advisory Committee (NAMAC), a technical advisory group to the Communicable Diseases Network of Australia (CDNA) of the Australian Department of Health; project overview presented by David Williams, 4th September 2019;

Scientific presentations at the 2019 Australian Association of Veterinary Laboratory Diagnosticians and the 2021 Arbovirus Research in Australia conference.

Online articles to promote the work undertaken and impacts of the project were also published by ACIAR and CSIRO, including:

'Zapping away mosquito-borne diseases in Papua New Guinea' published online on CSIROscope blog page on the 27th August 2020 (https://blog.csiro.au/mosquito-borne-diseases-papua-new-guinea/)

'Two threats highlighted in the shadow of COVID-19' published online on the ACIAR Blogs page, 17th September, 2020 (https://www.aciar.gov.au/media-search/blogs/two-threats-highlighted-shadow-covid-19)

'Improving animal health diagnostic capacity in PNG' published in the online News, 20th October 2020 (https://www.aciar.gov.au/media-search/news/improving-animal-health-diagnostic-capacity-png)

'Japanese encephalitis and zoonotic arboviruses in Papua New Guinea' Youtube video featuring project leader David Williams for International One Health Day, November 2020

(https://www.facebook.com/ACIARAustralia/videos/onehealthday-japanese-encephalitis-and-zoonotic-arboviruses-in-papua-new-guinea/678341809520135/)

'Our One Health approach to Japanese encephalitis' published online on the CSIRO website (https://www.csiro.au/en/research/animals/pests/Mosquito-borne-diseases_JapaneseEncephalitis).

In addition, regular updates of ASF project activities were provided at whole-of-government roundtable meetings on ASF in PNG organised by DFAT in 2020 and 2021, involving relevant stakeholders. Project updates were also provided at the monthly online STRIVE PNG Molecular Working group meetings to ensure coordination and knowledge sharing relevant to overlapping or complementary project activities.

Research for One Health System Strengthening (ROHSS) program meetings organised by ACIAR in 2020 and 2021 also provided a valuable forum to exchange project experiences and challenges with other ROHSS program project leaders and team member.

Finally, regular internal communications occurred via email or online meetings to highlight achievements and impacts of project staff and milestones.

9 Conclusions and recommendations

The main achievement of this pilot arbovirus surveillance study was the confirmation that flaviviruses are a potentially important zoonotic risk for the PNG population. Of these viruses, JE is overwhelmingly that of highest concern because of its propensity to spread and cause serious disease and outbreaks. The analysis on the spatiotemporal epidemiology of seroconversion in pigs suggests a marked seasonality of infection which varies across regions. A follow-on study is recommended to unravel seasonal infection dynamics, focussing on defining the window of seroconversion to JE virus in sentinel pigs and identifying temporal changes in mosquito abundance and rates of arbovirus infection. Such a study should focus on peri-urban areas and known arbovirus hot spots such as the Western province.

To understand the human disease burden of JE and zoonotic arboviruses, we also recommend human clinical surveillance as part of a holistic approach to future arbovirus surveillance. This may be readily incorporated into existing clinical sentinel sites already established for febrile disease surveillance for the PNG Department of Health or other surveillance projects (e.g., STRIVE PNG).

From our evaluation of sentinel animals, pigs proved to be a more sensitive animal than chickens for detecting antibodies to JE virus. Moreover, an advantage of using pigs as sentinels is the potential to leverage existing collaborations between NAQIA and commercial production farms located near major population centres, such as Lae and Port Moresby. Even though the presence of maternal antibody can complicate interpretation of seroconversion, the findings from this study indicate that a more focussed approach involving sampling from around 2-3 months of age will enable accurate tracking of waning maternal antibody in pigs and subsequent seroconversion from natural infection.

Although small numbers of MVE and KUN virus antibody positive samples were detected in chickens, albeit slightly higher than those detected in the sentinel pigs, the practical and resourcing challenges and high costs associated with establishing and maintaining dedicated sentinel chicken flocks (e.g., from housing, agistment and animal ethics requirements) argue against this option.

Future pig sentinel surveillance activities may be complimented by PCR testing of oro-nasal swabs taken from weaner pigs to directly detect JE virus RNA in oro-nasal secretions. The incorporation of a JE virus PCR for this purpose at NAQIA would build on and strengthen the molecular diagnostics established in this project for ASF. The use of pen-based rope chewing methods for regular sampling could potentially be employed for this purpose. Such an approach would represent a novel surveillance method for JE virus and would also provide the opportunity for surveillance of other swine pathogens such as ASF and influenza viruses.

From the study on comparisons of different mosquito traps for collecting and testing vectors of zoonotic arboviruses we demonstrated the effectiveness of the CDC_UV trap for capturing high numbers and a diverse range of *Culex* species. We therefore recommend the CDC_UV trap for future monitoring and surveillance of arboviral vector monitoring programs in PNG.

A potential extension of mosquito surveillance is its application to non-vector-borne pathogens. Xenosurveillance is a novel surveillance technique that leverages and extends mosquito surveillance activities to detect pathogens that are circulating in mammalian or avian hosts on which mosquitoes feed. This approach utilises blood-fed mosquitoes and PCR testing as an alternative to direct sampling of hosts (such as sentinel animals), with mosquitoes acting as ‘syringes’. Xenosurveillance could readily be applied for future mosquito surveillance activities in PNG for pathogens of humans and animals.

In conclusion, we consider there is the potential to deliver for the PNG public health authorities a follow-on One Health study integrating human and animal health coupled

with a deeper understanding of the environmental drivers of mosquito-borne disease emergence.

In terms of laboratory capacity building, this project succeeded in developing and establishing new diagnostic test methods and expertise at NAHFTL for flavivirus serology and ASF PCR testing. In doing so, QA standards and practices were incorporated into training and development activities. It is recommended that competency in these tests is maintained at NAHFTL through regular testing following a QA framework and should include ILCT or proficiency testing. Expanding training in these tests to include other members of NAHFTL staff will also provide additional depth and capacity.

It is strongly recommended that PCR testing capability is maintained at NAHFTL and, if possible, expanded to other targets. It is expected that much-needed dedicated PCR laboratory infrastructure will be provided through the recent DFAT funding announcement for NAHFTL. However, the building or refurbishment of new laboratories may take many months to years to be completed and commissioned. It is therefore important that the newly acquired PCR training and skills are maintained over this time, so that testing can continue, as well being readily transferred to the new laboratories once they are put into operation. For this to occur, further support from PNG IMR may be required. Alternative options such as a temporary molecular laboratory at NAHFTL utlilising point-of-care molecular equipment may also be explored, which is decommissioned once the full suite housing dedicated laboratory PCR equipment becomes operational.

Finally, several recommendations were made following the LMT assessments of the NAHFTL (see section 7.1.3), including recommendations to establish PCR testing capacity addressed above. Additional recommendations included:

- infrastructure support to upgrade and maintain the laboratory to international standards
- support to implement best practice QA following ISO 17025 guidelines, and international standards of laboratory biosafety/safety to allow safe and secure handling and storage of infectious materials
- support to implement a Quality Management (QM) system, including appointment of a QA manager and Biosafety Officer, together with the formation of a QA/Biosafety committee to implement and manage QA/Biosafety at NAHFTL

A final recommendation is that the LMT-C and LMT-S are undertaken on a regular basis (e.g., annually) and national LMT experts and laboratory focal points are established. This will enable progress in addressing gaps to be tracked and provides a basis for funding and support from PNG government and donor organisations.

10 References

10.1 References cited in report

Anga, G., Barnabas, R., Kaminiel, O., Tefuarani, N., Vince, J., Ripa, P., Riddell, M., Duke, T., 2010. The aetiology, clinical presentations and outcome of febrile encephalopathy in children in Papua New Guinea. Annals of tropical paediatrics 30, 109-118.

Amben, S., Dom, M., Ayalew, W. 2017. A survey of pig production in Morobe Province of Papua New Guinea, In: Glatz, P. (Ed.) Local feed resources for pig, poultry and fish production in Papua New Guinea. Australian Centre for International Agricultural Research, Canberra.

Ayalew, W. 2013. Challenges and opportunities of smallholder livestock production in PNG. In 15th Australasia/Oceania CVA Conference on Sustainable Animal Health and Production in Australasia/Oceania: the role of veterinary education, disease control, food safety and security and animal welfare (Nadi, Fiji).

Ayalew, W., Danbaro, G., Dom, M., Amben, S., Besari, F., Moran, C., Nidup, K., 2011. Genetic and cultural significance of indigenous pigs in Papua New Guinea and their phenotypic characteristics. Animal Genetic Resources 37-46.

Barton, A.J., Bielefeldt-Ohmann, H., 2017. Clinical Presentation, Progression, and Management of Five Cases of Ross River Virus Infection in Performance Horses Located in Southeast Queensland: A Longitudinal Case Series. Journal of Equine Veterinary Science 51, 34-40.

Bernard K.A., Maffei J.G., Jones S.A., Kauffman E.B., Ebel G., Dupuis A.P., Ngo K.A., Nicholas D.C., Young D.M., Shi P.Y., Kulasekera V.L., Eidson M., White D.J., Stone W.B., Kramer L.D., 2001. West Nile virus infection in birds and mosquitoes, New York State. Emerg Infect Dis 7(4):679-85.

Boukraa S., de La Grandiere M.A., Bawin T., Raharimalala F.N., Zimmer J.Y., Haubruge E., Thiry E., Francis F., 2016. Diversity and ecology survey of mosquitoes potential vectors in Belgian equestrian farms: A threat prevention of mosquito-borne equine arboviruses. Prev Vet Med 124: 58-68.

Bourke, R.M. and Harwood, T. 2009. Food and Agriculture in Papua New Guinea. ANU E Press, Canberra, 665 p

Brioudes, A., Gummow, B., 2017. Understanding Pig and Poultry Trade Networks and Farming Practices Within the Pacific Islands as a Basis for Surveillance. Transboundary and emerging diseases 64, 284-299.

Campbell, G.L., Hills, S.L., Fischer, M., Jacobson, J.A., Hoke, C.H., Hombach, J.M., Marfin, A.A., Solomon, T., Tsai, T.F., Tsu, V.D., Ginsburg, A.S., 2011. Estimated global incidence of Japanese encephalitis: a systematic review. Bull World Health Organ 89, 766-774.

Cappelle, J., Duong, V., Pring, L., Kong, L., Yakovleff, M., Prasetyo, D.B., Peng, B., Choeung, R., Duboz, R., Ong, S., Sorn, S., Dussart, P., Tarantola, A., Buchy, P., Chevalier, V., 2016. Intensive Circulation of Japanese Encephalitis Virus in Peri-urban Sentinel Pigs near Phnom Penh, Cambodia. PLoS Neg Trop Dis, 10(12), e0005149.

Chaiphongpachara T., Laojun S., Kunphichayadecha C., 2019. Effectiveness of ultraviolet (UV) insect light traps for mosquitoes control in coastal areas of Samut Songkhram province, Thailand. Journal of Animal Behaviour and Biometeorology 7: 25-30.

Claflin, S.B., Webb, C.E., 2015. Ross River Virus: Many Vectors and Unusual Hosts Make for an Unpredictable Pathogen. PLoS Pathog 11, e1005070.

Di Francesco J., Choeung R., Peng B., Pring L., Pang S., Duboz R., Ong S., Sorn S., Tarantola A., Fontenille D., Duong V., Dussart P., Chevalier V., Cappelle J., 2018. Comparison of the dynamics of Japanese encephalitis virus circulation in sentinel pigs between a rural and a peri-urban setting in Cambodia. PLoS Negl Trop Dis, 12(8):e0006644.

Dwyer, P.D., 2006. People, pigs and parasites in New Guinea: relational contexts and epidemiological possibilities. Parasitol. Int., 55: 167-173.

Essed, W.C., Van, T., 1965. Arthropd-borne virus infections in Western New Guinea. I. Report of a case of Murray Valley encephalitis in a Papuan woman. Trop Geogr Med 17, 52-55.

Farajollahi A., Kesavaraju B., Price D.C., Williams G.M., Healy S.P., Gaugler R., Nelder M.P., 2009. Field efficacy of BG-Sentinel and industry-standard traps for Aedes albopictus (Diptera: Culicidae) and West Nile virus surveillance. J Med Entomol 46: 919-25.

Fick S.E., and Hijmans R.J. (2017). WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. Int J Climatology 37:4302-4315.

French, E.L., Anderson, S.G., Price, A.V., Rhodes, F.A., 1957. Murray Valley encephalitis in New Guinea. I. Isolation of Murray Valley encephalitis virus from the brain of a fatal case of encephalitis occurring in a Papuan native. Am J Trop Med Hyg 6, 827-834.

Gorsich E.E., Beechler B.R., van Bodegom P.M., Govender D., Guarido M.M., Venter M., Schrama M., 2019. A comparative assessment of adult mosquito trapping methods to estimate spatial patterns of abundance and community composition in southern Africa. Parasit Vectors 12: 462.

Haines F.J., Hofmann M.A., King D.P., Drew T.W., Crooke H.R., 2013. Development and validation of a multiplex, real-time RT PCR assay for the simultaneous detection of classical and African swine fever viruses. PLoS One, 8(7):e71019.

Hanson, J.P., Taylor, C.T., Richards, A.R., Smith, I.L., Boutlis, C.S., 2004. Japanese encephalitis acquired near Port Moresby: implications for residents and travellers to Papua New Guinea. Med J Aust 181, 282-283.

Hii, J., Dyke, T., Dagoro, H., Sanders, R.C., 1997. Health impact assessments of malaria and Ross River virus infection in the Southern Highlands Province of Papua New Guinea. P N G Med J 40, 14-25.

Hou J., Wu Y., Mao Z., Zhu X., Wu Y., Liu Q., Wang J., Li T., Gong Z., Dong X., Wang Z., 2021. Field evaluation of two mosquito traps in Zhejiang Province, China. Sci Rep. 11(1):294.

Johansen, C., van den Hurk, AF, Ritchie, SA, Zborowski, P, Nisbet, D, Paru, R, Bockarie, MJ, MacDonald, J, Drew, AC, Khromykh, TI, Mackenzie, JS, 1997. The search for Japanese encephalitis virus in the western province of Papua New Guinea, 1996. Arbovirus Research in Australia 7, 131-136.

Johansen, C.A., van den Hurk, A.F., Ritchie, S.A., Zborowski, P., Nisbet, D.J., Paru, R., Bockarie, M.J., Macdonald, J., Drew, A.C., Khromykh, T.I., Mackenzie, J.S., 2000. Isolation of Japanese encephalitis virus from mosquitoes (Diptera: Culicidae) collected in the Western Province of Papua New Guinea, 1997-1998. Am J Trop Med Hyg 62, 631-638.

Johansen, C.A., Nisbet, D.J., Foley, P.N., Van Den Hurk, A.F., Hall, R.A., Mackenzie, J. S., Ritchie, S.A., 2004. Flavivirus isolations from mosquitoes collected from Saibai Island in the Torres Strait, Australia, during an incursion of Japanese encephalitis virus. Med Vet Entomology 18(3), 281–287.

Johansen, C.A., Power, S.L., Broom, A.K., 2009. Determination of Mosquito (Diptera: Culicidae) Bloodmeal Sources in Western Australia: Implications for Arbovirus Transmission. J Med Entomol, 46(5), 1167–1175.

Jonduo, M.H., Bande, G., Horwood, P.F., 2012. Arboviruses of human health significance in Papua New Guinea. P N G Med J 55, 35-44.

Johnson T., Braack L., Guarido M., Venter M., Gouveia Almeida A.P., 2020. Mosquito community composition and abundance at contrasting sites in northern South Africa, 2014-2017. J Vector Ecol 45: 104-117.

King D.P., Reid S.M., Hutchings G.H., Grierson S.S., Wilkinson P.J., Dixon L.K., Bastos A.D., Drew T.W., 2003. Development of a TaqMan PCR assay with internal amplification control for the detection of African swine fever virus. J Virol Methods. 2003;107(1):53-61.

Kline D., 2006. Mosquito population surveillance techniques. Technical Bulletin 6: 2-8.

Krockel U., Rose A., Eiras A.E., Geier M., 2006. New tools for surveillance of adult yellow fever mosquitoes: comparison of trap catches with human landing rates in an urban environment. J Am Mosq Control Assoc 22: 229-38.

Li Y., Su X., Zhou G., Zhang H., Puthiyakunnon S., Shuai S., Cai S., Gu J., Zhou X., Yan G., Chen X.G., 2016. Comparative evaluation of the efficiency of the BG-Sentinel trap, CDC light trap and Mosquito-oviposition trap for the surveillance of vector mosquitoes. Parasit Vectors 9: 446.

Luhken R., Pfitzner W.P., Borstler J., Garms R., Huber K., Schork N., Steinke S., Kiel E., Becker N., Tannich E., Kruger A., 2014. Field evaluation of four widely used mosquito traps in Central Europe. Parasit Vectors 7: 268.

Lyons, A.C., Huang, Y.S., Park, S.L., Ayers, V.B., Hettenbach, S.M., Higgs, S., McVey, D.S., Noronha, L., Hsu, W.W., Vanlandingham, D.L. (2018). Shedding of Japanese Encephalitis Virus in Oral Fluid of Infected Swine. Vector Borne Zoonotic Dis, 18(9), 469–474.

Mackenzie, J.S., Lindsay, M.D., Coelen, R.J., Broom, A.K., Hall, R.A., Smith, D.W. (1994). Arboviruses causing human disease in the Australasian zoogeographic region. Arch Virology, 136(3-4), 447–467.

Mackenzie, J.S., Williams, D.T., Smith, D.W., 2007. Japanese encephalitis virus: the geographic distribution, incidence and spread of a virus with a propensity to emerge in new areas, In: Tabor, E. (Ed.) Perspectives in Medical Virology: Emerging Viruses in Human Populations. Elsevier, Amsterdam, 201-268.

Marshall, I.D. 1988. Murray Valley and Kunjin encephalitis, In: Monath, T.P. (Ed.) The arboviruses: epidemiology and ecology. CRC Press, Boca Raton, Florida, 151-189.

Mboera L.E.G., Kihonda J., Braks M.A.H., Knols B.G.J., 1998. Short report: Influence of Centers for Disease Control light trap position, relative to a human-baited bed net, on catches of Anopheles gambiae and Culex quinquefasciatus in Tanzania. American Journal of Tropical Medicine and Hygiene 59: 595-596.

Mohlmann T.W.R., Wennergren U., Talle M., Favia G., Damiani C., Bracchetti L., Koenraadt C.J.M., 2017. Community analysis of the abundance and diversity of mosquito species (Diptera: *Culicidae*) in three European countries at different latitudes. Parasit Vectors 10: 510.

Moore S., Zunwei D., Z H., Xuezhong W., L H., Yujiang X., Hill N., 2001. The efficacy of different mosquito trapping methods ina forest-fringe village, Yunnan Province, Southern China. Southeast Asian J Trop Med Public Health 32: 282-9.

Mouillé B., Dauphin G., Wiersma L., Blacksell S.D., Claes F., Kalpravidh W., Kabore Y., Hietala S., 2018. A Tool for Assessment of Animal Health Laboratory Safety and

Biosecurity: The Safety Module of the Food and Agriculture Organization's Laboratory Mapping Tool. Trop Med Infect Dis., 3(1):33.

Mwanga E.P., Ngowo H.S., Mapua S.A., Mmbando A.S., Kaindoa E.W., Kifungo K., Okumu F.O., 2019. Evaluation of an ultraviolet LED trap for catching Anopheles and Culex mosquitoes in south-eastern Tanzania. Parasit Vectors 12: 418.

Newhouse V., Chamberlain R., JG J., Sudia W., 1966. Use of dry ice to increase mosquito catches of the CDC miniature light trap. Mosquito News 26: 282-289.

Niazi, A.U.R. The Role of Genetic Diversity in the Replication, Pathogenicity and Virulence of Murray Valley Encephalitis Virus. Ph.D. Thesis, Curtin University, Perth, WA, Australia, 2013.

OIE, 2020. African swine fever, Papua New Guinea. World Animal Health Information System. https://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?page_refer=MapFullEventReport&reportid=33803

PNG National Strategic Plan Taskforce. 2009. Papua New Guinea Vision 2050. Government of Papua New Guinea. 82 p. actnowpng.org/sites/default/files/png%20version%202050.pdf

Ponlawat A., Khongtak P., Jaichapor B., Pongsiri A., Evans B.P., 2017. Field evaluation of two commercial mosquito traps baited with different attractants and colored lights for malaria vector surveillance in Thailand. Parasit Vectors 10: 378.

Pyke A.T., Smith I.L., van den Hurk A.F., Northill J.A., Chuan T.F., Westacott A.J., Smith G.A., 2004. Detection of Australasian Flavivirus encephalitic viruses using rapid fluorogenic TaqMan RT-PCR assays. J Virol Methods. 2004 May;117(2):161-7.

Ricklin M.E., García-Nicolás O., Brechbühl D., Python S., Zumkehr B., Nougairede A., Charrel R.N., Posthaus H., Oevermann A., Summerfield A., 2006. Vector-free transmission and persistence of Japanese encephalitis virus in pigs. Nat Commun, 7:10832.

Roiz D., Roussel M., Munoz J., Ruiz S., Soriguer R., Figuerola J., 2012. Efficacy of mosquito traps for collecting potential West Nile mosquito vectors in a natural Mediterranean wetland. Am J Trop Med Hyg 86: 642-8.

Sane, J., Kurkela, S., Levanov, L., Nikkari, S., Vaheri, A., Vapalahti, O., 2012. Development and evaluation of a real-time RT-PCR assay for Sindbis virus detection. J Virol Methods, 179(1), 185–188.

Sastre P., Gallardo C., Monedero A., Ruiz T., Arias M., Sanz A., Rueda P., 2016. Development of a novel lateral flow assay for detection of African swine fever in blood. BMC Vet Res, 12:206.

SCHERER W.F., MOYER J.T., IZUMI T., 1959. Immunologic studies of Japanese encephalitis virus in Japan. V. Maternal antibodies, antibody responses and viremia following infection of swine. J Immunol, 83:620-626.

Shao, N., Li, F., Nie, K., Fu, S.H., Zhang, W.J., He, Y., Lei, W.W., Wang, Q.Y., Liang, G.D., Cao, Y.X., Wang, H.Y., 2018. TaqMan Real-time RT-PCR Assay for Detecting and Differentiating Japanese Encephalitis Virus. Biomed Environ Sci, 31(3), 208–214.

Sriwichai P., Karl S., Samung Y., Sumruayphol S., Kiattibutr K., Payakkapol A., Mueller I., Yan G.Y., Cui L.W., Sattabongkot J., 2015. Evaluation of CDC light traps for mosquito surveillance in a malaria endemic area on the Thai-Myanmar border. Parasites & Vectors 8: 636.

Staunton K.M., Goi J., Townsend M., Ritchie S.A., Crawford J.E., Snoad N., Karl S., Burkot T.R., 2021. Effect of BG-Lures on the Male Aedes (Diptera: Culicidae) Sound Trap Capture Rates. J Med Entomol. 58(6):2425-2431.

Sudia W.D., Chamberlain R.W., 1962. Battery-operated light trap, an improved model. . Mosquito News 22: 126-129.

Tennekes MJJoSS, 2018. tmap: Thematic Maps in R. 84:1-39.

Tesh, R.B., Gajdusek, D.C., Garruto, R.M., Cross, J.H., Rosen, L., 1975. The distribution and prevalence of group A arbovirus neutralizing antibodies among human populations in Southeast Asia and the Pacific islands. Am J Trop Med Hyg 24, 664-675.

van den Hurk A.F., Nisbet D.J., Johansen C.A., Foley P.N., Ritchie S.A., Mackenzie J.S., 2001. Japanese encephalitis on Badu Island, Australia: the first isolation of Japanese encephalitis virus from *Culex gelidus* in the Australasian region and the role of mosquito host-feeding patterns in virus transmission cycles. Trans R Soc Trop Med Hyg 95(6):595-600.

Victor O.A., Adekunle A.J., Tahiru I.K., David O.O., 2017. Influence of Meteorological Variables on Diversity and Abundance of Mosquito Vectors in Two Livestock Farms in Ibadan, Nigeria: Public Health Implications. J Mosq Res 7: 70-78.

Williams, D.T., Daniels, P.W., Lunt, R.A., Wang, L.F., Newberry, K.M., Mackenzie, J.S., 2001. Experimental infections of pigs with Japanese encephalitis virus and closely related Australian flaviviruses. Am J Trop Med Hyg, 65(4), 379–387.

Yamada, M., Nakamura, K., Yoshii, M., Kaku, Y., 2004. Nonsuppurative encephalitis in piglets after experimental inoculation of Japanese encephalitis flavivirus isolated from pigs. Vet Pathol 41, 62-67.

Zepner L., Karrasch P., Wiemann F., Bernard L., 2021. ClimateCharts.net – an interactive climate analysis web platform. International Journal of Digital Earth 14:338-356.

Zsak L, Borca MV, Risatti GR, Zsak A, French RA, Lu Z, Kutish GF, Neilan JG, Callahan JD, Nelson WM, Rock DL., 2005. Preclinical diagnosis of African swine fever in contact-exposed swine by a real-time PCR assay. J Clin Microbiol, 43(1):112-9.

10.2 List of publications produced by project

Goi, J., Koinari, M., Vinit, R., Sakur, M., Pomat, W., Williams, D.T., Karl, S., Comparison of Different Mosquito Traps for Zoonotic Arbovirus Vectors in Papua New Guinea. Am J Trop Med Hyg. 2022 Jan 17:tpmd210640. doi: 10.4269/ajtmh.21-0640. Epub ahead of print. PMID: 35026726.

Williams, DT, Paradkar, P, Karl, S. (2021). Chapter 14: Arbovirus Detection in Vectors. In: Genetically Modified and other Innovative Vector Control Technologies: Eco-bio-social Considerations for Safe Application"; Ed. Brij Kishore Tyagi. Springer Nature, Singapore. Published 14th December, 2021 (457 pages; ISBN-10: 9811629633; ISBN-13: 978-9811629631).

Di Rubbo, A. A Simplified Approach to Serodiagnosis of Zoonotic Flaviviruses. 14th Mosquito Control Association of Australia and 13th Arbovirus Research in Australia Online Symposium. 30th Aug to 1st Sep, 2021; oral presentation.

Goi, J. Comparison of BGS and CDC Light Traps for Vector Mosquitoes Surveillance in Papua New Guinea. 14th Mosquito Control Association of Australia and 13th Arbovirus Research in Australia Online Symposium. 30th Aug to 1st Sep, 2021; oral presentation.

DiRubbo A., Cook, J., Newberry, K., Bowden T., Williams, D. A simplified approach to zoonotic flavivirus serodiagnosis in sentinel pigs and chickens. Fifteenth Annual Meeting of the Australian Association of Veterinary Laboratory Diagnosticians. Brisbane, 28th-29th November 2019; oral presentation (T. Bowden).

11 Appendixes

11.1 Appendix 1: Provincial map and satellite images of sentinel sites for mosquito collections and animal sampling

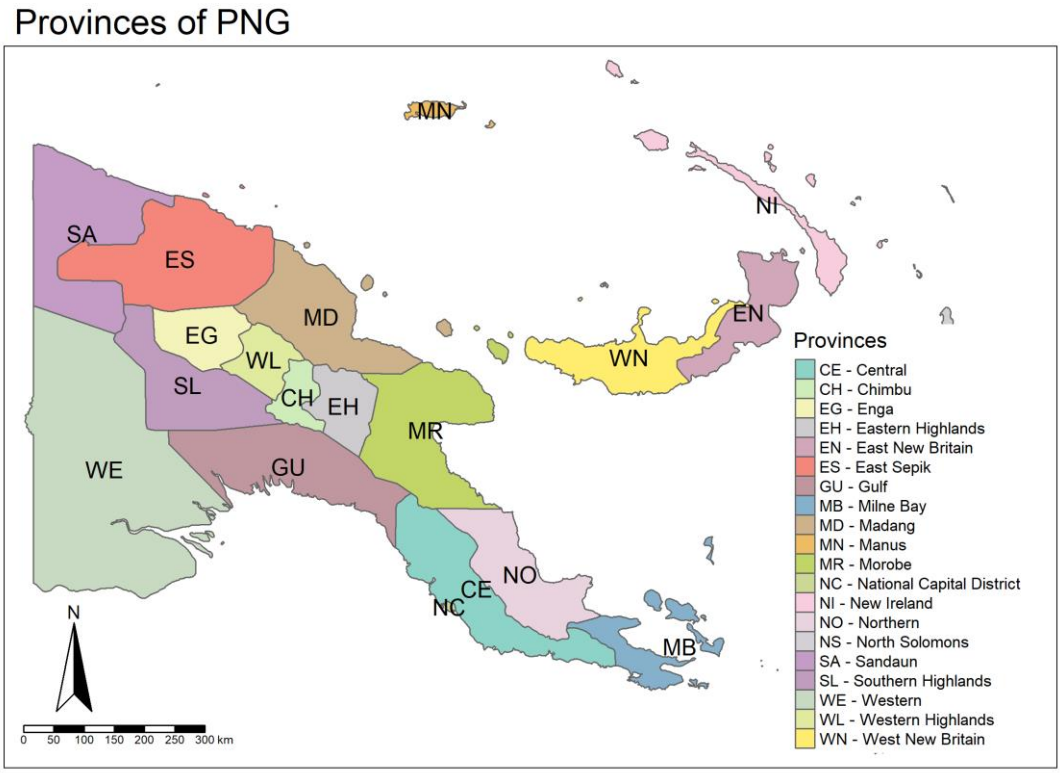


Figure A1. Provincial map of Papua New Guinea constructed from data for provincial map downloaded from the Natural Earth website (https://www.naturalearthdata.com/)

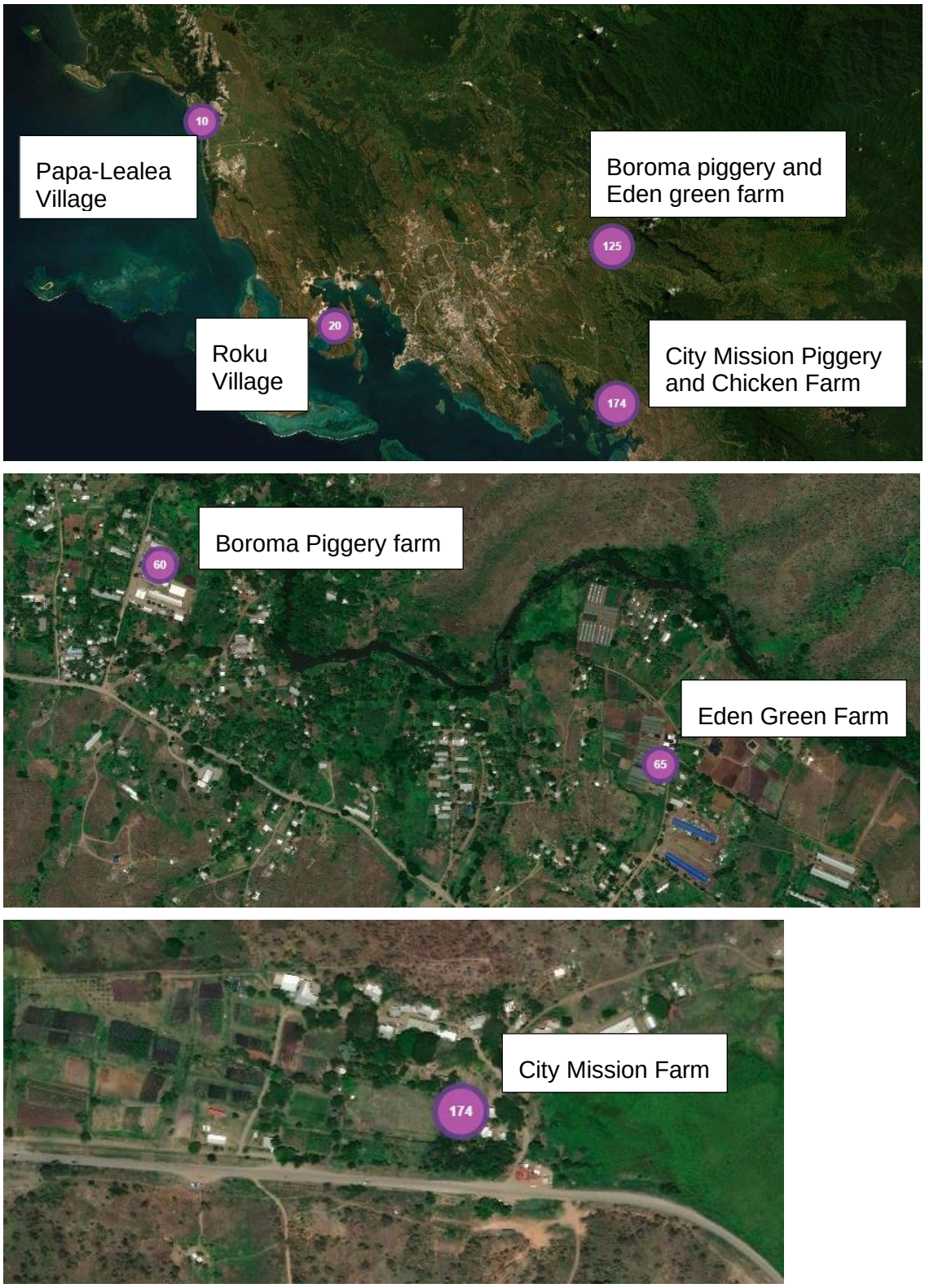


Figure A2. National Capital District and Central province sites.

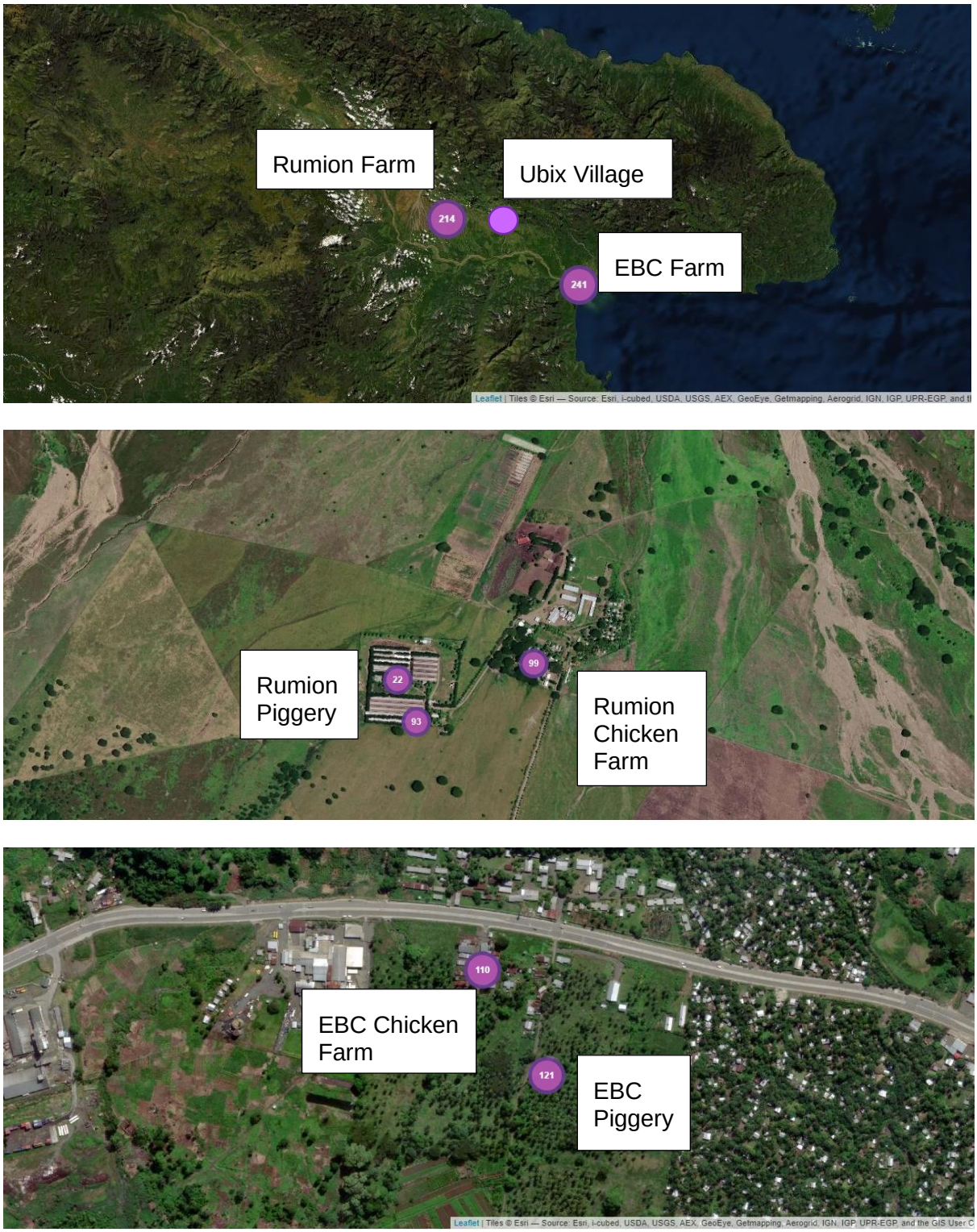


Figure A3. Morobe province sites.

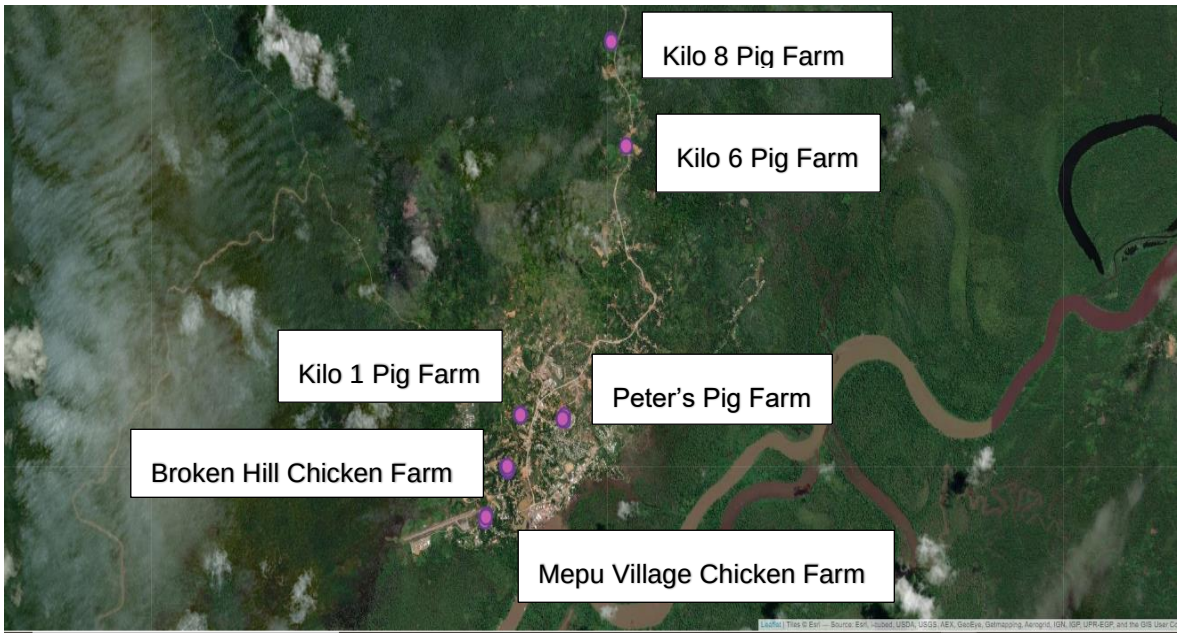


Figure A4. Western Province (Kiunga) mosquito collection sites.

11.2 Appendix 2: Template used for CSIRO Animal Welfare Statement – Use of Privately Owned Animals

Project Title: A one health approach to establish surveillance strategies for Japanese encephalitis and zoonotic arboviruses in Papua New Guinea

Chief Investigator: David Williams

Number and common name of animals to be used: Chickens (up to 50 with up to 25 replacement animals); pigs (up to 110 with up to 55 replacement animals)

Dear *[Insert name of animal owner]*

Thank you for agreeing to allow your animals to be used for the above research project. CSIRO is required to ensure that all research involving animals is conducted in accordance with the requirements specified under the *Australian code for the care and use of animals for scientific purposes (the Code)* and any relevant state and territory legislation. These requirements aim to ensure that the highest standards of animal welfare are maintained at all times. They include a number of responsibilities for the research team, CSIRO, yourself (as the owner of the animals) and any of your employees who will be involved in the care and handling of the animals as part of the above research project. A copy of the Code is attached for your reference.

This letter seeks to outline the nature of the research being conducted on your animals and your responsibilities under the Code and the Victorian State Prevention of Cruelty to Animals Act 1986 Act.

Project details:

- This project aims to sample animals for laboratory testing in order to detect signs of infection by viruses that can cause sickness in people (but not animals), and which are carried by mosquitoes that bite both animals and people.
- Blood Sampling will be undertaken at least weekly for chickens and pigs, by trained National Agriculture Quarantine and Inspection Authority (NAQIA) officers.
- For chickens, blood will be collected from the brachial wing vein. As well as blood collection using a syringe and needle, we will also evaluate small volume (~0.1 ml) collection onto filter paper. For this, 2-3 drops of blood will be collected onto filter paper from needle puncture of the brachial vein of restrained chickens. Blood will then be allowed to dry on the filter paper before shipment back to the laboratory at ambient temperature. This potential advantages of this method is that cold shipment of samples is not required and the procedure may be less invasive than larger volume collection using a syringe.
- For pigs, blood will be collected from the jugular vein or anterior vena cava, depending on the size of the pig. In addition, oral swabs will be collected at the same time for laboratory testing.

Location of animals:
[Various - to be completed by NAQIA animal health officer upon agreement by the private owner to use their animals.]

Project duration:

September 1st to December 21st, 2019.

(NOTE: research will only commence once approval from the CSIRO Animal Ethics Committee has been obtained).

Your responsibilities (and those of your employees who are involved in the care and handling of animals as part of this research project):

Daily monitoring, feeding, and husbandry, including the provision of adequate accommodation. The owner will immediately notify NAQIA animal health officers of any adverse events or issues by contacting *[insert contact number]*. Veterinary services will be provided as required.

Possible risks to your animals:

Stressed animals: to reduce this risk, only healthy animals that handle blood sampling well will be used.

Haematoma (bruising) at bleed site: to reduce this risk experienced animal health officers will collect specimens and handle animals. Animals that develop haematoma will either be samples at an alternative site (e.g. the other wing for chickens) or be rested from subsequent sampling until this heals.

Animal care: In the event that an animal suffers adverse welfare as a direct result of this research, CSIRO will cover the costs of Veterinary care. If the health of animals is normal within 24 hours of blood collection is normal, CSIRO accepts no liability for any subsequent illness of these animals.

Project Contact: Mr Andy Yombo, PNG National Agriculture Quarantine and Inspection Authority

+675 71003392 / 79445537

E: xxxxxxxxxxxxgmail.com

Please acknowledge your acceptance of these responsibilities by signing and returning a copy of this form to Mr Yombo or the relevant NAQIA officer. This research will be subject to ethical review by a licensed CSIRO Animal Ethics Committee. Research will only commence once approval from this Committee has been obtained. The Committee may specify additional requirements regarding animal welfare as part of its approval. You will be advised of these requirements in writing before the commencement of sampling. Should you have any concerns or questions regarding the ethical treatment of animals in this research please contact: CSIRO's Animal Ethics Co-ordinator – AnimalEthicsCoordinator@csiro.au.

Kind regards,

Dr. David Williams

Project Leader

T: +61-3-5227-5364

E: d.williams@csiro.au

Signed (Animal owner):_____

Date: _____

11.3 Appendix 3: Summary of travel undertaken for the project.

Dates	Staff involved	Purpose
5th-10th May 2019	D Williams (ACDP)	Meet NAQIA staff for planning animal surveillance and laboratory testing Attended STRIVE PNG Molecular Detection of Pathogens Workshop Meet PNG IMR staff at Madang for mosquito surveillance planning and Divine Word University about potential student involvement
24th-28th June 2019	D Williams, Peter Durr, Tim Bowden (ACDP), S Karl, D Meyer Steiger (JCU), S McEwan (Burnet), M Koinari (DWU)	First project meeting and technical workshop on PCR and ELISA diagnostic testing Dr Meyer Steiger visited PNG IMR entomology laboratory in Madang mosquito surveillance planning. D Williams meetings with CPHL and PNG Dept. of Health
8th-14th September 2019	D Williams (ACDP)	Establish animal surveillance sites with NAQIA officers Meeting with NAHFTL staff on diagnostic capability for animal surveillance, recruitment of a project officer, and PCR capacity building. Meeting with STRIVE PNG partners at Harbour City ABT Office Meeting with Doreen Iga, ACIAR in-country manager, at the Australian High Commission to review project
20th-26th October 2019	D Williams (ACDP)	Undertake the FAO LMT-C at NAHFTL Attend STRIVE PNG Vector Workshop (24-25 Oct) Meetings with: Dr Ilagi Puana, new Chief Veterinary Officer, to brief him on the ACIAR ZAPPA project Dr Stephan Karl, on planning and resourcing for upcoming mosquito trapping activities. Dr Nime Kapo (FAO PNG, One Health Regional Initiative) to brief him on the ZAPPA project and explore potential linkages NAHFTL staff on design of a new PCR suite and workflow
12th-20th January 2020	D Williams (ACDP) S Karl (JCU)	Meet with NAQIA and PNG IMR staff to review and conduct planning for remaining animal and mosquito surveillance activities Interview and appoint the project officer role, based at NAQIA. Meetings held with: Ms Edwina Tago, General Manager of Corporate Services at NAQIA Mr David Duran, General Manager of Rumion Farm, Lae Ms Doreen Iga, ACIAR in-country manager

11.4 Appendix 4: FINAL AGENDA of the first workshop of partners of the ACIAR project 'A one health approach to establish surveillance strategies for Japanese encephalitis and zoonotic arboviruses in Papua New Guinea'

Date: June 26th-27th

Location: NAQIA Headquarters, Corner of Morea-Tobo Road and Saraga Street, Six Mile, National Capital District, Port Moresby, National Capital District, Papua New Guinea

The overall aims of the first project meeting are to:

- Provide an opportunity for project partners and relevant associates to meet and learn about our different institutes/organisations
- Provide an overview of zoonotic arboviruses in Australasia, with a focus on surveillance and laboratory diagnosis
- Review specific objectives and goals
- Finalise plans for specific activities for each major objective

Emergency contacts:

David Williams: +(61)-405-815-358

Andy Yombo: +(675)-7944-5537

Participants: ***(full list and contact details provided on page 3)***

CSIRO, Australian Animal Health Laboratory: David Williams, Peter Durr, Tim Bowden

PNG National Agriculture Quarantine and Inspection Authority: Joel Alu, David Tenakanai, Andy Yombo, Orlando Mercado, Martin Paina, Pius Clement, Estelo Quimbo, Fova Naoka

PNG Institute of Medical Research: Willie Pomat, Stephan Karl

James Cook University: Dagmar Steiger Meyer

Burnet Institute: Peter Eloni, Samuel McEwen

Central Public Health Laboratory: Willie Porau

Divine Word University: Melanie Koinari

Australian Centre for International Agricultural Research: Francette Dusan

National Department of Health: TBC

Apologies: Prasad Paradkar, Moses Laman, Paul Horwood, Leanne Robinson

Transfers: Will be provided to/from the Holiday Express Inn Hotel to NAQIA HQ and the workshop dinner. Please contact David Williams, Andy Yombo or Peter Eloni for specific requests.

Day 1 – Wednesday 26th June

8:45-9:00	Arrival
9:00	Opening Welcome

9:05	Word of prayer
First Session: Introduction of partner organisations (10 min presentations) *Chair: David Williams*	
9:10	David Williams – CSIRO Australian Animal Health Laboratory
9:20	Andy Yombo - NAQIA
9:30	Willie Pomat – PNGIMR
9:40	Willie Porau - CPHL
9:50	Melanie Koinari – Divine Word University
10:00	TBC – ACIAR
10:10	Peter Eloni – STRIVE partners
10:20-11:00 Morning Tea and group photo	
2nd Session: ACIAR project and arbovirus surveillance *Chair: Andy Yombo*	
11:00	David Williams - Epidemiology of zoonotic arboviruses in PNG
11:20	David Williams – ACIAR One Health project
11:40	Stephan Karl – Vector-borne disease surveillance and mosquito trapping
11:55	Orlando Mercado – Laboratory diagnostic capability at Kila Kila
12:10	Sam McEwan – An Introduction to STRIVE-PNG
12:30 – 1:15 Lunch break	
Technical sessions: One Health strategies *Chair: Francette Dusan*	
1:15 pm	Francette Dusan – One health strategies for disease control
1:30	Peter Durr – One health experiences of pig zoonotic diseases in Laos
1:45	Andy Yombo/Willie Porau/Willie Pomat – existing links between animal and public health sectors (10 mins each)
2:15 (30 mins)	Whiteboard session on establishing reporting links between NAQIA-PNGIMR-CPHL for detection of arboviruses, review specific objectives for One Health linkages
2:45-3:00 Afternoon tea break	
Technical session: Data Management and Integration	

Chair: Peter Durr	
3:00	Peter Durr – Introduction to science data management systems
3:15	Stephan Karl – Data management systems in use at PNGIMR
3:25	Orlando Mercado – Data management systems in use at Kila Kila
3:35	Willie Porau – Data management systems in use at CPHL
3:45	Sam McEwan – STRIVE PNG data capture and aggregation software platform
3:55 (30 mins)	Whiteboard session on gaps and needs, review specific objectives and activities
End of Day 1 (around 4:30pm)	

6:30pm Workshop dinner for partners

Imperial Chinese Restaurant, Section 64, Lot 1-4, Waigani Drive, *Port Moresby*

Day 2 – Thursday 27th June

8:45-9:00am	Arrival
Technical sessions: Mosquito surveillance *Chair: Stephan Karl*	
9:00	David Williams – Mosquito surveillance in Australia and laboratory diagnosis
9:15	Dagmar Meyer – Review current and novel mosquito trapping methods
9:35	Stephan Karl – Surveillance and mosquito trapping strategy for ACIAR project
10:20	Peter Durr – Data management proposal/summary for mosquito surveillance
10:30-11:00 Morning tea break	
Technical session: Animal Surveillance *Chair: Orlando Mercado*	
11:00	David Williams – Sentinel animal surveillance in Australia
11:15	Andy Yombo – Animal surveillance in PNG
11:30	Whiteboard session on animal surveillance for ACIAR project.
12:15	Peter Durr – Data management proposals for animal surveillance
12:25	Final wrap-up and next steps – David Williams
12:40 Lunch break and end of workshop	

11.5 APPENDIX 5: African swine fever diagnostic workshop draft programme

AFRICAN SWINE FEVER (ASF) DIAGNOSTIC WORKSHOP

12-14 October 2021

PROGRAMME

TUESDAY 12 OCTOBER 2021 (DAY 1: THEORY COMPONENT)			
WELCOME			
Time	Activity	Venue	Participant
0800 - 0830	Arrival and registration of participants	NAQIA HQ	Ms. Elaine Kagena Mr. Daniel Kelly Ms. Valentia Yamanea
0830 - 0840	Welcome and opening remarks	NAQIA HQ	NAQIA MD / GMTASD
0840 - 0845	Prayer	NAQIA HQ	Mr. Pius Clement
0845 - 0850	House-keeping/announcements	NAQIA HQ	Ms. Elaine Kagena
0850 - 0900	Introduction and program	NAQIA HQ	CVO

Topic 1: Minimum protective clothing for pig disease surveillance teams

- Types of Personal Protective Equipment (PPE)
- Donning of personal protective equipment (PPE)
- Doffing of personal protective equipment (PPE)
- Contraindications

Time: 0900 - 0930 Hours

Venue: NAQIA HQ

Presenter: DAWE

Method of delivery: Pre-recorded Power-Point Presentation

Questions & Answers (Microsoft Teams)

Topic 2: Basic hygiene practices for pig disease surveillance teams

a) Types of disinfectants and applications

b) Procedure for disinfection/decontamination

- PPE
- Clothes
- Field gear
- Vehicles
- Personnel

c) Procedure for disposal of used PPE, sampling items including syringes and needles, RTKs on-site and off-site

- Burial
- Incineration

Time: 0930-1000 Hours

Venue: NAQIA HQ

Presenter: Mr. Thompson Walambo & Ms. Elaine Hevoho (NAQIA)

Method of delivery: Power-point Presentation

Questions & Answers

TEA/COFFEE BREAK (10 MINUTES)

TUESDAY 12 OCTOBER 2021 (DAY 1: THEORY COMPONENT)

Topic 3: Porcine diseases of interest *[disease summary]*

a) African Swine Fever (ASF)[1]
b) Classical Swine Fever (CSF)[1]
c) Porcine Anthrax[2]

Time: 1010-1050 Hours

Venue: NAQIA HQ

Presenter: Dr. David Williams[1] and Dr. Anthony Keyburn[2] (CSIRO ACDP)

Method of delivery: Pre-recorded Power-point Presentation
Questions & Answers (Microsoft Teams)

Topic 4: Diagnostic tests

a) Ingezim ASF Antigen Rapid Test Kits
b) Ingezim ASF Antibody Rapid Test Kits
c) Ingezim ASF Antigen Enzyme Linked Immunosorbent Assay (ELISA)
d) Ingezim ASF Antibody Enzyme Linked Immunosorbent Assay (ELISA)
e) Enzyme Linked Immunosorbent Assay (ELISA) for CSF
f) Quantitative Polymerase Chain Reaction (qPCR) for ASF and Porcine Anthrax
g) Virus isolation

Time: 1050-1120 Hours

Venue: NAQIA HQ

Presenter: Dr. David Williams (CSIRO ACDP)

Method of delivery: Pre-recorded Power-point Presentation
Questions & Answers (Microsoft Teams)

Topic 5: Sample matrices required for diagnostic testing

a) Whole blood in EDTA anticoagulant
b) Plasma (EDTA anticoagulant)
c) Serum (from plain tubes or tubes with clot activator/coagulant)
d) Nasopharyngeal swabs
e) Post-mortem tissues and fluids
f) Pork meat and processed pork products for human consumption
g) Commercially produced stock-feed/stock-feed ingredients
h) Labelling, handling and storage[3]
i) Contraindications

Time: 1120-1150 Hours

Venue: NAQIA HQ

Presenter: Dr. David Williams (CSIRO ACDP)
Ms. Bridgit Kavana (NAQIA)[3]

Method of delivery: Pre-recorded Power-point Presentation
Questions & Answers (Microsoft Teams)
Power-Point Presentation

TUESDAY 25 OCTOBER 2021 (DAY 1: THEORY COMPONENT)

Topic 6: Laboratory Sample Submission and Test Request Form

a) Introduction and explanation of the contents of the form
b) How to complete the form
c) Laboratory accession, inspection and registration (workflow)

Time: 1150-1210 Hours

Venue: NAQIA HQ

Presenter: Ms. Ellenica Kingal (NAQIA)

Method of delivery: Power-point Presentation
Questions & Answers

LUNCH BREAK (1 HOUR)

Topic 7: Sampling of live pigs

a) Procedure
- Equipment and preparation
- Systemic field reference ID and field sample ID
  - Site, animal, sample dentification, history and traceability records
- Pig restraining techniques
- Aseptic techniques
- Bleeding for whole-blood, plasma and serum samples
- Swabbing using COPAN Flog and Genotubes for ASF and CSF
- Swabbing (nasopharyngeal) for porcine anthrax

b) Sample preservation, handling and storage
c) Contraindications

Time: 1310-1340 Hours

Venue: NAQIA HQ

Presenter: DAWE

Method of delivery: Pre-recorded Power-Point Presentation
Questions & Answers (Microsoft Teams)

TUESDAY 25 OCTOBER 2021 (DAY 1: THEORY COMPONENT)

Topic 8: Post-mortem examination for pig disease diagnosis

a) Procedure

- Equipment and preparation
- Systemic field reference ID and field sample ID
  - Site, animal, sample dentification, history and traceability records
- Euthanasia
- Post-mortem

- Aseptic techniques
- Collection of blood for Ingezim ASF Antigen RTK
- Collection of blood and tissues for ASF Antigen Enzyme Linked Immunosorbent Assay (ELISA)
- Collection of tissues for virus isolation
- Collection of tissues for Polymerase Chain Reaction (PCR) testing
- Swabbing using COPAN FLOK and Genotubes for ASF and CSF
- Swabbing (nasopharyngeal) for porcine anthrax

b) Sample preservation, handling and storage

c) Contraindications

Time: 1340-1410 Hours

Venue: NAQIA HQ

Presenter: DAWE

Method of delivery: Pre-recorded Power-Point Presentation

Questions & Answers (Microsoft Teams)

Topic 9: Sampling of commercially produced stock-feed and ingredients

a) Procedure

- Equipment and preparation
- Systemic field reference ID and field sample ID
  - Site, animal, sample dentification, history and traceability records
- Sample preservation, handling and storage
- Contraindications

Time: 1410-1440 Hours

Venue: NAQIA HQ

Presenter: DAWE

Method of delivery: Pre-recorded Power-Point Presentation

Questions & Answers (Microsoft Teams)

Topic 10: Identifying and confiscation/detention of pork meat and processed pork products at ports of entry and post offices and record keeping

Time: 1440-1510 Hours

Venue: NAQIA HQ

Presenter: DAWE

Method of delivery: Pre-recorded Power-Point Presentation

Questions & Answers (Microsoft Teams)

TUESDAY 25 OCTOBER 2021 (DAY 1: THEORY COMPONENT)

Topic 11: International Air Transport Association (IATA) Dangerous Goods (DG) packaging requirements

a) Introduction

b) Classes of Dangerous Goods

c) UN compliant commercial packaging and sample transport boxes

d) UN labels

e) Labelling of transport boxes

Time: 1510-1540 Hours

Venue: NAQIA HQ

Presenter: Mr. Fova Naoka (NAQIA)

Method of delivery: Power-Point Presentation

Questions & Answers

TEA/COFFEE

END OF DAY 1

PRACTICAL COMPONENT - LIST OF TRAINERS AND PARTICIPANTS PER GROUP – DAY 2

A. VFS practical activities 1

Group A1 - Dr. Ilagi Puana (Trainer), Josephine Inguba (Assistant), Joel Alu, Manape Kum, Wilson Guhe, Fova Naoka, Ellenica Kingal
Group A2 - Dr. Estelo Quimbo (Trainer), Sapon Suman (Assistant), Gurume Toka, Godwin Mongoi, Valentina Yamanea.
Group A3 - Dr. Ian Enriquez (Trainer), Dr. Tania Areori (Assistant), David Tenakanai, Martin Paina, Dorothy Pagru, Daniel Kelly.
Group A4 - Heni Nigani (Trainer), Elaine Kagena (Assistant), Andy Yombo, Noki Mokrui, Bridgit Kavana, Eric Makui.
Group A5 - Paskalis Ominipi (Trainer), Elaine Hevoho (Assistant), Pius Clement, Robert Lomba, Thompson Walambo, Nathan Momba.

PRACTICAL COMPONENT - LIST OF TRAINERS AND PARTICIPANTS PER GROUP – DAY 3

VLS practical activities 2 & 4

Group B1 - Mr. Fova Naoka (Trainer), Dr. Ilagi Puana, Josephine Inguba, Joel Alu, Manape Kum, Wilson Guhe, Valentina Yamanea.
Group B2 - Bridgit Kavana (Trainer), Heni Nigani, Elaine Kagena, Andy Yombo, Noki Mokrui, Eric Makui, Godwin Mongoi.
Group B3 - Elaine Hevoho (Trainer), Paskalis Ominipi, Pius Clement, Robert Lomba, Thompson Walambo, Nathan Momba, Godwin Mongoi, Gurume Toka, Dr. Estelo Quimbo.
Group B4 - Ellenica Kingal (Trainer), Dr. Ian Enriquez, Ellenica Kingal, David Tenakanai, Martin Paina, Dorothy Pagru, Daniel Kelly, Sapon Suma.

WEDNESDAY 26 OCTOBER 2021 (DAY 2: THEORY COMPONENT)			
REGISTRATION & REVISION			
Time	Activity	Venue	Participant
0800 – 0830	Arrival and registration of participants	NAQIA HQ	Ms. Elaine Kagena Mr. Daniel Kelly Ms. Valentia Yamanea
0830 – 0900	Summary revision Session 1 topics	NAQIA HQ	Dr. Tania Areori

Topic 12: Domestic consignments

a) Communicating with NAHFTL staff

b) UN compliant packaging and labelling

c) Verification of compliance by licenced IATA DG Packer

d) Completed Laboratory Sample Submission and Test Request Form

e) Sample packing list (sample inventory list)

f) TNT Consignment Note and Airway Bill No.

Time: 0900-0930 Hours

Venue: NAQIA HQ

Presenter: Elaine Bridgit Kavana (NAQIA)

Method of delivery: Power-Point Presentation

Questions & Answers

Topic 13: Export of diagnostic samples to OIE reference laboratory (i.e., CSIRO ACDP)

a) Communicating with ACDP staff

b) Veterinary diagnostic sample consignment letter from OCVO

c) CSIRO ACDP Specimen Advice Note (SAN)[4]

d) Department of Agriculture Environment and Water Australia (DAWE) import permit for veterinary diagnostic samples

e) DAWE import permit for pork meat products (including intercepted pork products)

f) DAWE import permit of animal stock-feed

g) NAQIA Customs Invoice

h) NAQIA - NAHFTL Manufacturer's Declaration

i) NAHFTL Sample packing list (Sample inventory list)

j) TNT Consignment Note and Airway Bill No.

Time: 0930-1000 Hours

Venue: NAQIA HQ

Presenter: Ms. Bridgit Kavana and Ms. Elaine Hevoho (NAQIA)

Dr. Mark Ford (CSIRO ACDP)[4]

Method of delivery: Power-Point Presentation

Questions & Answers (Microsoft Teams)[4]

TEA/COFFEE BREAK (10 MINUTES)

WEDNESDAY 26 OCTOBER 2021 (DAY 2: THEORY COMPONENT)

Topic 14: Use and interpretation of Ingezim Rapid Test Kits (lateral flow device) for ASF virus antigen and antibody detection

a) Introduction to ASF Ingezim antigen RTK and ASF antibody RTK

b) Procedure

- Setting up the tests
- Reading the tests
- RTK record sheet (i.e. site, animal, sample dentification, history and traceability records)

Time: 1010-1040 Hours

Venue: NAQIA HQ

Presenter: Dr. David Williams (CSIRO ACDP) and DAWE

Method of delivery: Pre-recorded Power-Point Presentation

Questions & Answers (Microsoft Teams)

Topic 15: Reporting process of field RTK test results and decision-making process on-site.

- Decision tree
- Official channel of communication and reporting

Time: 1040-1110 Hours

Venue: NAQIA HQ

Presenter: CSIRO ACDP and DAWE

Method of delivery: Pre-recorded Power-Point Presentation

Questions & Answers (Microsoft Teams)

LUNCH BREAK (1 HOUR)

WEDNESDAY 26 OCTOBER 2021 (DAY 2: PRACTICAL COMPONENT)

Activity 1: Sampling of live pigs

- Sample types

- African Swine Fever (ASF)
- Classical Swine Fever (CSF)
- Porcine Anthrax

- Documentation and records

Time: 1300-1500 Hours

Venue: NAHFTL

Trainers: 1. Dr. Ilagi Puana, 2. Dr. Tania Areori, 3. Dr. Estelo Quimbo, 4. Dr. Ian Enriquez and 5. Mr. Heni Nigani

Method of delivery: Hands-on

Questions & Answers

TEA/COFFEE

END OF DAY 2

THURSDAY 27 OCTOBER 2021 (DAY 3: PRACTICAL COMPONENT)			
REGISTRATION			
Time	Activity	Venue	Participant
0800 - 0830	Arrival and registration of participants	NAQIA NAHFTL	Ms. Elaine Kagena Mr. Daniel Kelly Ms. Valentia Yamanea

Activity 2: ASF Rapid Antigen and Antibody Test Kit Testing (Lateral Flow Device)

- Sample requirements
- Setting-up of ASF antigen and antibody RTKs
- Reading of reaction lines and interpretation
- Documentation and records

Time: 0900-1000 Hours

Venue: NAHFTL

Trainers: 1. Mr. Fova Naoka, 2. Dr. Ms. Ellenica Kingal, 3. Ms. Elaine Hevoho, 4. Ms. Bridgit Kavana

Method of delivery: Hands-on

Questions & Answers

TEA/COFFEE BREAK (10 MINUTES)

Activity 3: NAQIA protocol for pork products and commercial pig-feed

- Sampling, sample handling and storage and requirements for:
- Pork meat products (including intercepted pork products) for ASF
- Commercially produced stock-feed and pig biological matrix adulteration for ASF
- Documentation and records

Time: 1010-1210 Hours

Venue: NAHFTL

Chair: CVO

Method of delivery: Group discussion and proposals

Questions & Answers

LUNCH BREAK (1 HOUR)

Activity 4: International Air Transport Association (IATA) Dangerous Goods (DG) Packing

- Sample types
- Veterinary diagnostic samples
- Pork products including intercepted pork products
- Stock-feed
- IATA DG requirements and associated documentation

Time: 130-1410 Hours

Venue: NAHFTL

Trainers: 1. Mr. Fova Naoka, 2. Dr. Ms. Ellenica Kingal, 3. Ms. Elaine Hevoho, 4. Ms. Bridgit Kavana

Method of delivery: Hands-on

Questions & Answers

TEA/COFFEE

END OF DAY 3

11.6 APPENDIX 6: UNRE Paravet training course report from Mr Eric Makui

NATIONAL AGRICULTURE QUARANTINE & INSPECTION AUTHORITY
ANIMAL HEALTH SECTION VETERINARY FIELD SERVICES
LOCATION: NBPOL Numundo Plantation/cattle Farm, Along Talasea Highway, Kimbe WNBP

P O BOX 459
KIMBE
WEST NEW BRITAIN PROVINCE
PAPUA NEW GUINEA

TEL : (675) 9835172/9834707
FAX : (675) 9835172
MOBILE : (675) 79030525/78325971
A/EMAIL : ericmakui97@gmail.com

MINUTE

To : **Dr David Williams - ACIAR**

From : Animal Health Officer - Kimbe Port

Date : 04th March 2021

Subject : **COURSE STUDY REPORTS**

Dear Dr Williams,

First of all, like to personally commend you and the ACIAR Team for funding this very important Para Vet course. The first of its kind in Papua New Guinea, and giving me the opportunity of a lifetime to participate with other students. Thank you so much. Really appreciated it.

The course topics and outlines (attached) are new and very interesting as well. The theory sessions in classroom, followed by practical out in the farm and the laboratory analysis, really equipped me a lot. And, after successful completion of the courses, I can go confident to use these knowledge and skills back at my own organization/workplace, etc.

The courses, also designed in way of up-skilling of appropriate human resources, specifically those in the Animal Health and Disease Management arena is a way forward to promote biosecurity and ensure food safety and security in Papua New Guinea.

NAQIA CVO Dr. Ilagi Puana is commended as well for his input in nominating and sending me to participate, including GM Tech Mr. David Tanekanai, AHPMFS Mr. Clement Pius and A/HRM Ms Hale Baida for their continued support during my three months of studies.

Thank you all.

FYI and record.
Regards,

Eric Makui (Mr)
AHO-Technical Division Kimbe

Cc: CVO, GM Tech, AHPMFS, A/HRM

Sustaining Development through Better Plant and Animal Health
Keep It Growing