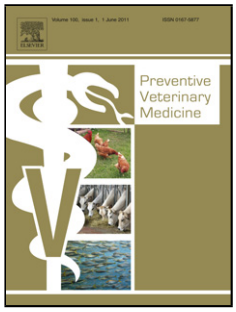

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Highlights

- Seroprevalence varied widely at various levels of spatial aggregation
- Pigs positive for CSFV antibody in areas with no vaccination or reported cases
- Levels of herd immunity inadequate for disease control

Title

An investigation of classical swine fever virus seroprevalence and risk factors in pigs in East Nusa Tenggara, eastern Indonesia

Authors

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Abstract

Classical swine fever virus (CSFV) is a highly infectious disease of pigs. It has had significant impacts on East Nusa Tenggara, eastern Indonesia since its introduction in 1997. In spite of its importance to this region, little is known about its seroprevalence and distribution, and pig-level and farmer-level factors that may have an impact on the serological status of an individual pig. To address this knowledge deficit, a cross-sectional seroprevalence survey was conducted in 2010 involving 2160 pigs and 805 farmers from four islands in the region. Farmer questionnaires and pig record forms were used to collect data about the farmers and pigs surveyed. Blood was collected from each pig to determine its CSFV serological status. Apparent and true prevalence were calculated for each island, district, subdistrict, and village surveyed. CSFV serological status was used as an outcome variable in mixed effects logistic regression analyses.

Overall true CSFV seroprevalence was estimated at 17.5% (lower CI 16.0%; upper CI 19.5%). Seroprevalence estimates varied widely across the islands, districts, subdistricts, and villages. Manggarai Barat, a district on the western end of Flores Island, contained pigs that were positive for antibody to CSFV. This result was unexpected, as no clinical cases had been reported in this area. Older pigs and pigs that had been vaccinated for CSFV were more likely to test positive for antibody to CSFV. The final multivariable model accounted for a large amount of variation in the data, however much of this variation was explained by the random effects with less than two percent of the variation explained by pig age and pig CSFV vaccination status.

In this study we documented the seroprevalence of CSFV across four islands in East Nusa Tenggara, eastern Indonesia. We also identified risk factors for the presence of antibody to CSFV. Further investigation is needed to understand why clinical CSFV has not been

69 reported on the western end of Flores Island, and to identify additional risk factors that
70 explain CSFV serological status to inform disease control strategies.
71
72

73 **Keywords**

74 Classical swine fever virus; East Nusa Tenggara; Indonesia; Epidemiology; Risk factors;
75 Seroprevalence

76

Introduction

Classical swine fever virus (CSFV), or hog cholera, is a *pestivirus* associated with high morbidity and mortality rates in pigs. CSFV has been eradicated from several countries including the United States, Australia, and New Zealand, and a number of countries in Central and Western Europe (Artois et al., 2002; Edwards et al., 2000; World Organisation for Animal Health, 2014). However, recent outbreaks in countries previously free of CSFV in domestic pigs have had significant economic and animal health consequences (Elbers et al., 1999; Moennig et al., 2003; World Organisation for Animal Health, 2014), and CSFV remains endemic in parts of Asia, Central America and South America.

East Nusa Tenggara (NTT) is the province with the highest level of pork consumption in Indonesia, and the largest pig population with an estimated size of 1.8 million animals (BPS Statistics, 2013). Smallholder pig farmers (total herd sizes of ≤20 pigs) are the predominant producers in this region, with 85% of households raising pigs (Johns et al., 2009; Santhia et al., 2006) and agriculture is the primary income source for the majority of households (Wang, 2007). In NTT, pigs provide a food source and financial security, and are highly valued socially and culturally (Santhia et al., 2006; Leslie et al., 2014). Therefore, morbidity and mortality events in the pig population impact a large proportion of the human population.

CSFV is a highly contagious transboundary disease. Pigs are generally infected oronasally, and spread is both direct via horizontal and vertical transmission, and indirect via contaminated fomites and pork products. Clinical disease caused by CSFV is classified as acute, subacute, or chronic, and is determined by CSFV strain, as well as host factors, including pig age, breed, stage of pregnancy, previous CSFV exposure status, and CSFV vaccination status. There are no pathognomonic signs for CSFV, and therefore laboratory diagnostics are required to make a diagnosis (Moennig et al., 2003).

CSFV was confirmed in NTT in 1998. It subsequently spread across the province largely through uncontrolled live pig movement, causing substantial losses. It continues to limit pig production in the region (Tri Satya et al., 1999; Christie, 2007). In NTT, districts are classified by CSFV infection status, which is based on clinical case reports to the NTT Livestock Office and limited government-led serological surveys. In 2010, all districts on West Timor and Sumba Island and one district on the eastern end of Flores Island were classified as infected; one district in east Flores Island was classified as suspect; and the rest of Flores Island plus Lembata Island classified as not infected (Figure 1). Vaccination campaigns are conducted in districts with the highest pig densities and annual reports of cases in an attempt to control disease. However, fluctuations in the size of the pig population have continued, and the NTT Livestock Office has documented an increase in the number of annual reported cases (Dinas Peternakan Propinsi, 2011).

Live attenuated 'Chinese' strain (C-strain) vaccine CSFV vaccine is used in NTT to control disease. Its effectiveness has been demonstrated by a number of studies, and protection lasts at least 6-18 months and may be life long (van Oirschot, 2003). Neutralizing antibody usually appears within two weeks and increases until at least 4-12 weeks post vaccination (van Oirschot, 2003). Antibody can persist many years after inoculation with a single dose, but also disappears in some individuals and may disappear at a higher rate under 'real' field conditions compared to field trial conditions (van Oirschot, 2003). It is generally accepted that the presence of neutralizing antibody confers CSFV protection (Suradhat et al., 2007). Similarly, pigs that recover from acute CSFV infection develop neutralizing antibody as early as two weeks post infection (Moennig, 2000). These animals are protected against future infection for several years and immunity may be life long (Moennig, 2000).

In spite of the importance of CSFV to NTT, little is known about the seroprevalence and distribution. No serological surveys have been conducted in many parts of NTT,

127 including the western half of Flores Island. Inconsistencies have been noted between the
128 number of CSFV cases reported by the NTT Livestock Office and the few published studies
129 (Santhia et al., 2003; Dinas Peternakan Propinsi, 2011). It has been recognised that as a result
130 of government decentralisation, communication between and within different government
131 sectors is lacking, which may be the cause of data inconsistencies (Brandenburg et al., 2002).
132 However, previous studies have also noted that farmers across NTT are reluctant to report
133 CSFV cases (Robertson et al., 2010; Deveridge, 2008). Moreover, Santhia et al. (2003) stated
134 that farmers and animal health workers on Alor Island in NTT were not reporting all CSFV
135 cases.

136 The overarching aim of the presented study was to better understand CSFV
137 seroprevalence and distribution in NTT to provide information to support decisions on CSFV
138 control. The objectives of this study were: 1) to determine CSFV seroprevalence in West
139 Timor and Sumba islands, both classified as CSFV infected; 2) to detect the presence of
140 CSFV antibody in CSFV suspect and not infected districts on Flores island, and in Lembata
141 island, which was classified as not infected in 2010, and; 3) to investigate pig-level and
142 farmer-level factors to determine their impact on pig CSFV serological status in the islands
143 surveyed.

144

145 ## Material and methods

146 The survey was conducted as described from April to September 2010 following
147 approval the University of Sydney's Human Research Ethics Committee (08-2009/11866).

148

149 **Questionnaire design**

150 A questionnaire was developed to record information on farmer demographics, farm
151 structure and performance, pig husbandry, reproductive management, pig movements, pig

health history and response, and farmer CSFV knowledge and awareness, and vaccination practices. A pig record sheet was developed to record information regarding the sex, age, health in the last three months, source, body condition score (BCS), and CSFV vaccination status of the pigs from which a blood sample was collected. Both consisted of open and closed questions. Closed questions included multiple choice, checklist, or short answer type questions. Throughout the farmer questionnaire and pig record forms, CSFV was referred to as hog cholera as this term is used commonly in Indonesia. It took approximately 30 minutes to complete the farmer questionnaire with each participant. The farmer questionnaire and pig record form are provided as online supplements (S1-2).

The documents were developed initially in English and then translated into Bahasa Indonesia by Dr. Maria Geong, Director of Livestock Services NTT and a native speaker of local origin. Veterinarians from each island attended a joint training event during which the farmer questionnaire and pig record form were pilot tested with 12 pig owners in Kupang, which allowed question refinement.

Sampling strategy

A multi-stage approach to sampling was used to select the districts, subdistricts, villages and farmers.

Selection of districts

Purposive sampling was used to select districts within each island. District inclusion was based on reported clinical cases of CSFV (Dinas Peternakan Propinsi, 2011), geographical diversity, and perceived high importance of pig production and trade within the district according to Livestock Services NTT veterinarians.

On West Timor Island, the districts Belu and Kota Kupang were included. Both have a history of reported CSFV and vaccination campaigns for CSFV. Belu borders Timor Leste and pig trading across the border is known to occur. Kota Kupang is the main pig-producing district on West Timor Island and includes the provincial capital Kupang.

On Sumba Island, the districts Sumba Barat Daya and Sumba Timur were included. Both have a history of clinical cases of CSFV and CSFV vaccination campaigns.

On Flores Island, the districts Manggarai Barat in west Flores and Sikka in central Flores were included. Manggarai Barat is the most western district on Flores Island. It is considered not infected with CSFV based on no CSFV clinical case reports. There has been no CSFV vaccination in this district, and therefore CSFV seroprevalence was expected to be very low, or zero. Sikka is a CSFV suspect district as there have been very few reported cases with only one case reported from 2002 to 2009, and therefore CSFV seroprevalence was also expected to be low.

For Lembata Island, a district in itself, clinical CSFV had not been reported prior to the study and there had been no CSFV vaccination campaigns.

Selection of subdistricts and villages

Simple random sampling was used to select two subdistricts per district in Flores, West Timor, and Lembata, and one subdistrict per district in Sumba. Subdistricts considered remote, unsafe, or unlikely to co-operate were excluded from the sampling frame. The number of subdistricts sampled was based on logistical issues relating to time and funds available. For each selected subdistrict, simple random sampling was used to select three villages per subdistrict in Flores and Lembata and two villages per subdistrict in Sumba and West Timor.

Selection of farmers

In West Timor and Sumba, 30 farmers from each village completed the farmer questionnaire, while in Flores and Lembata 20 farmers from each village completed the farmer questionnaire. For each selected village, a sampling frame was constructed by obtaining a list of pig farmers from the Village Head. Livestock Services veterinarians requested this information during a preliminary visit to each selected village. At the same time permission to conduct the survey in the village was obtained.

Simple random sampling was used to select twenty to 50 percent more farmers than required from each village. Extra farmers were selected to ensure a sufficient number of farmers were surveyed. Farmers had to be present in the village on the day of the interview team visit to participate. In Flores and Lembata, farmers also had to own at least three pigs over the age of three months. In Sumba, pigs owned by multiple individuals were often grouped in pens and under the care of a single farmer. Therefore in Sumba, farmers had to have at least three pigs over the age of three months under their care to participate. In West Timor, farmers had to own a minimum of one pig over three months of age to participate. If a selected farmer did not meet the selection criteria or was unwilling to participate the next farmer selected during the random sampling process who met the criteria replaced them. Farmers were informed of their selection on the day prior to the village visit, and therefore a high rate of farmer attendance in the village was expected. Farmers were provided with a free health check of their pigs and administration of medications as required as an incentive.

Selection of pigs

In Sumba, three pigs three months of age or older were selected for blood sample collection from each interviewed farmer using convenience sampling for a total of 90 pigs sampled per village. In West Timor, 1-4 pigs greater than three months of age were selected

from each interviewed farmer using convenience sampling. In each village in West Timor, at the end of the interview process there were fewer than 90 pigs sampled and therefore the decision was made to sample pigs greater than three months of age from farmers who did not take part in the farmer interview. In Flores and Lembata, three pigs greater than three months of age were selected from each interviewed farmer using convenience sampling for a total of 60 pigs sampled per village. Previous studies have shown that maternally derived antibody levels reach a minimum level by about 10 weeks of age, and therefore it was assumed that maternal derived antibody would not be present in pigs greater than three months of age (Klinkenberg et al., 2002a).

Table 1 lists by island the names of the study districts, subdistricts, and villages, and the number of farmers and pigs sampled.

Estimation of required sample size

In Sumba and West Timor, the number of pigs required to estimate CSFV antibody prevalence was calculated with Epitools (Sergeant, 2010) using: 1) an expected seroprevalence of 20-30% based on the expert opinion of Dr. Maria Geong; 2) a village pig population of 2000 pigs based on the expert opinion of Dr. Maria Geong; 3) a level of precision of 10%; 4) a level of confidence of 95%; and 5) an imperfect test with 95% sensitivity and 95% specificity. The sample size required ranged from 81 to 99 pigs depending on the expected prevalence, and therefore the midrange value was chosen.

In Flores and Lembata, the number of pigs required to detect CSFV antibody was calculated with Epitools (Sergeant, 2010) using: 1) a minimum expected prevalence in the selected villages of 5%; 2) a village pig population ranging from 500 to 5000 pigs; 3) a level of confidence of 95%; and 4) an imperfect test with 95% sensitivity and 100% specificity.

These inputs resulted in a sample size ranging from 59 to 62 pigs depending on the village pig population size, and therefore the midrange value was chosen.

Data and sample collection

The farmer questionnaire was completed during each participant interview. This process was conducted prior to blood collection from the sampled pigs. The pig record form was completed after sample collection. Each farmer and pig was assigned a unique identification code.

Pigs were manually restrained with a nose snare and 3 ml of blood was collected from the jugular vein using a serum vacutainer and 20-gauge needle. When blood could not be collected from the jugular vein, a 23-gauge needle and 3 ml syringe were used to collect blood from the lateral ear vein. This sample was then immediately transferred to a serum vacutainer.

Vacutainers were labeled with the corresponding pig identification code from the pig questionnaire and stored on ice during the sample collection period and transportation to the Livestock Services Department Laboratory. Serum separation was performed within 12 hours of sample collection – samples were centrifuged for 5 minutes at 80 000 rpm and the serum transferred to a serum vacutainer and stored at -5°C. Samples were later transported on ice to the Animal Biomedical and Molecular Biology Laboratory, University of Udayana, Denpasar, and stored at -20°C until serological analysis.

Serological analysis

Serum samples were analysed using a commercial CSFV enzyme-linked immunosorbent assay (ELISA) kit (PrioCHECK® CSFV Ab, Lleydstat, Netherlands). ELISAs were performed according to the manufacturer's instructions. ELISA plates were

read using a 450 nm filter on an ELISA reader to determine optical density and these values were used to calculate percent inhibition (PI) (Colijn et al., 1997). A sample was considered positive for CSFV antibody when PI was ≥50%, inconclusive when PI was 31-50% and negative when PI was <30% (Colijn et al., 1997). Samples that had haemolysed or appeared contaminated were included in the analysis.

Serum samples that were classified as inconclusive or positive by the PrioCHECK CSFV Ab, or for which the result was missing, were reanalyzed using the PrioCHECK® CSFV Ab 2.0. This second generation ELISA is more specific for CSFV antibody compared to other pestivirus antibody, and therefore was used to reduce the likelihood that positive results were due to cross reaction with antibody to another pestivirus. A sample was considered positive for CSFV antibody when PI ≥40%, negative when PI was <40% according to the manufacturer's instructions.

A sample that was positive for CSFV antibody on the PrioCHECK CSFV Ab but for which there was insufficient serum to conduct the PrioCHECK CSFV Ab 2.0 was considered positive for CSFV antibody. Samples that had no result available for the PrioCHECK CSFV Ab but tested positive with PrioCHECK CSFV Ab 2.0 were deemed positive, while samples that had no result available for the PrioCHECK CSFV Ab but tested negative on the PrioCHECK CSFV Ab 2.0 were deemed negative. Samples that had an inconclusive test result with PrioCHECK CSFV Ab but had no result on the PrioCHECK CSFV Ab 2.0 were deemed to have no result.

Data management

Data were entered into two databases created in Epi Info™ Software (version 3.5.1, CDC, www.cdc.gov/epiinfo, Atlanta, GA, USA), one for the farmer questionnaire and one for the pig record form. These databases were exported to Microsoft Excel, and merged by

matching on farmer identification code. The data were cleaned in Microsoft Excel and exported for analysis in R (version 3.0.2. © 2013, The R Foundation for Statistical Computing).

Calculation of apparent and true seroprevalence

Apparent seroprevalence and confidence intervals, using the normal approximation interval, were calculated for each island, district, subdistrict, and village in the R statistical package (prevalence, v 0.2.0). Estimated true seroprevalence and confidence intervals were calculated in the R statistical package (prevalence, v.0.2.0) (Rogan and Gladen, 1978). Test sensitivity was set at 89% and specificity at 100%. These test performance parameters were calculated in Epitools (Sergeant, 2014) for use in series of the PrioCHECK CSFV Ab (sensitivity 98% and specificity 99% determined by Colijn et al., (1997) and Moser et al., (1996)) and the PrioCHECK CSFV Ab 2.0 (sensitivity 91% and specificity 100% determined by Schroeder et al., (2012)).

Risk factor analysis

Outcome and explanatory variables

The unit of interest was the individual pig. The outcome variable was CSFV serological status. Pigs were classified as either CSFV antibody positive or CSFV antibody negative as previously described.

Twenty-six explanatory variables were derived from the questionnaires: 20 farmer-level variables and six pig-level variables. Number of pigs on the farm was the only continuous variable. The remaining explanatory variables were categorical, 20 of which were binary variables. All explanatory variables are presented in Table 2.

Descriptive analyses

Contingency tables were created to explore the relationship between each of the categorical explanatory variables and CSFV serological status. In addition, summary statistics were calculated for the number of pigs on farm (the only continuous explanatory variable), both alone and according to CSFV serological status.

Univariable analysis

The association of each explanatory variable with the binary outcome variable was assessed using univariable mixed effects logistic regression analyses in the R statistical package (lme4, v.1.0-5). To control for the effect of clustering, farmer, village, subdistrict, district, and island were fitted separately as random effects. Based on the association between each explanatory variable and the outcome variable, all explanatory variables with a *p*-value of ≥0.20 were excluded from the multivariable analyses. In addition, variables with more than 10% of missing values were excluded from multivariable analyses (Dohoo et al., 2009, pp. 369).

According to expert opinion from Livestock Services NTT, farmers with herd sizes of 1-3 pigs generally keep pigs for home consumption or use in traditional ceremonies, while farmers with larger herds were responsible for the majority of pig movements into and out of a village. Therefore, the decision was made to exclude the data from West Timor from the univariable and multivariable analysis. The data from Lembata were also excluded as all samples tested negative for CSFV antibody.

Multivariable analyses

A multivariable mixed effects logistic regression model was constructed using the R statistical package (lme4, v.1.0-5) with a manual backward stepwise approach to evaluate the

association of explanatory variables with the outcome variable after adjusting for each other. Variables that were statistically significant (p-value <0.05) were retained in the final model. The correlation between covariates was evaluated using a chi-square test and deemed significant at a p-value of less than 0.05. A 2-way interaction between age and vaccinated for CSFV was tested within the multivariable model.

To control for the effect of clustering, farmer, village, subdistrict, district, and island were fitted separately as random effects. Goodness-of-fit of the final logistic regression model was assessed by calculating conditional R^2 for the final model ($R^2_{GLMM(c)}$). The amount of variation in the data explained by the fixed effects was assessed by calculating marginal R^2 for the fixed effects ($R^2_{GLMM(m)}$) (Nakagawa et al., 2013).

Intra-class correlation coefficient

Intra-class correlation (ICC) was calculated for each random effect using the latent variable approach to quantify the amount of clustering between units at each of the different levels of clustering (Browne et al., 2005). Clustering was deemed high for random effects that had an ICC greater than 0.3 (Dohoo et al., 2009, pp. 537, 583).

Results

Seven hundred and twenty farmers and 2160 pigs from 805 farmers were surveyed across the four islands. Herd size ranged from 1 to 48 pigs, with an average of 4.6 pigs. One thousand four hundred fifty-two (67.2%) of the 2160 pigs included in the survey were born in the farmer's herd. Approximately 42% (898/2160) of the pigs surveyed were 3-5 months of age, 30% (652/2160) were 6-11 months of age, and the remaining 28% (610/2160) were equal to or greater than 12 months of age. Five percent (113/2160) of the pigs had been sick in the three months prior to the time of the survey.

375

376 **Seroprevalence**

377 Three hundred and twenty two samples tested positive for CSFV antibody with
378 PrioCHECK CSFV Ab, 1761 samples tested negative, and 46 samples had an inconclusive
379 result. For 31 samples there was no result with PrioCHECK CSFV Ab. Of the 46 samples
380 that had an inconclusive test result with PrioCHECK CSFV Ab, 19 tested positive and 25
381 tested negative on the PrioCHECK CSFV Ab 2.0. There was no result for two samples that
382 had an inconclusive result on the PrioCHECK CSFV Ab. Of the 322 samples that tested
383 positive on the PrioCHECK CSFV Ab, 315 tested positive and 7 tested negative with
384 PrioCHECK CSFV Ab 2.0. Of the 31 samples for which there was no result on the
385 PrioCHECK CSFV Ab, two tested negative and two tested positive on the PrioCHECK
386 CSFV Ab 2.0, while 27 had no result available. Therefore serological findings were available
387 for 2131 of the 2160 sample collected. Overall apparent CSFV seroprevalence across the four
388 islands was 15.8% (95%CI 14.3, 17.4), while overall true CSFV seroprevalence was
389 estimated at 17.5% (95%CI 16.0, 19.5). Apparent prevalence and true prevalence estimates
390 across the islands, districts, subdistricts, and villages are presented in Table 3.

391

392 **Univariable mixed effects logistic regression analyses**

393 Eleven variables were associated with CSFV serological status at the univariable cut-
394 off *p*-value of <0.20, six variables at the pig level and five variables at the farmer level
395 (Tables 4-7). The variables 'Use own boar for breeding' and 'Body condition score' were
396 excluded due to too many missing responses. A total of eleven variables were considered in
397 multivariable analyses.

398

399 **Multivariable mixed effects logistic regression analyses**

The final model for CSFV serological status is presented in Table 8. The two variables in the final model were both pig-level characteristics. Pigs with a previous history of vaccination for CSFV were 3 times more likely to test positive for antibody to CSFV. Pigs equal to or greater than 12 months of age were 2.5 times as likely to test positive for antibody to CSFV compared to pigs 3-5 months of age, while pigs 6-11 months of age were equally as likely to test positive for antibody to CSFV compared to pigs 3-5 months of age (Table 8).

The variables 'Age' and 'Vaccinated for CSFV' were significantly correlated. However, pigs 3-5 months of age were more likely to be vaccinated for CSFV compared to the other two age categories, and the proportion of pigs 6-11 months of age vaccinated for CSFV was similar to that of pigs ≥12 months of age. Therefore both variables were left in the multivariable model. The interaction term for age and vaccinated for CSFV was not significant. None of the other variables in the final model were significantly correlated.

The conditional R^2 value for the overall model was 0.638, though the marginal R^2 value for the fixed effects was 0.0181, indicating that the fixed effects accounted for 1.8% of the variation in the data.

Intraclass correlation coefficient

The variances and ICCs for the five random effect terms are shown in Table 9. The data were highly clustered at the subdistrict, village, and farmer levels.

Discussion

CSFV is a highly infectious disease of pigs with major animal health and economic consequences. In regions where CSFV is endemic, the first step to controlling the disease is to understand its seroprevalence and distribution. Therefore we undertook a CSFV seroprevalence and risk factor study in the NTT province of eastern Indonesia.

In the sample size calculations for CSFV antibody prevalence, the inputs for test sensitivity and specificity were estimated to be lower than the values reported in the literature (Colijn et al., 1997; Moser et al., 1996). The decision to use more conservative estimates was based on the fact that the performance of first-generation CSFV antibody ELISAs has not been evaluated in Indonesia, and the desire to ensure that a sufficient number of pigs were sampled during each village visit. The decision to use the first- and second-generation CSFV antibody ELISAs in series was made after the unexpected result from Flores Island. The number of pigs required to estimate CSFV antibody prevalence was sufficient at a level of precision of 10 percent and a level of confidence of 95 percent given that test sensitivity was 89 percent and test specificity was 100 percent for the tests in series (Sergeant, 2014). In 2010, Epitools assumed a test specificity of 100 percent when calculating the sample size required for disease detection, which is the reason that test specificity was set at 100 percent when determining the number of pigs to be sampled on Flores and Lembata. A sample size of 60 was sufficient for detecting CSFV antibody at a minimum expected prevalence of six percent, assuming a village pig population of 5000, a level of confidence of 95 percent, and an imperfect test with 89 percent sensitivity and 100 percent specificity (Sergeant, 2014).

In West Timor, farmers had to own at least one pig over three months of age to participate, while in Flores, Lembata, and Sumba farmers had to care for at least three pigs over three months of age to participate. This selection bias toward larger herds in Flores, Lembata, and Sumba could have impacted the seroprevalence calculations. Given that larger herds are responsible for the majority of pig movements into and out of a village, seroprevalence estimates for the islands of Flores, Lembata and Sumba may be higher than those that would have been calculated had farmers with fewer than three pigs over three months of age been eligible to participate.

True seroprevalence varied widely between the islands, districts, subdistricts, and villages (Table 3). For example, even in the district of Kota Kupang, an area with a history of clinical reports of CSFV and CSFV vaccination campaigns, village-level true seroprevalence estimates ranged from five percent in Sikumana to 42 percent in Oebufu. This finding is significant because it shows that CSFV seroprevalence is dissimilar within and between various levels of spatial aggregation. Further, it demonstrates that in areas where CSFV vaccination campaigns have been undertaken, the levels of herd immunity required to control disease are not being achieved (Klinkenberg et al., 2002b).

Of the 1080 pigs included in the univariable and multivariable analysis, 152 were reported vaccinated for CSFV. Of the pigs reported vaccinated, only 46% (70/152) tested positive for CSFV antibody (Table 4). There are a number of factors that could be contributing to this low seroconversion rate. Maternally derived antibody is the most common cause of CSFV vaccination failure, particularly in highly endemic areas (Suradhat et al., 2007), and therefore piglets that have circulating maternal antibody may not seroconvert when vaccinated. This interference may be particularly important in NTT where farmers may not actively wean piglets, prolonging the time during which piglets nurse from the sow. Alternatively, vaccine storage and delivery may not be adequate for achieving the high levels of efficacy reported for the C-strain vaccine in the literature (van Oirschot, 2003). Further, it is possible that farmers in the region are not accurately reporting the CSFV vaccination status of their pigs. Finally, co-infection with other pathogens (e.g., pseudorabies, porcine reproductive and respiratory syndrome virus), as well as mycotoxins and chemicals are known to interfere with CSFV vaccination (Suradhat et al., 2007), and their negative impact in this region cannot be ruled out.

None of the farmer-level variables were significant determinants of CSFV serological status, and only two pig-level factors were included in the final multivariable model (Table

8). Pigs equal to or greater than 12 months of age were more likely to test positive for antibody to CSFV. This result is expected, as older animals are both more likely to have been exposed to CSFV and vaccinated during a campaign. Pigs that were reported vaccinated for CSFV were three times as likely to test positive for CSFV antibody compared to those that were not reported vaccinated. This result is expected, and importantly suggests that the vaccination campaigns undertaken by NTT Livestock Services are contributing to CSFV seroprevalence in the region.

In contrast to the expectation that older pigs are more likely to be reported vaccinated, in this study pigs 3-5 months were more likely to be reported vaccinated compared to pigs in the other two age categories. This finding could be the result of reporting bias whereby farmers are more likely to recall the vaccination of young pigs compared to older pigs, in particular because vaccination of younger pigs would have had to occur in the more recent past and therefore might be more memorable. Alternatively, vaccinated pigs may be healthier compared to unvaccinated pigs and therefore may be more likely to be slaughtered once they reach the 6-11 month or ≥12 month age category. Finally, farmers could assume that any injection is a CSFV vaccination, and therefore 'CSFV vaccination status' may in fact represent 'Treatment by NTT Livestock Services'.

The final multivariable model accounted for a large amount of the variation in the data, however the fixed effects, pig age and CSFV vaccination status, accounted for only 1.8% of the variation. High ICCs at the farmer, village, and subdistrict indicate the data were highly clustered. It may be that unmeasured factors at any one of these three levels of spatial aggregation better explain CSFV serological status compared to the pig and farmer-level factors explored in this study. This finding is of relevance to CSFV control efforts in the region because it suggests that interventions may be best implemented at the subdistrict level to account for the high level of clustering at this level, as well as the level of village and

farmer. A recent social network analysis found that the majority of formal and informal pig movements occur between subdistricts, lending further support for intervention at the subdistrict level (Leslie, 2012).

The C-strain vaccine has a number of advantages, including early onset of CSFV immunity and full protection against vertical transmission (Suradhat et al., 2007; Schroeder et al., 2012). However, one of its disadvantages is that the antibody response it induces cannot be differentiated from that caused by CSFV infection. While marker vaccines have been developed in the hope of enabling differentiation of infected from vaccinated animals, these vaccines are less protective and the immune response is delayed when compared to the C-strain vaccine (Suradhat et al., 2007; Schroeder et al., 2012). Additionally, the antibody ELISAs developed as accompanying marker tests have been shown to lack sensitivity (Schroeder et al., 2012). These characteristics of CSFV vaccines and ELISAs impact significantly our ability to understand the epidemiology of the virus in the field. Future studies should consider using additional detection techniques, including polymerase chain reaction, virus genotyping, and sentinel pigs, to better understand CSFV herd immune status, pathogenesis, and epidemiology in NTT.

Clinical cases of CSFV have not been reported in Manggarai Barat district on the west end of Flores Island. Based on this history, CSFV seroprevalence in the district was expected to be very low to non-existent. A minimum expected prevalence of 5% was used to calculate the sample size required to detect the presence of CSFV in this district. The true prevalence of CSFV in Manggarai Barat was 13.1%, with all seropositive pigs detected in Lembor subdistrict (Table 3). This result could be due to one or a combination of several factors. Underreporting of clinical CSFV has been reported in NTT (Santhia et al., 2003). In addition, the virulence of the strain or strains of CSFV circulating in NTT is unknown, and therefore infected pigs may show few clinical signs and recover. CSFV vaccination is not

524 permitted in this area (Tri Satya et al., 1999), however its occurrence cannot be ruled out.
525 Finally, while movement of pigs from CSFV infected to suspect or uninfected areas is not
526 permitted in Indonesia (Tri Satya et al., 1999), illegal movements from central districts to
527 western districts on Flores are known to occur (Leslie, 2012). While such movements could
528 result in the introduction of CSFV into Manggarai Barat, it could also result in the presence
529 of vaccinated pigs in the region.
530

531 ## Conclusions

532 In 2010, Lembata Island was confirmed free of antibody to CSFV. However, the
533 district of Manggarai Barat on the west end of Flores Island contained pigs that were
534 seropositive for CSFV, in spite of no reports of clinical CSFV and no government-led
535 vaccination programs in this region. Pig age and CSFV vaccination status were associated
536 with CSFV serological status, with older pigs and pigs vaccinated for CSFV more likely to
537 test positive for antibody to CSFV. Our results indicate that further research to identify the
538 strains of circulating CSFV and determine the effectiveness of disease control strategies is
539 required. Such activities would contribute to a better understanding of CSFV epidemiology in
540 NTT.
541
542

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552 ## References

553 Artois, M., Depner, K.R., Guberti, V., Hars, J., Rossi, S., Rutili, D., 2002. Classical swine
554 fever (hog cholera) in wild boar in Europe. Rev. Sci. Tech. 21, 287-303.
555 BPS Statistics, 2013. Nusa Tenggara Timur Dalam Angka 2013, Integration processing and
556 statistical dissemination division. Publication Number 53000.06.02. Badan Pusat Statistik
557 Office of Nusa Tenggara Timur Province, Indonesia.
558 Brandenburg, B., Sukobagyo, P., 2002. An update on livestock sector performance in
559 response to the economic crisis, government decentralization and local autonomy. Indonesia
560 Livestock Sector Study, Document 37565. Director General of Livestock Services, Jakarta,
561 Indonesia.
562 Browne, W.J., Subramanian, S.V., Jones, K., Goldstein, H., 2005. Variance partitioning in
563 multilevel logistic models that exhibit overdispersion. J. Roy. Stat. Soc. A. Sta. 168, 599-613.
564 Christie, B. (Ed.), 2007. ACIAR Technical Reports 65: A review of animal health research
565 opportunities in Nusa Tenggara Timur and Nusa Tenggara Barat Province, Eastern Indonesia.
566 ACIAR, Canberra, Australia.
567 Colijn, E.O., Bloemraad, M., and Wensvoort, G., 1997. An improved ELISA for the
568 detection of serum antibodies directed against classical swine fever virus. Vet. Microbiol. 59,
569 15-25.
570 Deveridge, A., 2008. Challenges in the development of a livestock disease surveillance
571 program on Alor, eastern Indonesia. Honours Thesis. School of Natural Sciences, University
572 of Western Sydney, Sydney.

573 Dinas Peternakan Propinsi, 2011. Dinas Peternakan (Provincial Livestock Service) annual
574 report 2011. Dinas Peternakan Propinsi, Kota Kupang, Indonesia.
575 Dohoo, I., Martin, W., Stryhn, H., 2009. Veterinary Epidemiologic Research. VER Inc.,
576 Charlottetown, Canada.
577 Elbers, A.R.W., Stegeman, A., Moser, H., Ekker, H.M., Smak, J.A., Pluimers, F.H., 1999.
578 The classical swine fever epidemic 1997-1998 in the Netherlands: descriptive epidemiology.
579 Prev. Vet. Med. 42, 157-184.
580 Johns, C., Cargill, C., Patrick, I., Geong, M., Ly, J., Shearer, D., 2009. Smallholder
581 commercial pig production in NTT - opportunities for better market integration, SADI Final
582 Report. Australian Centre for International Agricultural Research, Canberra.
583 Klinkenberg, D., Moormann, R.J.M., de Smit, A.J., Bouma, A., de Jong, M.C.M., 2002a.
584 Influence of maternal antibodies on efficacy of a subunit vaccine: transmission of classical
585 swine fever virus between pigs vaccinated at 2 weeks of age. Vaccine. 20, 3005-3013.
586 Klinkenberg, D., de Bree, J., Laevens, H., de Jong, M.C., 2002b. Within- and between-pen
587 transmission of Classical Swine Fever Virus: a new method to estimate the basic
588 reproduction ratio from transmission experiments. Epidemiol. Infect. 128, 293-299.
589 Leslie, E.C.C., 2012. Pig movements across eastern Indonesia and associated risk of classical
590 swine fever transmission. Doctor of Philosophy Ph.D. University of Sydney, Sydney.
591 http://hdl.handle.net/2123/9316
592 Leslie, E.E.C., Geong, M., Abdurrahman, M., Ward, M.P., and Toribio, J.-A.L.M.L., 2014. A
593 description of smallholder pig production systems in eastern Indonesia. Prev. Vet. Med. [In
594 review].
595 Moennig, V., 2000. Introduction to classical swine fever: virus, disease and control policy.
596 Vet. Microbiol. 73, 93-102.

597 Moennig, V., Floegel-Niesmann, G., Greiser-Wilke, I., 2003. Clinical signs and
598 epidemiology of classical swine fever: A review of new knowledge. Vet. J. 165, 11-20.
599 Moser, C., Ruggli, N., Tratschin, J.D., and Hofmann, M.A., 1996. Detection of antibodies
600 against classical swine fever virus in swine sera by indirect ELISA using recombinant
601 envelope glycoprotein E2. Vet. Microbiol. 51, 41-53.
602 Nakagawa, S., Schielzeth, H., 2013. A general and simple method for obtaining R2 from
603 generalized linear mixed-effects models. Methods. Ecol. Evol. 4, 133-142.
604 R Development Core Team, 2008. R: A language and environment for statistical computing.
605 R Foundation for Statistical Computing, Vienna, Austria.
606 Robertson, I., Holyoake, C., Ramsay, G., Mesiti, L., Geong, M., Pullingomang, D., 2010. The
607 development of a national surveillance system for classical swine fever, avian influenza, and
608 foot and mouth disease. Annual Report, Project AH/2004/020. Australian Centre for
609 International Agricultural Research (ACIAR), Canberra.
610 Rogan, W.J., Gladen, B., 1978. Estimating prevalence from the results of a screening test.
611 Am. J. Epidemiol. 107, 71-76.
612 Santhia, K.A.P., Purnatha, N.I., Sunarno, C., Wulan, S.S.N., Pullingomang, D., 2003.
613 Epidemiological study of Hog Cholera in Alor District, East Nusa Tenggara Province. Balai
614 Penyidikan dan Pengujian Veteriner Regional VI Denpasar, Disease Investigation Centre,
615 Denpasar, Bali, Indonesia.
616 Santhia, K.A.P., Dibia, I., N. , Purnatha, N., I., Sutami, N.N., 2006. Surveillance for Hog
617 Cholera eradication in Alor District, Nusa Tenggara Timur. Balai Penyidikan dan Pengujian
618 Veteriner Regional VI Denpasar, Disease Investigation Centre, Denpasar, Bali, Indonesia.
619 Schroeder, S., von Rosen, T., Blome S., Loeffen, W., Haegeman, A., Koenen, F., Uttenthal,
620 A., 2012. Evaluation of classical swine fever virus antibody detection assays with an

emphasis on the differentiation of infected from vaccinated animals. Rev. Sci. Tech. 31, 997-1010.

Sergeant, E.S.G., 2010. Epitools epidemiological calculators. AusVet Animal Health Services and Australian Biosecurity Cooperative Research Centre for Emerging Infectious Disease. Available at: http://epitools.ausvet.com.au.

Sergeant, E.S.G., 2014. Epitools epidemiological calculators. AusVet Animal Health Services and Australian Biosecurity Cooperative Research Centre for Emerging Infectious Disease. Available at: http://epitools.ausvet.com.au.

Suradhat, S., Damrongwatanapokin, S., Thanawongnuwech, R., 2007. Factors critical for successful vaccination against classical swine fever in endemic areas. Vet. Microbiol. 119, 1-9.

Tri Satya, P., Hutabarat, Santhia, K., 1999. The distribution and control strategies of classical swine fever in Indonesia. In: Blacksell, S. (Ed.), Classical Swine Fever and Emerging Diseases in Southeast Asia. ACIAR Proceedings No. 94. Vientiane, Loa, pp 111-115.

van Oirschot, J.T., 2003. Vaccinology of classical swine fever: from lab to field. Vet. Microbiol. 96, 367-384.

Wang, Y., 2007. Rural poverty in Indonesia - Enabling the rural poor to overcome poverty in Indonesia. International Fund for Agricultural Development (IFAD), Rome, Italy.

World Organisation for Animal Health, 2014. World Animal Health Information Database (WAHID) Interface. World Organisation for Animal Health, Paris, France. Accessed online on 20/10/2014 at http://www.oie.int/wahis_2/public/wahid.php/Wahidhome/Home.

642

643 **Figure captions**

644 Figure 1: The distribution and spread of CSFV across Nusa Tenggara Timur, eastern
645 Indonesia up until the end of 2010. The numbers correspond to the following events: 1) the
646 first suspected CSFV cases reported in Sumba Timur and Flores Timur districts in mid 1997;
647 2) the first diagnostic laboratory confirmed cases of CSFV in NTT in March 1998; 3)
648 additional cases identified in 1999; 4) the first suspected CSFV cases from clinical reports
649 from in Sikka district in 2000; and 5) the first case of CSFV detected on Alor Island in July
650 2002. Adapted from information obtained from Tri Satya et al., (1999), Christie (2007),
651 Santhia et al., (2003), and Geong, M (pers comm., 2011). The classification of districts
652 according to CSFV infection status is illustrated using boxes – red boxes contain districts
653 classified as infected, yellow boxes contain districts classified as suspect, and green boxes
654 contain districts classified as not infected.

655

655
656 **Tables**

657 Table 1: Total number of farmers and pigs surveyed across the islands, districts, subdistricts,
658 and villages in East Nusa Tenggara, eastern Indonesia, from April to September 2010.

Island	District	Subdistrict	Village	# of farmers (# of pigs)
Flores	Sikka	Alok	Kota Uneng	20 (60)
			Madawat	20 (60)
			Nangalimang	20 (60)
		Nita	Bloro	20 (60)
			Tilang	20 (60)
			Tebuk	20 (60)
	Manggarai Barat	Komodo	Wae Kelambu	20 (60)
			Batu Cermin	20 (60)
			Golo Bilas	20 (60)
		Lembor	Tangge	20 (60)
			Amba	20 (60)
			Poco Rutang	20 (60)
			Island total	240 (720)
Lembata	Lembata	Nubatukan	Selandoro	20 (60)
			Lewoleba	20 (60)
			Bakalerek	20 (60)
		Lebatukan	Lamatuka	20 (60)
			Merdeka	20 (60)
			Waienga	20 (60)
			Island total	120 (360)
Sumba	Sumba Timur	Haharu	Rambangaru	30 (90)
			Praibakul	30 (90)
	Sumba Barat Daya	Loura	Waitabula	30 (90)
			Rada Mata	30 (90)
			Island total	120 (360)
West Timor[1]	Belu	Tasifeto Barat	Naitimu	37 (90)
			Naekasa	41 (90)
		Atambua Selatan	Fatukbot	50 (90)
			Lidak	42 (90)
	Kota Kupang	Maulafa	Sikumana	39 (90)
			Oepura	32 (90)
		Oebobo	Oebobo	39 (90)
			Oebufu	45 (90)
			Island total	325 (720)
			Total	805 (2160)

659 [1]Thirty farmers per village in West Timor completed the farmer questionnaire. The number
660 of farmers in the table indicates the number of farmers that owned the sampled pigs.

661

661

662 Table 2: Explanatory variables analysed for associations with CSFV serological status

663 amongst 1080 pigs from 360 farmers surveyed in East Nusa Tenggara, eastern Indonesia,

664 from April to September 2010.

Variable group	Variables
Pig-level variables	Sex[b]; Age[a]; Vaccinated for CSFV; Sick in the last three months; Source of pig[b]; Body condition score[a]
Farmer level variables	Cattle on farm; Goats on farm; Buffalo on farm; Number of pigs[c]; Pigs free to roam; Pigs fed swill; Pigs fed agricultural waste; Cook swill; Litters with dead piglets before weaning[b]; Pigs introduced in the last 12 months; Pigs exited in the last 12 months; Use own boar for breeding; Pigs slaughtered at home; Sudden death of pigs in the last three months; Sick pigs that died in the last three months; Livestock services contacted in the event of a sick pig; Pigs have contact with other pigs outside the herd[b]; Heard of CSFV; Pigs vaccinated for CSFV

665 All variables are binary (1 – Yes, 2 – No) unless otherwise indicated.

666 [a] Ordinal variable

667 [b] Categorical variable

668 [c] Continuous variable

Table 3: Apparent prevalence and true prevalence of CSFV antibody across the islands, districts, subdistricts, and villages surveyed in East Nusa Tenggara, eastern Indonesia, from April to September 2010.

Island	District	Subdistrict	Village	Number of samples with a test result	Number positive	Apparent prevalence	True prevalence
Flores				706	55	7.8 (5.9, 10.1)	8.9 (6.8, 11.2)
	Sikka			357	15	4.2 (2.5, 7.0)	5.0 (2.9, 7.7)
		Alok		179	6	3.4 (1.4, 7.5)	4.3 (1.8, 8.0)
			Kota Uneng	60	1	1.7 (0.087, 10.1)	3.6 (0.46, 10.0)
			Madawat	60	0	0 (0, 7.5)	1.8 (0, 5.4)
			Nangalimang	59	5	8.5 (3.2, 19.4)	11.1 (4.3, 20.7)
		Nita		178	9	5.1 (2.5, 9.7)	6.3 (3.0, 10.6)
			Bloro	59	4	6.8 (2.2, 17.3)	9.2 (3.1, 18.0)
			Tilang	59	0	0 (0, 7.6)	1.8 (0, 5.5)
			Tebuk	60	5	8.3 (3.1, 19.1)	10.9 (4.1, 20.3)
	Manggarai Barat			349	40	11.5 (8.4, 15.4)	13.1 (9.6, 17.2)
		Komodo		177	0	0 (0, 2.6)	0.63 (0, 1.9)
			Wae Kelambu	59	0	0 (0, 7.6)	1.8 (0, 5.4)
			Batu Cermin	59	0	0 (0, 7.6)	1.8 (0, 5.4)
			Golo Bilas	59	0	0 (0, 7.6)	1.8 (0, 5.4)
		Lembor		172	40	23.3 (17.3, 30.4)	26.4 (19.7, 33.7)
			Tangge	56	12	21.4 (12.0, 34.8)	25.3 (14.4, 38.4)
			Amba	59	22	37.3 (25.3, 50.9)	42.3 (29.3, 56.2)
			Poco Rutang	57	6	10.5 (4.4, 22.2)	13.3 (5.6, 23.8)
Lembata				360	0	0 (0, 1.3)	0.31 (0, 0.94)
	Lembata			360	0	0 (0, 1.3)	0.31 (0, 0.94)
		Nubatukan		180	0	0 (0, 2.6)	0.6 (0, 1.8)
			Selandoro	60	0	0 (0, 7.5)	1.8 (0, 5.4)
			Lewoleba	60	0	0 (0, 7.5)	1.8 (0, 5.4)
			Bakalerek	60	0	0 (0, 7.5)	1.8 (0, 5.4)
		Lebatukan		180	0	0 (0, 2.6)	0.6 (0, 1.8)

			Lamatuka	60	0	0 (0, 7.5)	1.8 (0, 5.4)
			Merdeka	60	0	0 (0, 7.5)	1.8 (0, 5.4)
			Waienga	60	0	0 (0, 7.5)	1.8 (0, 5.4)
Sumba				358	139	38.8 (33.8, 44.1)	43.7 (38.2, 49.5)
	Sumba Timur			179	60	33.5 (26.8, 41.0)	37.8 (30.2, 45.9)
		Haharu		179	60	33.5 (26.8, 41.0)	37.8 (30.2, 45.9)
			Rambangaru	89	35	39.3 (29.3, 50.3)	44.5 (33.7, 56.0)
			Praibakul	90	25	27.8 (19.1, 38.4)	31.7 (21.9, 42.7)
	Sumba Barat Daya			179	79	44.1 (36.8, 51.7)	49.7 (41.7, 58.0)
		Loura		179	79	44.1 (36.8, 51.7)	49.7 (41.7, 58.0)
			Waitabula	90	36	40.0 (30.0, 50.9)	45.2 (34.4, 56.8)
			Rada Mata	89	43	48.3 (37.7, 59.1)	54.3 (42.7, 66.0)
West Timor				707	142	20.1 (17.2, 23.3)	22.7 (19.5, 26.1)
	Belu			352	83	23.6 (19.3, 28.4)	26.6 (21.8, 31.7)
		Tasifeto Barat		178	49	27.5 (21.2, 34.8)	31.2 (24.2, 38.7)
			Naitimu	90	18	20.0 (12.6, 30.0)	23.2 (14.6, 32.9)
			Naekasa	88	31	35.2 (25.5, 46.2)	39.9 (29.1, 51.5)
		Atambua Selatan		174	34	19.5 (14.1, 26.4)	22.4 (16.2, 29.4)
			Fatukbot	87	22	25.3 (16.8, 35.9)	29.1 (19.6, 39.5)
			Lidak	87	12	13.8 (7.6, 23.2)	16.4 (9.1, 25.4)
	Kota Kupang			355	59	16.6 (13.0, 21.0)	18.9 (14.7, 23.5)
		Maulafa		178	9	5.1 (2.5, 9.7)	6.2 (3.0, 10.5)
			Sikumana	89	3	3.4 (0.87, 10.2)	4.9 (1.4, 10.6)
			Oepura	89	6	6.7 (2.8, 14.6)	8.6 (3.6, 15.6)
		Oebobo		177	50	28.2 (21.9, 35.6)	32.0 (24.8, 39.7)
			Oebobo	88	17	19.3 (12.0, 29.4)	22.5 (13.9, 32.7)
			Oebufu	89	33	37.1 (27.3, 48.0)	42.0 (31.3, 53.4)

Table 4: Descriptive results for pig-level explanatory variables significantly associated (p <0.20)[a] with CSFV serological status amongst 1080 pigs from 360 farmers surveyed in East Nusa Tenggara, eastern Indonesia, from April to September 2010.

Variables	Categories	CSFV serological status		Total
		Negative (Row%)	Positive (Row%)	
Age				
	3 to 5 months	242 (80%)	59 (20%)	301
	6 to 11 months	310 (88%)	44 (12%)	354
	≥12 months	318 (78%)	91 (22%)	409
Sex				
	Male	224 (84%)	42 (15%)	226
	Female	516 (80%)	128 (20%)	664
	Male castrated	123 (85%)	22 (15%)	145
Sick in the last three months				
	Yes	37 (70%)	16 (30%)	53
	No	829 (82%)	177 (18%)	1006
Pig source				
	Born in your herd	507 (82%)	109 (18%)	616
	Other	362 (81%)	83 (19%)	445
Body condition score				
	1	28 (65%)	15 (35%)	43
	2	95 (75%)	32 (25%)	127
	3	228 (90%)	25 (10%)	253
	4	392 (91%)	41 (9%)	433
	5	27 (93%)	2 (7%)	29
Vaccinated for CSFV				
	Yes	82 (54%)	70 (46%)	152
	No	787 (87%)	120 (13%)	907

CSFV – classical swine fever virus.

[a]All pig-level variables had p-values <0.20.

Table 5: Descriptive results for farmer-level explanatory variables significantly associated (p <0.20)[a] with CSFV serological status amongst 1080 pigs from 360 farmers surveyed in East Nusa Tenggara, eastern Indonesia, from April to September 2010.

Variables	Categories	Total herds	CSFV serological status		Total pigs
			Negative (Row%)	Positive (Row %)	
Cook swill					
	Yes	114	268 (79%)	72 (21%)	340
	No	241	558 (83%)	121 (17%)	709
Pigs slaughtered at home					
	Yes	125	274 (73%)	100 (27%)	374
	No	231	588 (87%)	90 (13%)	678
Sick pigs that died in the last three months					
	Yes	17	33 (65%)	18 (35%)	51
	No	338	825 (83%)	173 (17%)	998
Livestock services contacted in the event of a sick pig					
	Yes	193	512 (90%)	57 (10%)	569
	No	164	350 (72%)	136 (28%)	486
Pigs vaccinated for CSFV					
	Yes	59	98 (56%)	77 (44%)	175
	No	299	767 (87%)	116 (13%)	883

CSFV – classical swine fever virus.

[a]Variables with p >0.20 not included in this table: Cattle on farm; Buffalo on farm; Goats on farm; Number of pigs; Pigs free to roam; Pigs fed swill; Pigs fed agricultural waste; Litters with dead piglets before weaning; Pigs introduced in the last 12 months; Pigs exited in the last 12 months; Sudden death of pigs in the last three months; Pigs have contact with other pigs outside the herd; Heard of CSFV.

Table 6: Univariable mixed effects logistic regression results for pig-level variables associated with CSFV serological status (p <0.20)[a] amongst 1080 pigs from 360 farmers surveyed in East Nusa Tenggara, eastern Indonesia, from April to September 2010.

Variables and categories	β	SE (β)	Odds ratio	LCL (OR)	UCL (OR)	p-value[a]
Vaccinated for CSFV						<0.0001
No	-	-	1.0	-	-	
Yes	1.26	0.32	3.51	1.87	6.58	
Age						<0.001
3-5 months	-	-	1.0	-	-	
6-11 months	0.0160	0.304	1.02	0.560	1.843	
≥12 months	1.023	0.298	2.78	1.551	4.989	
Sick in the last three months						0.005
No	-	-	1.0	-	-	
Yes	-0.38	0.42	0.68	0.298	1.555	
Body condition score						<0.0001
1	-	-	1.0	-	-	
2	-0.252	0.446	0.78	0.32	1.86	
3	0.889	0.610	2.43	0.735	8.05	
4	1.144	0.727	3.14	0.756	13.05	
5	0.521	1.117	1.68	0.189	15.03	
Source of pig						0.023
Other	-	-	1.0	-	-	
Born in your herd	-0.00146	0.280	0.999	0.577	1.73	
Sex						0.0116
Female	-	-	1.0	-	-	
Male	-0.124	0.249	0.884	0.542	1.44	
Castrated male	-0.766	0.314	0.465	0.251	0.861	

[a] All pig-level variables had p-values <0.20.

[b] p-values based on likelihood ratio X^2-test of significance.

Table 7: Univariable mixed effects logistic regression results for farmer level variables associated with CSFV serological status (p <0.20)[a] amongst 1080 pigs from 360 farmers surveyed in East Nusa Tenggara, eastern Indonesia, from April to September 2010.

Variables	β	SE (β)	Odds ratio	LCL (OR)	UCL (OR)	p-value[b]
Pigs vaccinated for CSFV						0.000186
No	-	-	1.0	-	-	
Yes	0.988	0.306	2.686	1.474	4.893	
Livestock services contacted in the event of a sick pig						0.0032
No	-	-	1.0	-	-	
Yes	-0.284	0.316	0.752	0.405	1.39	
Sick pigs that died in the last three months						0.00892
No	-	-	1.0	-	-	
Yes	0.0498	0.446	1.051	0.438	2.52	
Pigs slaughtered at home						0.00040
No	-	-	1.0	-	-	
Yes	0.380	0.277	1.25	0.850	2.52	
Cook swill						0.0020
No	-	-	1.0	-	-	
Yes	-0.029	0.424	0.971	0.423	2.23	

[a] Variables with p >0.20 not included in this table.

[b] p-values based on likelihood ratio X^2-test of significance.

Table 8: Final multivariable mixed effects logistic regression model (p <0.05) for CSFV serological status amongst 1080 pigs from 360 farmers surveyed in East Nusa Tenggara, eastern Indonesia, from April to September 2010.

Variables	β	SE (β)	Odds ratio	LCL (OR)	UCL (OR)	p-value[a]
Fixed Effects						
Intercept	-3.31	1.02	-	-	-	0.0012
Age						<0.001
3-5 months	-	-	1.0	-	-	
6-11 months	-0.044	0.31	0.96	0.524	1.75	
≥12 months	0.925	0.30	2.52	1.40	4.54	
Vaccinated for CSFV						<0.001
No	-	-	1.0	-	-	
Yes	1.15	0.32	3.17	1.68	5.98	

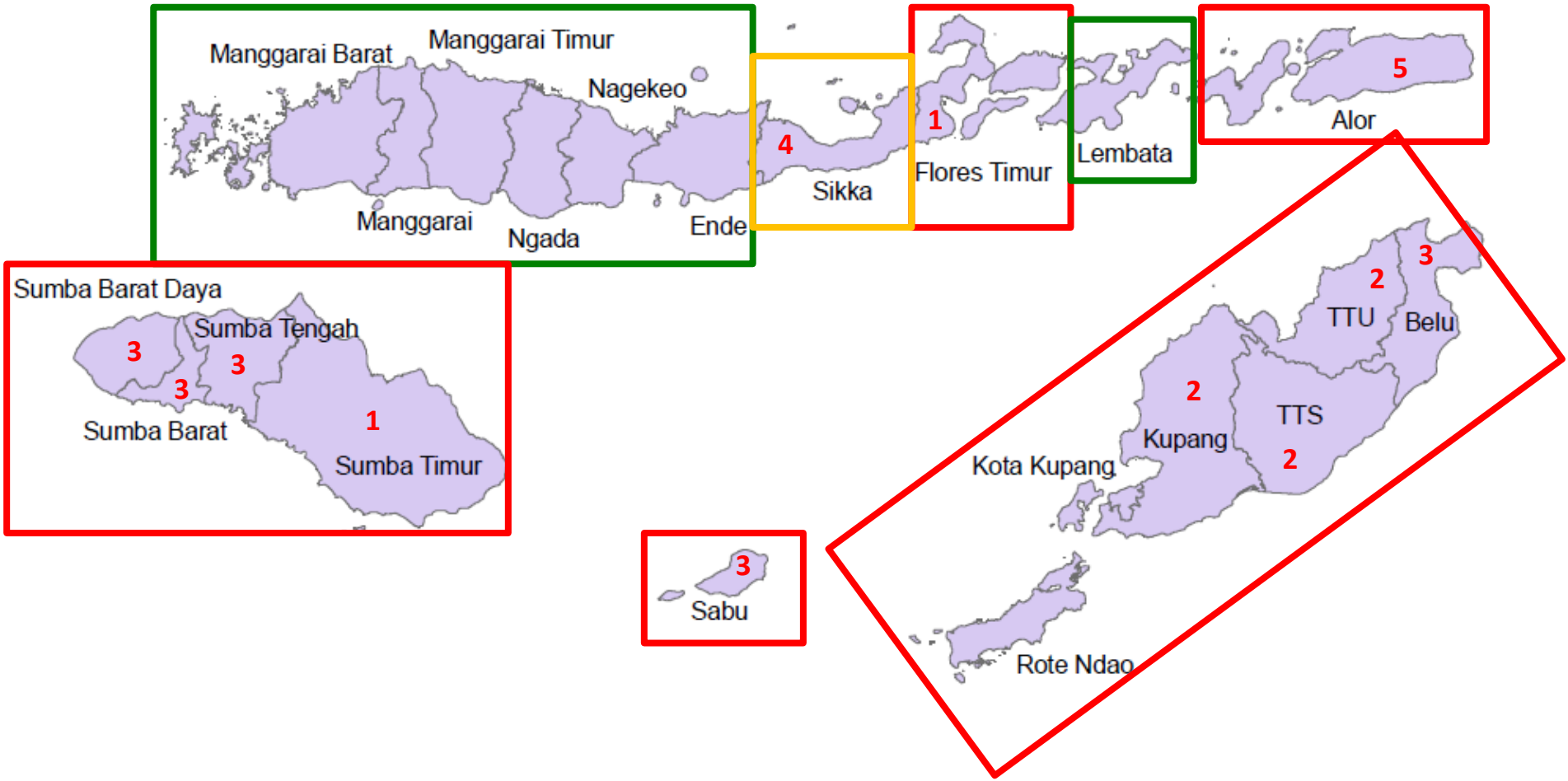
N = 1059, Log-likelihood = -374.41; d.f. = 9; p <0.001; Goodness-of-fit R_2-test statistic $R^2_{GLMM(c)}$ = 0.64.

[a] p-values based on likelihood ratio X^2-test of significance.

Table 9: Variances and intraclass correlation (ICC) coefficients for each random effect term in the final multivariable mixed effects logistic regression model for CSFV serological status amongst 1080 pigs from 360 farmers surveyed in East Nusa Tenggara, eastern Indonesia, from April to September 2010.

Random effect term	Number in level	Variance	ICC
Island	2	0.591	0.0645
District	4	0.00056	0.0646
Subdistrict	6	3.504	0.447
Village	16	0.687	0.522
Farmer	359	1.090	0.641

Figure 1


Manggarai Barat
Manggarai Timur
Nagekeo
Manggarai
Ngada
Ende
4
Sikka
1
Flores Timur
Lembata
5
Alor
Sumba Barat Daya
Sumba Tengah
3
3
3
Sumba Barat
1
Sumba Timur
3
Sabu
2
TTU
3
Belu
2
Kupang
TTS
2
Kota Kupang
Rote Ndao