

Surveillance for One Health and high consequence veterinary pathogens (Brucellosis, Coxiellosis and Foot and Mouth Disease) in Southeast Asia: Lao PDR and Cambodia in focus and the importance of international partnerships

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Abstract. Animal disease surveillance in limited-resource countries is challenging but critical in providing epidemiological information to inform disease prevention and control programmes. Despite multiple international agencies and partnerships supporting Lao PDR and Cambodia's animal disease surveillance activities over many years, cost-effectiveness and sustainability remain significant constraints. Here we describe the development and implementation of national abattoir-based surveillance networks in Laos and central Cambodia consisting of an information exchange platform and sample collection and submission systems. The networks enhanced the national surveillance capacity and provided snapshot information of seroprevalence for selected One Health and high consequence veterinary pathogens, including Q fever, brucellosis, and Foot and Mouth Disease (FMD). Despite abattoir survey data revealing that the seroprevalence of Q fever and brucellosis was generally low, the true impact on public health for these diseases remains unclear due to low levels of awareness and diagnostic capacity. FMD antibodies derived from natural infection rather than vaccination were noted in greater than 40% of the animal sampled in both countries, which suggests significant underreporting of outbreak events. Such networks will continue to be refined to improve their cost-effectiveness and sustainability, including the introduction of a simple online application for reporting animal disease outbreaks as well as expanding to other relevant One Health pathogens and species.

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Introduction

Animal disease surveillance and control in lower-middle-income countries has been a challenge due to low human and financial resources¹. Lao People's Democratic Republic (Lao PDR, also referred to as Laos) and the Kingdom of Cambodia have large agricultural sectors estimated to comprise approximately 40–50% of the countries' Gross Domestic Product². The majority of farmers in both countries remain as smallholders rearing livestock as an additional source of income^{3,4}, although in some cases, the raising livestock is relied upon for subsistence purposes. Backyard farming with little or no biosecurity is common practice for smallholders⁴, meaning that incursions of major endemic animal diseases such as foot and mouth disease (FMD), classical swine fever, porcine respiratory and reproductive syndrome and African swine fever, as well as zoonoses including brucellosis, Q fever and anthrax have the potential to significantly impact the wealth of smallholders and the countries' public health and food security.

Beyond the local impact of such diseases, it is in the interest of other countries to be cognisant of the disease situation, especially those that have geographical, trading or strategic links. Projects have been managed and coordinated by the World Organisation for Animal Health (OIE), the Australian Centre for International Agricultural Research (ACIAR), the Australian Aid programme (AusAID), the European Union, the Government of South Korea, and The Japan Trust Fund⁵. In addition, the United States government has played a significant role in disease surveillance via the United States Agency for International Development (USAID), which largely focussed on pandemic threat reduction via the PREDICT program⁶ and the Biological Threat Reduction Program of the Defense Threat Reduction Agency (BTRP-DTRA) who provide resources and technical expertise to strengthen surveillance infrastructure worldwide to identify biological threats and future pandemic risks. More recently, the AusAID's Indo-Pacific Centre for Health Security initiative has partnered with governments in Indo-Pacific region countries and other stakeholder organisations to

address infectious disease surveillance and response infrastructure requirements⁷.

FMD is a contagious animal disease endemic in mainland Southeast Asia (SEA) and has been regularly reported in wild and domestic animals in Laos and domestic animals in Cambodia, but not reported in wildlife according to the OIE-WAHIS database⁸. FMD would cause severe biosecurity and economic consequences if it were to be introduced to Australia, and government agencies, including ACIAR have supported FMD projects in Thailand, Laos, and Cambodia for more than 30 years⁵ through projects managed by the CSIRO Australian Animal Health Laboratory (now known as the Australian Centre for Disease Preparedness)⁹ and the University of Sydney veterinary school^{10–12}. Another long-running project with multilateral support is the SEA Foot and Mouth Disease Campaign (SEAFMD)^{13,14} that has focused on the coordinated control of FMD within eight countries in the ASEAN region, including Cambodia, Indonesia, Laos, Malaysia, Myanmar, the Philippines, Thailand and Vietnam. The campaign is coordinated through a Regional Coordination Unit of OIE in Bangkok, with support over the years from the Australian, Swiss and New Zealand Governments. In 2010, with Brunei Darussalam and Singapore as FMD-free countries and the People's Republic of China (PR China) joined the campaign whereby it was renamed the Southeast Asia and PR China FMD campaign (SEACFMD)⁵.

In addition to high consequence animal diseases, neglected zoonotic diseases such as Q fever (caused by *Coxiella burnetii*) and brucellosis (caused by *Brucella spp.*) are endemic in many countries worldwide^{15–17}; however, their situation is under-recognised in Laos and Cambodia based on the OIE's WAHIS database reporting⁸. Such diseases not only present a problem in terms of animal health but also in terms of public health impacts, including acute and chronic ill-health for those individuals who may be occupationally exposed to such pathogens, such as farmers or abattoir workers. Furthermore, both pathogens have bioterrorism potential and, as such, are subject to significant biosecurity controls such as Select Agent requirements in the US¹⁸. Transmission routes of Q fever are inhalation of contaminated particles and direct contact with infected animals. Primary reservoirs of Q fever are ruminants; however, ticks, wildlife¹⁹, and domestic animals such as dogs and cats can also be reservoirs¹⁵. Interestingly, *Coxiella spp.* has been previously detected in many tick species in Laos²⁰. *Brucella spp.* can be transmitted via direct contact, ingestion and inhalation, and wildlife could be reservoirs.

Development of abattoir-based surveillance networks in Laos and Cambodia

There is evidence from OIE's WAHIS database⁸ and numerous previous studies that there are significant livestock and zoonotic

pathogens that circulate in livestock in Laos and Cambodia. Therefore, there is imperative to support national animal disease surveillance and reporting networks to provide epidemiological information to better inform decision-making for disease prevention and control. In both Laos and Cambodia, there have been several animal disease surveillance programs supported by multiple international agencies; however, the programs were often sporadic and subject to the donor's interests. Since 2019, national animal disease abattoir-based surveillance networks have been established as part of BTRP-DTRA-funded projects managed by the Mahidol Oxford Tropical Medicine Research Unit (MORU) in conjunction with Laos and Cambodian governments with key objectives to strengthen veterinary surveillance capacity and provide a snapshot of the seroprevalence of selected zoonoses and high impact animal diseases circulating in the countries.

In 2019 and 2020, the main objectives during the implementation of the networks were (1) capacity building of field officers in animal sample collection and laboratory officers on diagnostic techniques, and (2) data collection and establishing communication platforms using available social networking platforms. Training sessions and sample collection manuals were also developed to assist with the optimal collection of specimens in the field and from abattoirs (Figure 1). The abattoir-based survey methodology was selected due to its affordability and practicality compared with active surveillance techniques. In both countries, social networking-based communication platforms (Laos used WhatsApp – 36 members, and Cambodia used Telegram – 27 members) for information exchange regarding disease outbreaks, training, sample collection activities, sample shipping and notifications were implemented.

The abattoir survey in Laos was performed in all 18 (100%) provinces, while seven (28%) provinces in Cambodia participated in the survey. Samples were collected twice a month, aiming to collect at least 30% of available animals in each province during the abattoir visit. IDvet (Grabels, France) ELISA kits that detected antibodies against *Brucella spp.* (ID Screen[®] Brucellosis Serum Indirect), *C. burnetii* (ID Screen[®] Q Fever Indirect) and FMD non-structural protein (NSP, which detects antibodies against natural infection rather than vaccine-induced antibodies) (IDScreen[®] FMD NSP Competition) were used for the serological testing. Rose Bengal Test was also used for confirmation and diagnosis of brucella antibodies. A total of 5192 and 839 large ruminant samples were collected from the abattoir surveys in Laos and Cambodia, respectively, with summary results presented in Table 1. Full epidemiological analyses of the surveillance outcomes are published elsewhere or submitted for publication^{5,21–23}.

The seroprevalence results for both *Brucella spp.* and *C. burnetii* antibodies in large ruminant populations sampled were low, which



Figure 1. Training and surveillance activities.

Table 1. Overall seroprevalence of *Brucella* spp., *C. burnetii* and FMD non-structural protein antibodies in Laos and Cambodia during 2019–2020.

Type	n	% Seroprevalence (95% confidence interval)		
		<i>Brucella</i> spp	<i>C. burnetii</i>	FMD NSP
Lao PDR				
Buffalo	1610	0.06 (0.00, 0.35)	0.93 (0.57, 1.53)	46.52 (44.10, 48.96)
Cattle	3582	0.84 (0.59, 1.19)	1.90 (1.50, 2.40)	49.97 (48.34, 51.61)
Cambodia				
Cattle	839	0.60 (0.25, 1.39)	0.36 (0.12, 1.05)	42.79 (39.48, 46.16)

correlates with previous surveys^{24,25}. The results revealed that for Laos, the brucellosis seroprevalence in cattle was significantly higher than in buffaloes, while the seroprevalence in cattle in both countries were the same. However, the limitation of the abattoir-based surveys is that small ruminants are not normally processed, and in Laos, evidence of Q fever ‘hotspots’ has been previously reported in Xayabury province and *Brucella* spp. antibodies in goats around Vientiane capital (both provinces share a border with Thailand) using active surveillance²⁶. These are the first investigations of *Brucella* spp. and *C. burnetii* antibodies in Cambodia, and the diseases have never been formally reported; however, this is not surprising given the low levels of awareness and limited capacity for diagnosis and detection. It is likely that with further investigations, additional disease hotspots may be detected, as was demonstrated in the recent study in a Thai province bordering Cambodia that reported *Brucella* spp. and *C. burnetii* antibody seropositivity in cattle of 2.6% and 4.3–27.3%, respectively, and small ruminants (goat and sheep) of 13.3% and 33% respectively²⁷. The natural history of *Brucella* spp. and *C. burnetii* disease in both countries is under-investigated and certainly warrants further studies. From a public health viewpoint, both of these diseases remain truly neglected in

these countries given the dearth of case reports; however, this is not surprising given that both diseases are notoriously difficult to clinically diagnose due to the wide differential diagnosis and the requirement for specialised laboratory expertise to provide an accurate diagnosis^{28,29}.

The FMD serology results demonstrated that greater than 40% of large ruminants in both countries processed in the abattoirs had been exposed to FMD prior to slaughter; however, the high level of seropositivity is not reflected in the official WAHIS FMD outbreak reports. This is not surprising given the limitations of government resources and the possible reluctance of farmers to report disease to the authorities as there is often little to no compensation. The results correlate with previous studies in Cambodia between 2008–2011, where 76% of provinces reported FMD³⁰, and the overall seroprevalence of FMD in the southern provinces was 30%³¹. Similarly, in Laos, an abattoir-based nationwide animal disease surveillance program, similar to that described here, reported FMD seroprevalence between 1999–2001 of 32.2% in buffalo and 26.5% in cattle³². The consequence of this under-reporting is difficult to quantify; however, modelling of these serological results compared with outbreak information will provide valuable insights into the

economic and cultural consequences of FMD in low resource and subsistence farmer communities. For example, spatial modelling could identify spatial correlation, disease clusters and socio-economic factors associated with the disease distribution.

Conclusions

So far, a great deal of experience has been gathered regarding the opportunities and challenges presented by abattoir-based disease surveillance systems. Strengths include recognising the potential of informal social network communications as early disease detection platforms and leveraging the potential of the existing abattoir-based sampling network. Challenges remain for official disease reporting systems within and between government sectors, which are due to inefficiencies resulting in under-reporting of disease and delayed control measures. Limitations of the abattoir-based studies remain in that there is an inherent bias towards large ruminants processed for slaughter and will require refinement to include small ruminants. Building on these lessons learnt and the disease data collected to date, the next phase of the project will continue to improve the cost-effectiveness and sustainability of the surveillance networks by utilising simple online apps for reporting animal disease occurrences.

Conflicts of interest

The authors declare no conflicts of interest.

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Biographies



Professor Stuart Blacksell is a Senior Principal Research Scientist at the University of Oxford based at the Mahidol-Oxford Tropical Medicine Research Unit (MORU) in Thailand where he has been since 2001. Stuart is a Professor of Tropical Microbiology at the University of Oxford and Visiting Professor at the Open University UK and Mahidol University, Thailand. In his current post at MORU, he is Biorisk and Zoonosis group leader where he leads DTRA-funded projects on One Health and high consequence veterinary pathogens focussing on surveillance, biosafety and biosecurity knowledge and capacity building in veterinary and human health laboratories in Thailand, Laos and Cambodia. Stuart is a Registered Biosafety Professional with the American Biological Safety Association since 2009 and a member of the WHO Biosafety Advisory Group and a member of the recently published *WHO Laboratory Biosafety Manual 4th edition* (LBM) editorial

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Dr Jarunee Siengsanon-Lamont is the LACATH4 project manager and senior veterinary consultant at MORU. She is a Thai veterinarian based in Perth, Western Australia, with a research background in veterinary epidemiology and emerging and zoonotic diseases. Jarunee completed her PhD at Murdoch University and had been working as a researcher at Mahidol university, technical officer at the World Organisation for Animal Health (OIE), Bangkok office, where she coordinated the programme for strengthening veterinary services in South East Asia. More recently, Jarunee has worked as a veterinary consultant and later project manager at MORU. Her current role at MORU is to manage the LACATH4 project funded by the Defense Threat Reduction Agency (DTRA), US government which is being applied in Lao PDR, Cambodia and Thailand. The previous phase of the LACATH4 project (formerly called CAMNN3 and also managed by Jarunee) was successfully completed and recently received continued funding from DTRA until 2023. Jarunee manages a multidisciplinary team, coordinating activities with senior government officers and facilitating animal disease surveillance programs and training. Jarunee is also an affiliate supervisor at Sydney University.



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