

1 **Title:** The impact of African Swine Fever Virus on smallholder village pig production: an outbreak  
2 investigation in Lao PDR

3 **Running head:** Impact of African Swine Fever on smallholder pig production in Laos

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25

## 26 **Summary**

27 African Swine Fever Virus (ASFV) causes a deadly disease of pigs which spread through southeast Asia in  
28 2019. We investigated one of the first outbreaks of ASFV in Lao Peoples Democratic Republic amongst  
29 smallholder villages of Thapangtong District, Savannakhet Province. In this study, two ASFV affected villages  
30 were compared to two unaffected villages. Evidence of ASFV-like clinical signs appeared in pig herds as early  
31 as May 2019, with median epidemic days on 1 and 18 June in the two villages, respectively. Using participatory  
32 epidemiology mapping techniques, we found statistically significant spatial clustering in both outbreaks ( $P <$   
33 0.001). Villagers reported known risk factors for ASFV transmission – such as free-ranging management  
34 systems and wild boar access – in all four villages. The villagers reported increased pig trader activity from  
35 Vietnam before the outbreaks; however, the survey did not determine a single outbreak source. The outbreak  
36 caused substantial household financial losses with an average of 9 pigs lost to the disease, and Monte Carlo  
37 analysis estimated this to be USD 215 per household. ASFV poses a significant threat to food and financial  
38 security in smallholder communities such as Thapangtong, where 40.6% of the district's population are affected  
39 by poverty. This study shows ASFV management in the region will require increased local government  
40 resources, knowledge of informal trader activity and wild boar monitoring alongside education and support to  
41 address intra-village risk factors such as free-ranging, correct waste disposal and swill feeding.

42  
43 **Keywords:** African Swine Fever; village; smallholder; pig production; animal health economics; Lao PDR

## 45 **Introduction**

46 African Swine Fever (ASF) is a disease of domestic pigs and wild suids caused by the African Swine Fever  
47 Virus (ASFV). ASFV is a DNA virus present in all secretions, blood and tissues of affected animals (Sánchez-  
48 Vizcaíno, Laddomada, & Arias, 2019). It can survive for an extended period in the environment and years in  
49 frozen meat products. ASFV can spread via direct and indirect contact (Fareiz & Morley, 1997; Sánchez-  
50 Vizcaíno et al., 2019). Warthogs, wild boar, feral pigs and soft ticks contribute to the sylvatic cycle of ASFV.  
51 This combination of extended survival and sylvatic cycles enable distant spread across landscapes, often

52 facilitated by human transportation or management practices (Burrage, 2013; Costard et al., 2009; Jori et al.,  
53 2013; Nurmoja et al., 2018). In naïve pigs and wild boar, clinical signs of ASFV generally follow the peracute or  
54 acute disease syndromes (Sánchez-Vizcaíno et al., 2019). The first sign of an ASFV outbreak in a pig herd may  
55 be a small number of animals displaying clinical signs of the peracute syndrome, including depression, pyrexia  
56 and cutaneous hyperaemia, followed by death 1–4 days later (Sánchez-Vizcaíno et al., 2019). Signs of the acute  
57 syndrome include pyrexia, cutaneous haemorrhages in the ears and extremities, loss of appetite and  
58 splenomegaly on post-mortem. In the acute syndrome, mortality rates can reach 100% within seven days of  
59 clinical signs' appearance (Sánchez-Vizcaíno et al., 2019).

60

61 Reports suggest that in 2018, contaminated swill feed carried ASFV to a Chinese pig farm, from where it spread  
62 throughout the country (Zhou et al., 2018). The disease affected all production systems, from smallholders to  
63 commercial piggeries (FAO, 2020). The disease then spread through South-East Asia, including Vietnam, in  
64 early 2019 and was first reported in Lao PDR at the start of June 2019 (FAO, 2020). This outbreak occurred in  
65 Toomlan District, Salavane province in southern Lao PDR (FAO, 2020). A month later, in July 2019,  
66 neighbouring villages in Thapangtong district, Savannakhet province (Figure 1) first confirmed cases of ASFV  
67 (FAO, 2020).

68

69 Informal trading, low biosecurity and swill feeding – all common in Lao smallholder pig farming – increase the  
70 risk of ASFV spread (Nantima et al., 2015). Smallholder pig-farming practices in Thapangtong are typical of  
71 lowland Lao PDR. In a previous survey of Savannakhet smallholder pig keeping practices, performed before the  
72 outbreak, median herd size was two pigs per household (Holt et al., 2019). Approximately one-third of pigs  
73 ranged freely, and the rest were penned or tethered. Almost all pigs in the villages were either a local breed or  
74 cross-breed (94.8%) (Holt et al., 2019).

75

76 In the six months from July to December 2019, ASFV spread to 17 provinces of Lao PDR, with new case  
77 numbers dramatically declining by the end of the year as the available naïve population fell (FAO, 2020). The

78 case fatality rate averaged 85%–100%, often with sudden death or elevated mortality as the presenting clinical  
79 sign (FAO, 2020).

80

81 Lao PDR's 2019 ASFV outbreak stretched the investigation capacity of the local veterinary services as they  
82 allocated their limited financial and human resources to national stamping out, movement controls and education  
83 programs. Globally, information on ASFV ecology and epidemiology among smallholders is sparse, particularly  
84 amongst naïve pig populations. The objective of this study was to fill this knowledge gap. As part of our  
85 activities, we allocated additional resources and time to investigate the July 2019 ASFV outbreak in  
86 Thapangtong district. In this paper we describe the ASFV outbreak's occurrence, estimate related household  
87 financial loss and conduct a preliminary descriptive investigation into risk factors associated with ASFV in the  
88 Lao smallholder pig sector using data from Thapangtong district.

89

## 90 **Materials and methods**

### 91 *Investigating the timeline of Lao government response*

92 The timeline of the local government response and the process for reporting (from village to the province level)  
93 was provided by the acting head of the Savannakhet Provincial Agriculture and Forestry Office (PAFO)  
94 Livestock division through semi-structured interviews conducted in English followed by a written survey.

95

96 The Lao government animal disease reporting system begins at the village level: farmers report unusual  
97 outbreaks to their Village Veterinary Worker (VFW), a layperson trained in basic animal health management  
98 who reports to their District Agriculture and Forestry Office (DAFO). The DAFO communicates with their local  
99 PAFO, which then informs the Department of Livestock and Fisheries (DLF) and the National Animal Health  
100 Laboratory (NAHL) in Vientiane. The DLF handled epidemiology and control measures, while the NAHL  
101 performed the laboratory-based diagnosis of ASFV (O. Samathmanivong, pers. comm., 2019). The NAHL used  
102 real-time polymerase chain reaction (rt-PCR). The Taqman real-time quantitative PCR assay was used with  
103 AgPath mastermix (King et al., 2003; Matsumoto et al., 2020).

104

105 Village Chiefs (VC) and Village Veterinary Workers (VW) first reported abnormal pig deaths in Densateung  
106 and Phouphanang-Khampia in late May–early June 2019 to the Thapangtong District Agriculture and Forestry  
107 Office (DAFO). The DAFO then reported these deaths to the Savannakhet Provincial Agriculture and Forestry  
108 Office (PAFO) on 25 June 2019. Together the PAFO and DAFO investigated the cases on 29 June 2019  
109 (O. Samathmanivong, pers. comm., 2019).

110

111 PAFO staff collected whole blood from between 1–4 pigs per village, using jugular venepuncture on live  
112 animals showing clinical signs. In each of the two affected villages, the PAFO team collected all samples from a  
113 single household. The samples were transported by land from the Savannakhet PAFO to the NAHL in Vientiane  
114 (O. Samathmanivong, pers. comm., 2019). Following formal diagnosis from NAHL, PAFO and DAFO staff  
115 began control activities on 3 July. They completed stamping out measures in the two affected villages by 6 July  
116 2019, and movement controls in the 5km surrounding the district continued until early August 2019 (O.  
117 Samathmanivong, pers. comm., 2019).

118

#### 119 *Outbreak investigation study site*

120 This study was conducted in the Thapangtong district of Savannakhet province, which was the second location  
121 in Lao PDR to report an ASFV outbreak and is adjacent to Salavane province, where the first outbreak occurred.  
122 For this study, an 'affected village' was defined as a village with one or more PCR-confirmed ASF cases. An  
123 'affected household' was defined as a household that owned one or more pigs with clinical signs of ASFV in an  
124 'affected village' during the high-risk period. Not all affected households were PCR-confirmed.

125

126 The 'high-risk period' was when the ASFV outbreak might have existed in the affected villages including the  
127 time before the first report from the Thapangtong District Agriculture and Forestry Office (DAFO) to  
128 Savannakhet Province Agriculture and Forestry Office (PAFO). Based on farmer interviews, clinical signs and  
129 laboratory findings as per the approach described in Nurmoja et al., 2018, this period was estimated to be 1 May  
130 to 2 July 2019.

131

132 Of the district's three villages with confirmed cases (as at mid-September 2019), two of similar size, Densateung  
133 and Phouphanang-Khampia, were chosen for this study. The NAHL had confirmed the Densateung and  
134 Phouphanang-Khampia outbreaks on 1 July 2019. Due to the high reported pig mortality rate in the affected  
135 villages, two unaffected villages, Napaxard and Xaysomboun, were selected as controls. The control villages had  
136 healthy pig populations at the time of the survey, were of similar human population size and close to the same  
137 major road as the affected villages. The sample size was calculated using the "Sample Size to Estimate a Simple  
138 Proportion" tool in EpiTools with 95% confidence, 5% margin of error (Sergeant, 2018) and a design prevalence  
139 based on the outbreak data submitted to the OIE (Table 1). We estimated how many households were required  
140 for the survey by dividing the number of pigs by the median herd size of 2 pigs per household taken from a  
141 survey in 2011 (IQR of 1—4 pigs) per household (Holt et al., 2019). The number of surveys per village was set  
142 at 25 for simplicity of study design as a protocol needed to be created for both case and control villages.

143

#### 144 *Household survey*

145 The survey had two phases: a pilot followed by a final questionnaire. Questions found to be poorly understood or  
146 in need of additional information in the pilot were adapted and included in the final questionnaire. An  
147 independent company, experienced in medical and agricultural translations, translated the questionnaire into  
148 Lao, then NAHL staff experienced in animal health extension programs back-translated the questionnaire into  
149 English for confirmation. The questionnaire included 28 questions on how many animals they owned and their  
150 value in Lao Kip (LAK); purchasing/selling behaviour; biosecurity practices; pig management practices and pig  
151 health practices. Where literature existed about possible answers (such as housing methods and feeding), the  
152 question styles were closed. Where no literature existed, a short structured-open question was used, such as  
153 "How do you normally dispose of household food scraps?" Instructions for the interviewers to guide the  
154 questioning style added clarity. The questionnaire covered the recent history of disease outbreaks in the village,  
155 including the number of animals affected and when they were affected.

156

157 The 'household' was the unit of interest for analysis, and subjects were chosen from all the pig-raising  
158 households in the selected villages. In Densateung and Phouphanang-Khampia, almost all households were  
159 ASFV-affected (Table 2), disease-free pig-owning households being extremely rare as reported by the  
160 Savannakhet PAFO. Households in the unaffected villages of Napaxard and Xaysomboun were selected as  
161 controls for comparison with the 'affected households' described in the previous section. Experienced animal  
162 health fieldworkers from the Savannakhet PAFO and the Thapangtong DAFO conducted the survey in late  
163 September 2019. Before the survey, they were trained in disease investigation and biosecurity practices. A few  
164 days before the planned field visit, the DAFO staff contacted the village to create a sampling frame with the  
165 Village Chief (VC) and VVW, allowing villagers time to make themselves available on the day of surveys. The  
166 two unaffected villages were surveyed on the first day, and the two ASFV-affected villages were surveyed on the  
167 second day. In ASFV-affected and control villages, the VC created a sampling frame by naming 50 pig-rearing  
168 households. The investigators randomly chose 25 representatives to interview from this list using a random  
169 number generator in Microsoft Excel (Microsoft, 2002). The VVWs and VCs also provided population-level  
170 outbreak data and generalised spatial data. The local DAFO staff and PAFO staff conducted the surveys in Lao,  
171 with the household pig carer where available and the household head when the pig carer was not available. The  
172 questionnaires were conducted face-to-face in the village hall and meeting areas rather than at each household.  
173 All four villages had members of the Kattan or Bru ethnic group, some of whom did not speak Lao. These  
174 individuals worked with their Village Chief to translate their questionnaire responses back to Lao. Most  
175 interviews took 10-15 minutes to complete, and each survey participant was given an educational t-shirt as  
176 remuneration for their time.

177

### 178 *Participatory mapping*

179 After the individual surveys, villagers worked with the investigators to map their village, marking their  
180 households' locations, significant landmarks and known areas of wild boar activity. This map was hand-drawn  
181 on a large sheet of paper, and each household represented in the survey contributed to the development of the  
182 maps.

183

#### 184 *Data management and analysis*

185 Data were translated into English by University-trained animal health and laboratory staff at NAHL, stored in  
186 Microsoft Excel, collated and cleaned in Microsoft Excel and RStudio (RStudioTeam, 2018). RStudio was also  
187 used to calculate descriptive statistics on the household demography, farm details (before the outbreak), farm  
188 management and biosecurity practices (RStudioTeam, 2018). The data were then analysed for primary  
189 epidemiologic metrics, such as epidemic curves for the survey populations and median epidemic day in RStudio  
190 using EpiR (RStudioTeam, 2018; Stevenson et al., 2019). Logistic regression was performed using the lme4  
191 package in RStudio with the *glmer()* function and the binomial logit method (Bates, Mächler, Bolker, & Walker,  
192 2014).

193

#### 194 *Financial modelling*

195 Household financial losses due to ASFV were estimated by combining the herd structure data with the estimated  
196 value of pigs, as provided by the farmers. The financial Monte Carlo simulation used the farmer-estimated value  
197 of the pigs, multiplied by the farmer-reported number of pigs lost. A gamma distribution (based on the survey  
198 data) was used as a prior in the *gamma.buster()* function from the EpiR package in RStudio (Stevenson et al.,  
199 2019). A Monte Carlo analysis was performed in RStudio (RStudioTeam, 2018) with 10,000 iterations to  
200 estimate the mean lost herd value with a 95% confidence interval.

201

#### 202 *Spatial outbreak modelling*

203 We mapped the outbreak to investigate the spatial component of disease spread in the village. The map data was  
204 analysed with a space-time permutation (STP) scan statistic (SaTScan; Kulldorff, 2010; Kulldorff et al., 2005).  
205 Space-time scan statistics place numerous theoretical circles of different sizes onto a map and calculate the ratio  
206 of how many disease cases are observed versus expected within each circle. The circles also extend upwards as  
207 cylinders to represent different lengths of time. The height and base are permuted across the map in all possible  
208 combinations, and all clusters are recorded (Kulldorff et al., 2005). Unlike many traditional spatial analyses, this  
209 study utilised resources from participatory epidemiology approaches. The spatial cluster analyses therefore used



210 the hand-drawn village maps, and the radii of the clusters used the grid (Cartesian) dimensions of the maps  
211 created.

212 For the SaTScan space-time analysis, the maximum cluster size was set to 50% of the study area. The maximum  
213 period of the scanning window was set to 10 days based on the average latent period reported in the literature  
214 (Guinat et al., 2014). Monte Carlo simulation was used to determine statistical significance by running 999  
215 replications.

216

## 217 **Results**

### 218 *Household survey*

219 In the ASFV-affected villages, households owned on average six piglets and two sows. None of these  
220 households owned a boar. In the control villages, households owned on average two piglets and one sow. Two of  
221 these households owned fattening pigs, and one owned a boar. All pigs in surveyed households were native  
222 breeds (Table 2).

223

224 Sampled farmers listed pig housing methods with a range of biosecurity levels, from all-day free-ranging (n =  
225 38) to full-time enclosures (n = 19), some of the latter being communal rather than private. Of note were the  
226 farmers who kept their pigs in enclosures near their rice paddies (n = 6) some distance from the village, which  
227 removed their pigs from the village ecosystem (Table 3). Reported contacts between pigs within the villages  
228 were numerous (n = 35 villagers confirmed contact), including with neighbours' pigs and wild pigs or wild boar.  
229 In Lao PDR, wild pigs and Eurasian wild boar are called *muu paa* (forest pig), and both closely resemble the  
230 domestic village pigs.

231

232 Only two farmers (n = 2) reported feeding pork or kitchen swill to their pigs. All surveyed farmers reported  
233 feeding a mixture of rice bran and the water used to prepare sticky rice as the pigs' primary diet. Water sources  
234 (other than the rice water) included household water supplies, communal wells and rivers. Of the farmers  
235 surveyed, 79 used a communal water source for their pigs and 17 used private water sources. When asked an

open-ended question about how they disposed of their kitchen rubbish, farmers gave various responses, including burying waste. However, the most common method was to burn kitchen waste. Most surveyed households butchered animals inside the house after slaughter, but 14.9% butchered animals outside the house. Many farmers gave the leftover bones to their dogs (50.7%). Another possible transmission source was using the same syringes and needles to treat multiple sick animals during the outbreak as reported by the VVWs. Several farmers attempted antibiotic therapy, and during a semi-structured interview a VVW explained that they sometimes washed the syringe with soap and water between uses rather than disposing of the syringe.

#### *Outbreak investigation*

We surveyed 49 ASFV-affected households and 50 control households. Of these 99 households, eight households surveyed in the 'affected villages' did not meet the definition of an 'affected household' (outlined in the Methods). Across the ASFV-affected households surveyed ( $n = 41$ ), 330 pigs died with clinical signs of ASFV during the high-risk period. No pigs died in the control villages during the same period.

#### *Outbreak characteristics*

During the household surveys, an obvious route of disease entry did not become apparent. Direct contact through the purchase of an infected pig seems unlikely as none of the affected farmers in this survey purchased new pigs in the high-risk period or the four weeks prior. However, all (both affected and unaffected) reported Vietnamese pork traders during the risk period. Farmers were asked about their initial diagnosis, and 21% identified the cause of the deaths as a seasonal disease. However, many were unsure of the cause of the sudden increase in pig deaths (51%). The VVWs were also uncertain about what disease was causing the outbreak. An average of nine pigs died or were culled in affected households surveyed ( $n = 41$ ).

Of the affected animals ( $n = 330$ ), the most common early clinical signs were depression (21.5%), fever (15%), inappetence (15%) and shivering/trembling (15%). Late disease clinical signs included seizures/convulsions (21.1%), shivering (11.1%) and "looking cold" (6.7%). Many farmers noted death or sudden death (28.9%). The

262 median clinical interval from onset of clinical signs to death was less than one day (IQR = 2 days), meaning that  
263 farmers observed their pigs becoming sick and dying within 24 hours. The mean clinical interval was 4.4 days  
264 (SD  $\pm$  6.1). In Densateung, the median epidemic day was 1 June 2019, with an interquartile range (IQR) of 35  
265 days. In Phouphanang-Khampia, the median epidemic day was 18 June 2019 with an IQR of 5.5 days (Figures 2  
266 and 3). Farmers and VVWs attempted treatments, including antibiotics (penicillin or oxytetracycline) and  
267 vitamin injections. However, farmers' records of dose, medication, frequency, age category and the route of  
268 administration were often incomplete (Supplementary information 1). In the affected villages, almost all pigs  
269 died from disease before the stamping out measures commenced. Pigs that survived ( $n = 6$  households) were  
270 kept in enclosures adjacent to rice paddies and were therefore not included in the stamping-out measures. These  
271 six households were retained for descriptive management statistics but excluded from descriptions of the disease,  
272 including clinical signs and intervals.

273

#### 274 *Risk factor analysis*

275 The quasi-complete separation of ASFV outcome by the villages made the data unsuitable for logistic regression  
276 (Bates et al., 2014). When including 'village' as a random effect in the logistic regression model, no significant  
277 association between the odds of being an ASFV-affected household and housing style, water source, butchering  
278 method or pig contact structure was found. The intraclass correlation attributable to the village effect was  $> 95\%$   
279 for all analyses. Smaller herds of three pigs or less approached statistical significance when taking village into  
280 account ( $p = 0.06$ ). This is likely because smaller herds were significantly associated with the two control  
281 villages, Napaxard ( $p < 0.05$ ) and Xaysomboun ( $p < 0.001$ ), while the two ASFV-affected villages had more  
282 households with large herds.

283

#### 284 *Financial loss modelling*

285 Modelling of the financial impact of ASFV in affected villages is presented in Figure 4 where the purple line  
286 represents the density of households' losses using the field data. The Monte Carlo simulation then drew from a  
287 gamma distribution (shape 1.85 and scale 1013712.97) created using the field data in *gamma.buster* in EpiR.

288 After 10,000 simulations, the mean financial loss estimated in the Monte Carlo analysis was USD215.00, 95%  
289 CI (31.19, 569.30) with SEM +/- USD26.85 (Figure 4).

290

#### 291 *Spatial outbreak modelling*

292 Three significant clusters of more than one household and three clusters of one household ( $p < 0.001$ ) were  
293 detected in Densateung village. The first cluster noted ASFV symptoms in the second week of May 2019 and  
294 was the earliest cluster affected in the Thapangtong region (Figure 5). Households 4, 5 and 22 accounted for 26  
295 of the affected pigs in Densateung. This cluster was at the eastern end of the major road running through the  
296 village, which runs west-east from Thapangtong to the Vietnam border, via Salavane province. The ensuing  
297 clusters of more than one household occurred sequentially north-west from the first reported cluster. The  
298 outbreak in Phouphanang-Khampia began almost a month after the outbreak in Densateung. It included two  
299 significant clusters of more than one household and four clusters of one household ( $p < 0.001$ ) (Figure 6). The  
300 first spatial cluster involved households 13, 5, 10, 24, 3, 6 and 18 over 15–18 June, which was after the first  
301 reported household in the village.

302

#### 303 **Discussion**

304 This study describes the epidemiologic characteristics, including financial losses, associated with ASFV  
305 outbreaks in selected villages in Lao PDR. The study highlights knowledge that could be implemented to reduce  
306 the impact of ASFV and similar transboundary animal diseases on smallholders in similar resource limiting  
307 contexts. By performing this study, we also explored extant challenges and preliminary strategies to reduce the  
308 opportunity for inter- and intra-village spread of ASF. These strategies will benefit policymakers and researchers  
309 beyond ASFV in the control of other high-impact and zoonotic diseases.

310

311 The major potential pathways for introducing ASFV discussed here include traders of live pigs/pig products,  
312 iatrogenic spread and wild boar. This study did not identify any single, obvious route of ASFV entry into the  
313 villages. However, many plausible hypotheses present themselves, and all should be addressed in future disease

314 prevention activities. The study made obvious that conditions within the villages were ideal for the spread of  
315 ASFV. A combination of inter- and intra-village control measures will be required in future to prevent the spread  
316 and establishment of ASFV in smallholder communities.

317

318 A putative source for the ASFV outbreak in south Lao PDR is the ASFV outbreak in Vietnam that began in early  
319 2019. Both Thapangtong district and the first-affected Toomlan district are on the same major road to Vietnam.  
320 Despite a lack of evidence that any ASFV infected live pigs were purchased from traders in the high-risk period  
321 or the month prior, the reports of Vietnamese traders suggest increased activity from a region known to have had  
322 ASFV in that same period. Whilst the traders did not sell the villagers any pigs, the traders would have been able  
323 to contaminate the villages with ASFV contaminated pork meat products, pig wastes from trucks or even by  
324 dropping off dead carcasses. Previous social network analyses in the Northern Province of Xayabouri suggest  
325 that semi-commercial piggeries interact almost exclusively with 1–2 traders (Poolkhet et al., 2019). The lack of  
326 information on trader behaviours that might cause ASFV warrants future investigation. In future studies, the  
327 social network of interactions between traders and villagers in the Southern region should be investigated to  
328 understand national and transboundary ASFV epidemiology better.

329

330 ASFV can be found in the meat, blood, urine and faeces of infected pigs and provides numerous opportunities  
331 for indirect spread (Sánchez–Vizcaíno et al., 2019). VVWs mentioned that they had tried treating many of the  
332 symptomatic pigs, which may have led to iatrogenic spread through shared needles or insufficient disinfection  
333 between uses. Further investigation into farmer and VVW medication practices is warranted. Butchering outside  
334 after slaughter can cause significant environmental contamination during an ASFV outbreak, and several farmers  
335 in this survey participated in this practice. The movement of wild boar bones by scavenging animals has been  
336 implicated in European ASFV outbreaks. In Lao PDR, roaming dogs could be a similar indirect transmission  
337 pathway. Many farmers reported feeding leftover bones to their dogs. Despite only two farmers reporting that  
338 they fed pork waste to their pigs, opportunities for pigs to access and cannibalise ASFV-contaminated remains  
339 resulted from household choices to bury rubbish, butcher pigs outside and spread kitchen wastes on gardens for

340 compost. Future village education should discourage unsafe swill-feeding practices and include safe methods of  
341 potentially infectious waste disposal and butchering.

342

343 Wild boar and wild pigs are a possible source of the ASFV outbreak described, as in European outbreaks,  
344 however current literature suggests this to be unlikely in Lao PDR (Boklund et al., 2020). In this study, two  
345 farmers noted that village pigs had contact with wild "forest pigs" and that the studied villages (ASFV-affected  
346 and control) were near forests with forest pig populations. For the disease to spread from Vietnam to  
347 Thapangtong, the disease would have to have circulated in wild boar populations over a distance of  
348 approximately 168 km without affecting any other villages before Salavane and Thapangtong District. In late  
349 2019, wild boar ASFV outbreaks were noted in the far northern province of Houaphan, meaning wild boar  
350 remain a potential future outbreak source in the wild boar-environmental contamination pathway (Denstedt et  
351 al., 2020). The authors of this investigation posited that the wild boar outbreak was due to a spillover from the  
352 domestic population, and not the other way around (Denstedt et al., 2020). A wild boar reservoir may remain an  
353 ongoing issue; however, a recent scoping review of ASFV transmission suggests that the wild boar to domestic  
354 pigs is generally unlikely. The speed of disease spread in 2019 is more suggestive of human involvement in the  
355 spread of ASFV (Barrett et al., 2020).

356

357 The whole-village affected nature of the outbreak made the data unsuitable for risk factor analysis at the  
358 household level as initially planned. Univariate logistic regression analysis showed that the strongest effect on  
359 the outcome the ASFV outbreaks in this study was the village because of the quasi-complete separation of the  
360 disease outcome by village and that the data was inappropriately structured for logistic regression analysis. Risk  
361 factors for ASFV transmission include free-ranging, swill feeding and poor farm-level biosecurity, many of  
362 which were present in both the case and the control villages. While these factors probably impact on ASFV  
363 outbreaks in Lao smallholders, it is likely that a whole village risk factor also exists. To estimate risk factors, we  
364 believe a village-level analysis must be performed, although we recognise the difficulty of finding enough  
365 affected and unaffected villages to perform such a study. In future, a spatial mapping approach using PCR  
366 confirmed villages may provide opportunities to perform such an analysis in the absence of survey data.

367

368 Once ASFV entered a village, factors such as wide-spread use of free-ranging and generally higher pig  
369 populations allowed for the spread of the virus. Within the affected villages, a combination of direct and indirect  
370 transmission pathways facilitated the spread of disease. Sick animals could make contact both within and  
371 between herds because two-thirds of pigs were either fully or partially free-range. Sick pigs can spread ASFV  
372 via direct contacts, such as a sow to her piglets. Other pigs may cannibalise a sick or dead pig, and healthy pigs  
373 can eat kitchen wastes containing contaminated pork scraps. Their rooting and investigating instincts can lead  
374 pigs to uncover shallow-buried contaminated waste or carcasses. As demonstrated in the epidemic curves  
375 (Figure 2 and Figure 3), the disease propagated through the free-ranging and non-free ranging pig populations  
376 once established. Of interest is the considerable difference in IQR for the epidemic days for Densateung (35  
377 days) and Phouphanang-Khampia (5.5 days). It appears that the smallholder village pigs in Densateung operate  
378 under a contact structure similar to those in a commercial style farm where the disease spreads slowly before  
379 causing serious fatalities. The spread of the disease amongst the pigs of Phouphanang-Khampia more closely  
380 resembles that of a single pen of affected animals (Guinat et al., 2014). Animals with ASFV become infectious  
381 when clinical signs develop. The modal period, from clinical signs to death, in this study was one day or less.  
382 This short symptomatic period is consistent with reports of ASFV in other Asian and European outbreaks  
383 (Guinat et al., 2014; Olesen et al., 2017; Sánchez-Vizcaíno et al., 2019; Tran et al., 2020). Future studies should  
384 estimate the  $R_0$  of ASFV transmission at the pig and household level in these villages and compare these  
385 estimates with those of commercial piggeries. The results suggest that preventing ASFV entry at the village level  
386 is likely the best strategy for protecting whole communities.

387

388 The aim of assessing the participatory data for a spatio-temporal relationship between outbreak locations was to  
389 quantify how the disease spread through the villages beyond the calculation of an epidemic curve. The  
390 statistically significant clustering of disease outbreaks implies that the outbreak sources were not randomly  
391 distributed or a universal exposure. In particular, the sequence of localised clusters in Densateung followed a  
392 pattern moving across the village in sequentially bigger groups, reflecting the epidemic curve's propagative  
393 nature. The STP approach employed in this study, requires only case data, whereas Poisson and Bernoulli spatio-

temporal analyses require both case and population at-risk or control data (Gatrell & Durr, 2004). For an outbreak of ASFV, the STP approach is appropriate because all pigs are affected in a village during a short period, and the population can be considered a closed cohort (Ward & Carpenter, 2000). In these low-biosecurity, free-ranging contexts, there are often no control households or animals. In future studies, these outputs could be adjusted by using disease parameters unique to this outbreak, estimated using Approximate Bayesian computation with sequential Monte Carlo technique. Based on the strong village effect detected in the logistic regression analysis, future spatial analyses could use villages as the analytical unit to further investigate the spread of ASFV through Lao PDR.

ASFV outbreaks require prompt and thorough investigation. The epidemiologic findings suggest that ASFV was well established in the two villages before local authorities were able to act. The disease notification system used by the DLF (outlined in the Timeline section of the methods) relies on VVWs to identify and report cases to the DAFO, reporting to the PAFO for investigation. There are no standardised processes across the provinces, and funding for disease outbreak investigation is limited to the private veterinary incomes of the PAFO and DAFO staff. Weaknesses in this "ground-up" reporting approach emerged in the 2015 Vientiane FMD outbreak where numerous FMD-affected villages that were presumed to be "FMD free" by DAFO due to no reports from VVWs, yet retrospective serology determine otherwise (Miller et al., 2018). Here we note the discrepancy in the number of ASFV cases reported by the PAFO to the OIE ( $n = 80$ ) and the number of animals with ASFV-like clinical signs in the 'high risk period' ( $n = 330$ ). The reported clinical signs, whilst typical of acute and peracute ASFV are also typical of Classical Swine Fever, Erysipelas, Salmonellosis, and highly pathogenic Porcine Respiratory and Reproductive Syndrome, all of which are endemic to Lao PDR. Only five animals were sampled and definitively diagnosed as ASFV cases using PCR, highlighting difficulties in a centralised testing system. In the pilot survey, several farmers reported that pig disease was common during June and July (Lao PDR' wet season). They initially thought the deaths were due to this endemic disease syndrome. The familiar clinical signs may have also delayed reporting and action. None of the villagers included ASFV in their initial diagnoses for the pig deaths despite information materials being made available to local authorities by the OIE in early 2019.



421 The sampling strategy described here allowed us to speak with many household representatives in each village;  
422 however, it has its limitations. Households were selected from a list provided by the village chief, who may  
423 have favoured owners that were more educated, more receptive to government communications, owned more  
424 pigs or had positive management traits, leading to selection bias. Herd size skewed right across all villages, with  
425 most households owning small herds of three to four pigs and a few exceptional individuals owning larger herds.  
426 The ASFV-affected villages had more households with large herds than the control villages (Table 2). The  
427 relationship between village and herd size suggests that disease entry into the village could be related to  
428 increased economic activity. This contradicts evidence from Uganda, where ASFV is endemic and  
429 socioeconomic impact surveys in 2014–2015 found that smallholder households with larger herds were  
430 significantly associated with larger economic outputs and lower incidences of ASFV (Chenais et al., 2017).

431

432 Discussions on the impact of the 2019 ASFV outbreak on global markets have focused on pork prices, demand  
433 for alternative sources of protein and demand for intensive livestock feed products such as soya beans (Mason-  
434 D'Croz et al., 2020). Here we have estimated the cost to the smallholders and their local communities, a  
435 neglected aspect of the epidemic. Of Laotians affected by poverty in 2018–2019, 20%–30% live in the  
436 neighbouring provinces of Savannakhet and Salavane (World Bank, 2020). Thapangtong district is located at the  
437 border between the two provinces, and 40.6% of its population were living in poverty in 2015 (Coulombe,  
438 Epprecht, Pimhidzai, & Sisoulath, 2016). In neighbouring Toomlan district, where the outbreak began, 73.1%  
439 are affected by poverty (Coulombe et al., 2016). The modelled losses from ASFV of USD 215 per household are  
440 a substantial portion of annual income for smallholders in the region, who are already at risk of food insecurity  
441 due to economic shocks and environmental disasters. The wide confidence intervals (USD 31.19, 569.30) with  
442 smaller SEM (+/- USD 26.85) suggest that the data set was not limited by size, but rather that there is substantial  
443 heterogeneity in the economic impacts and herd structures of smallholder farmers. However, this estimate is  
444 based solely on the sale value of pigs that died. The method calculated the minimum possible loss as it was  
445 restricted to the value of the pigs alone. It did not consider lost treatment costs, time, future value or social costs.  
446 A more extensive study could estimate gross margins by calculating the production costs, based on more  
447 extensive interviews with farmers and collecting data on inputs, outputs and uses for dead pigs. Other studies

448 have reported that dead or diseased pigs may not have immediately lost their monetary value – as farmers may  
449 have sold the meat or kept it for household consumption – but this behaviour was not reported in our survey  
450 (Chenais et al., 2017). During the survey, questions about medication costs, vaccination and feeding received  
451 few responses, suggesting a very low-output/low-input system. This might explain why farmers continue to  
452 purchase and raise smallholder pigs despite risks of transboundary emergency animal diseases.

453

454 The findings of this study should be utilised in future decisions about the management of ASFV in the region.  
455 Trader, VVW and villager behaviour must be managed and control measures put into place for contact between  
456 village pigs and wild boar. The resources available to local government authorities must be assessed for them to  
457 act promptly in cases of emergency disease outbreaks. When designing control and education strategies, local  
458 farming practices, as well as the disease ecology must be considered together in order to develop effective  
459 materials.

460

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470

#### 471 **Conflict of interest statement**

472 The authors declare that there were no conflicts of interest in the development of this work.

473

474 **Data availability statement**

475 The data that support the findings of this study are available on request from the corresponding author. The data  
476 are not publicly available due to privacy or ethical restrictions.

477

478 **Ethics statement**

479 The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have  
480 been adhered to. Ethics approval for the surveys was obtained from the University of Sydney Human Ethics  
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482

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