



# An investigation of classical swine fever virus seroprevalence and risk factors in pigs in Timor-Leste



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## ABSTRACT

Classical swine fever virus (CSFV) is a highly infectious pathogen of pigs and believed to be a major constraint to pig production in Timor-Leste. The Ministry of Agriculture and Fisheries conducts vaccination campaigns in an attempt to control clinical disease, however, there is no empirical data available concerning the seroprevalence and distribution of CSFV in Timor-Leste. To help address this knowledge deficit, a cross-sectional study to determine seroprevalence was conducted in the three districts that border Indonesia. Data on farmer- and pig-level factors were also collected to look at their impact on CSFV serological status. Overall, true CSFV seroprevalence was estimated at 34.4%. Seroprevalence estimates varied widely between and within districts, subdistricts, and villages. Older pigs and pigs that had been vaccinated for CSFV were more likely to test positive for CSFV antibody. Pigs owned by farmers that experienced the sudden death of pigs in the 12 months prior to the survey were more likely to test positive for CSFV antibody, while pigs that had been sick in the previous three months were less likely to test positive for CSFV antibody. 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 one percent of the variation explained by the fixed effects. This work further supports the need for a collaborative approach to whole-island CSFV control between West Timor, Indonesia and Timor-Leste. Further work is needed to better understand the risk factors for CSFV serological status in order to allocate resources for control. As CSFV is now endemic in Timor-Leste research involving a combination of serology, antigen detection and in-depth investigation of suspect cases over a period of time may be required.

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## 1. Introduction

Classical swine fever virus (CSFV) is a highly transmissible *Pes-tivirus* that causes morbidity and mortality in pigs. CSFV spreads directly via horizontal and vertical transmission, and indirectly via contaminated fomites and pork products. Clinical presentation is

classified as acute, subacute, or chronic, and is determined by CSFV strain and a range of host factors, including pig age, breed, stage of pregnancy, CSFV and other pestivirus exposure history, and CSFV vaccination status. There are no pathognomonic signs for CSF, and therefore, laboratory testing is necessary to confirm CSFV infection and detect the presence of antibody (Moennig et al., 2003).

The first suspected cases of classical swine fever (CSF), or hog cholera, on Timor island were reported around Dili during the middle of 1997. During the following 12 months, it spread across the island from east to west, and then to all larger islands of East Nusa Tenggara province, Indonesia and to all districts of Timor-Leste, primarily through the movement of live pigs (Tri Satya et al., 1999; Christie, 2007).

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In Timor-Leste, smallholder pig farmers (herd size of less than 20 pigs) predominant, with around 67% of households raising pigs (National Statistics Directorate Timor-Leste, 2011). Families raise pigs so they can be gifted at social occasions, sold when cash is required, and consumed on special occasions (Aube et al., 2007). Timor-Leste has one of the most malnourished human populations in Asia and the Pacific—more than 20% of the population is undernourished and approximately 50% of children under five years of age are underweight (Asian Development Bank, 2010). Therefore, morbidity and mortality events in the pig population have a significant impact on the human population, and contribute to hunger in Timor-Leste.

The demand for pork and live pigs in Timor-Leste is increasing (Do Karmo, personal communication, 2014), however the size of the pig population in Timor-Leste has stagnated over the last decade: the 2010 census estimated the size of the population at 330,000 pigs (National Statistics Directorate Timor-Leste, 2011), which is approximately equal to the 2004 estimate (National Statistics Directorate Timor-Leste, 2006). While CSFV is endemic to Timor-Leste and believed to be one of the main constraints to pig population growth, at the time this study was conducted there was no national system for reporting CSF cases, and as a result very little is known about the burden of disease.

In spite of the lack of data on CSF in Timor-Leste, the Ministry of Agriculture and Fisheries (MAF) has conducted vaccination campaigns since 2003 in an attempt to reduce its impact (Hall, 2013). Challenges to this control strategy include maintaining cold chain requirements and vaccine delivery, and morbidity and mortality events attributed to CSF still occur (Hall, 2013).

Previous research has documented extensive pig movement across West Timor to the borders with Timor-Leste (Leslie et al., 2014), and movements across the borders are known to occur (Do Karmo, personal communication, 2014). As a result, improved understanding of CSF in Timor-Leste and island-level coordination are necessary to achieve disease control. While a CSFV serological survey was conducted on West Timor in 2010 (Sawford et al., 2015), no serological studies have been published for Timor-Leste and risk factors that impact pig-level CSFV serostatus in Timor-Leste have not yet been identified.

The overarching aim of this study was to better understand CSFV seroprevalence and distribution in Timor-Leste to provide information to support decisions on CSFV control in this country. The objectives were: (1) to determine CSFV seroprevalence in the three districts of Timor-Leste that border Indonesia, and; (2) to investigate pig-level and pig farmer-level factors to determine their impact on pig CSFV serological status in the districts surveyed.

## 2. Material and methods

A cross-sectional study was conducted in three districts of Timor-Leste to investigate CSFV seroprevalence and risk factors associated with pig-level CSFV serological status. Face-to-face interviews were conducted with pig farmers in eight villages from March to April 2013 and blood was collected from 90 pigs in each village.

### 2.1. Sampling

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

#### 2.1.1. Estimation of required sample size

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. Antonino do Karmo; (2) a village pig population of 2000 pigs

based on the expert opinion of Dr. Antonino do Karmo; (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.

#### 2.1.2. Selection of districts, subdistricts, villages, and farmers

Purposive sampling was used to select the three districts within Timor-Leste that border Indonesia, namely Oecussi, Bobonaro, and Covalima. Simple random sampling with a random number generator enumerating every eligible subdistrict was used to select one subdistrict per district in Bobonaro and Covalima, and two subdistricts in Oecussi. Subdistricts considered remote, unsafe, or unlikely to co-operate were excluded from the initial list of subdistricts. The number of subdistricts sampled was based on logistical issues relating to time and funds available. For each selected subdistrict, simple random sampling with a random number generator enumerating every village was used to select two villages per subdistrict.

Thirty pig farmers from each village completed the farmer questionnaire. MAF staff requested a list of pig farmers from each Village Head during a preliminary visit to construct a sampling frame. Concurrently permission to conduct the survey was obtained.

Simple random sampling was used to select 20–50% more farmers than required from each village. Extra farmers were selected to ensure a sufficient number of eligible farmers were surveyed. Farmers had to be present on the day the interview team visited the village and own at least three pigs over three months of age to participate. Farmers were informed of their selection on the day prior to the village visit, and therefore, a high rate of participation was expected. 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 provided with a free pig health check and administration of medications as required as an incentive.

#### 2.1.3. Selection of pigs

Three pigs, three months of age or older were selected for blood sample collection from each interviewed farmer using convenience sampling as this technique was the only one that enabled blood to be collected from 90 pigs in each village during a single visit. 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 maternally derived antibody would not be present in the pigs sampled (Klinkenberg et al., 2002a).

### 2.2. Questionnaire and pig record form design

A questionnaire was developed to record information on farmer demographics, farm structure and performance, pig husbandry, pig reproductive management and performance, pig movements, pig health history, and CSF-related knowledge, awareness and vaccination practices. It consisted of open and closed questions. It took approximately 30 min to complete the questionnaire with each participant. A pig record form was developed to collect information on 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. Throughout the questionnaire and pig record forms, CSF was referred to as hog cholera as the latter is the commonly used term in Timor-Leste.

Both documents were developed in English, translated into Bahasa Indonesia, and finally into Tetum by Dr. Antonino Do Karmo, Chief of the Animal Health Department, National Directorate of Livestock and Veterinary Services, MAF, a native speaker of local origin, and fluent in Bahasa Indonesia. Staff from MAF attended a training event during which the questionnaire and pig record

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