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REGULAR PAPER

Regional Study

Seroprevalence, potential risk factors, and pathological observation of classical swine fever in pigs in Bali Province, Indonesia

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Abstract

Classical swine fever (CSF) is an important viral disease of pigs caused by classical swine fever virus (CSFV), with major impacts on animal health and the livestock industry. This study aimed to estimate the seroprevalence of CSFV antibodies in pigs in Bali, assess potential risk factors associated with seropositivity, and describe pathological changes in clinically suspected cases confirmed by rRT-PCR. From September to December 2023, 470 blood samples from pigs with no available vaccination records were collected across Bali and tested using C-ELISA. In addition, 15 pigs showing CSF signs were necropsied for pathological and rRT-PCR evaluation. Six were confirmed CSFV-positive. The overall seroprevalence was 40.8% (192/470, 95% CI: 36.4–45.4). The highest prevalence occurred in Klungkung regency (65.4%, 17/26). Pigs from urban areas were 1.76 times more likely to be seropositive (OR: 1.76, 95% CI: 1.18–2.64) than those from rural areas. By age, pigs under two years were 1.21 times more likely to be seropositive (OR: 1.21, 95% CI: 0.65–2.23), while crossbred pigs were 1.27 times more likely (OR: 1.27, 95% CI: 0.85–1.88) compared to purebreds; however, these differences were not statistically significant. Only location (urban vs. rural) showed a significant association. Pathological changes were observed in the skin, brain, trachea, lungs, kidney, spleen, and intestines. This study demonstrates a relatively high seroprevalence of CSFV in Bali. The limited vaccination records suggests minimal vaccine coverage, indicating that the high seroprevalence most likely reflects natural infection.

Key Words: classical swine fever, pathology, seroprevalence, swine

Introduction

Classical swine fever (CSF), also known as hog cholera, is a highly significant viral disease affecting pigs, with considerable implications for animal health and the livestock industry^{3,27}. Owing to its substantial economic impact, CSF is classified as a notifiable disease by the World Organisation for Animal Health³³. The causative

agent, classical swine fever virus (CSFV), belongs to the genus *Pestivirus* within the family *Flaviviridae*²⁸. Members of the *Suidae* family, including both domestic (*Sus scrofa domestica*) and wild pigs (*Sus scrofa scrofa*), serve as the natural reservoir and are the primary hosts of CSFV^{1,7}. Antigenically, CSFV is closely related to bovine viral diarrhea virus and border disease virus⁸.

Clinical symptoms and pathological changes

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in CSF vary widely. These variations are likely influenced by viral factors and individual conditions such as age, immune response, general health status, and concurrent infections with other agents²⁾. The clinical form of CSF can be categorized as follows: acute and chronic⁶⁾. The acute form is generally characterized by prolonged high fever up to 40°C^{10,31)}. Clinical signs may include conjunctivitis, constipation followed by severe diarrhea, tremors, movement disorders, and swaying of the hindquarters⁸⁾. Pathological features that may be observed include petechiae on the skin and mucosa, as well as cyanosis of the abdomen, ears, snout, and medial aspects of the legs^{15,20)}. In the chronic form, clinical manifestations include stunting, anorexia, and intermittent episodes of diarrhea and high fever¹⁸⁾. Pathological findings may include thymic atrophy and necrotic or ulcerative lesions known as “button ulcers” in the ileum and colon⁸⁾. This pathological findings considered one of the pathognomonic changes in CSF³⁰⁾. Necrotic ulcers can also be found in the epiglottis and larynx, sometimes accompanied by secondary bacterial infections⁵⁾.

CSF causes substantial economic losses due to its high mortality rate, reduced growth performance, impaired reproduction, and restrictions on international trade^{29,36)}. Seroprevalence of CSFV has been reported in various countries. In India, a national true seroprevalence was reported at 38.29% (2,253/5,848, 95% CI: 37.03–39.57)²¹⁾. In China, Zhao et al. reported an overall prevalence of the disease between 2017 and 2021 at 60.40% (24,456/40,489, 95% CI: 59.92–60.88), with higher levels observed prior to the outbreak of African swine fever (ASF)³⁵⁾. In Indonesia, CSF is designated as a National Strategic Animal Disease under the Regulation of the Ministry of Agriculture No. 4026/Kpts/OT.140/04/2013, due to its high morbidity and mortality rates and substantial economic repercussions¹¹⁾. The first suspected case of CSF in Indonesia was reported in North Sumatra in early 1995, followed by its spread to several other provinces including West Sumatra, Riau, Jakarta, Central Java, West Kalimantan, North Sulawesi, South Sulawesi, and Bali²³⁾.

There is limited data on the seroprevalence of CSFV antibodies and the risk factors associated with seropositivity in pigs in Bali, including their related information on the pathological changes experienced by the infected pigs. These data are important for Bali as it is the province with high demand for pork, largely driven by traditional and religious ceremonies in which pigs play a central role¹³⁾. According to data from the Directorate General of Livestock and Animal Health Services, Ministry of Agriculture cited by Central Statistics Agency of Bali Province, the pig population in Bali reached 517,311⁴⁾, with livestock systems ranging from traditional to semi-modern¹²⁾. Despite ongoing control efforts through vaccination programs, CSF outbreaks continue to occur annually with variable incidence¹⁶⁾. Based on personal communication with authorized animal health staff in Bali Province, although CSFV vaccination is available in Bali, it is provided only upon farmers and/or veterinarian request, and they are required to report vaccination to local authorized animal health staff then the staff input the report into the National Integrated Animal Health Information System or called iSIKHNAS (<https://isikhnas.pertanian.go.id/>). In the practice, the reporting has remained very limited, suggesting that vaccination coverage is minimal.

Therefore, the objectives of this study were to determine the seroprevalence and geographic distribution of antibodies against CSFV in Bali, evaluate potential risk factors associated with seropositivity, including age group, breed, and region, and describe the pathological changes in pigs confirmed with CSFV using rRT-PCR.

Materials and Methods

Ethics statement

This proposed research was previously approved by Research Ethics Committee Udayana University with approval number B/166a/UN14.2.9/PT.01.04/2023 before the research conducted. Informed consent was obtained from all pig owners prior to sample collection. The farmers were informed about the objectives of the study

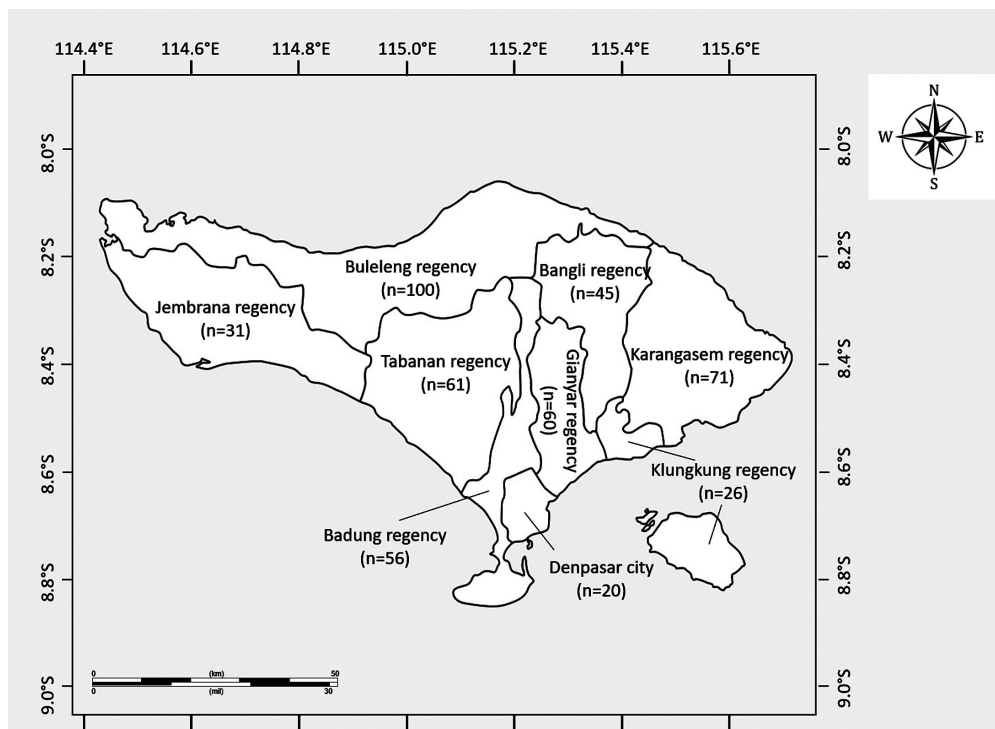


Fig. 1. Map of regions in Bali Province, Indonesia, indicating the locations where pig blood samples were collected for the study.

and the procedures involved in blood sampling. Blood collection of the farmers' pigs was carried out only after their voluntary agreement and consent had been secured.

Sample size

The sample size for this seroprevalence study was determined using the formula for estimating prevalence as described by Marami *et al.*: $n = Z^2 P(1 - P)/d^2$, where n is the required sample size, Z is the value for 95% confidence interval (CI) (1.96), P is the estimated prevalence, and d is the accepted absolute precision (5%)¹⁷⁾. Using a 95% CI, an expected disease prevalence (P) of 50% based on a previous study by Tenaya and Diarmita, and desired absolute precision (d) of 5%, the minimum required number of samples was calculated to be 384³²⁾.

Sample collection and study area

To improve the accuracy and precision of the estimated prevalence, the sample size was increased to 470 samples. Pig blood samples were collected using a convenience sampling

method. The samples were sourced from pig farms across eight regencies and one city in Bali Province, Indonesia (Fig. 1). The urban areas consist of Denpasar city, Badung regency, and Gianyar regency, whereas rural areas consist of Jembrana regency, Buleleng regency, Tabanan regency, Bangli regency, Klungkung regency, and Karangasem regency. Sample collection was conducted September to December 2023 and included pigs aged between 2 months and 5 years. Each pig was properly restrained, and approximately 2 mL of blood was aseptically drawn from the jugular vein using spuites 3-5 mL depending on the size of the pig samples. The collected blood was clotted and the serum was collected before it was transported and centrifuged. For analysis, pigs were grouped as < 2 years (including fattening pigs, approximately 2-23 month, and gilt candidates, approximately 5-8 month) and ≥ 2 years (adult breeding stock, sows/boars). This classification was intended to reflect different production stages that may influence exposure risk, as vaccination records were not available. In Bali, fattening pigs are often

kept for more than 1 year for religious ceremonial purposes.

Serum processing and C-ELISA test

All samples were transported to the Virology Laboratory, Disease Investigation Center Denpasar for further processing. In the laboratory, each of the collected sera were centrifuged at 2,500 rpm for 10 minutes, then aliquoted into sterile tubes and stored at -20°C until the serological analysis was performed. Serum samples were tested for the presence of antibodies against CSFV using a commercial competitive enzyme-linked immunosorbent assay (C-ELISA) kit (VDPro[®] CSFV Ab, JENO Biotech Inc., South Korea). The assay was performed according to the manufacturer's instructions. Absorbance was measured using an ELISA plate reader at a wavelength of 450 nm.

Pathological examination

For the pathological examination, 15 pigs exhibiting clear CSF clinical symptoms were selected. Pigs that were sick with clinical signs suspected to be CSFV infected were subsequently subjected to necropsy for gross pathological evaluation. During necropsy, major internal organs were collected and immediately fixed in 10% neutral buffered formalin for histopathological analysis. The organs sampled included such as the brain, trachea, esophagus, lungs, heart, liver, spleen, kidneys, stomach, and intestines. Fixed tissues were processed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for microscopic examination. Organs such as the pharynx, spleen, and lymph nodes were also collected for rRT-PCR assay.

rRT-PCR Assay

Real time reverse transcriptase (rRT)-PCR assay was conducted at the Virology Laboratory, Disease Investigation Center, Denpasar. Samples of pharynx, spleen, and lymph nodes were finely chopped, homogenized, pooled in an Eppendorf tube, and suspended in PBS. The homogenates were centrifuged to obtain the supernatant, which was used for

RNA extraction. RNA isolation was performed using Trizol (Invitrogen, USA) according to the Standard Operating Procedure of the Disease Investigation Center, Denpasar. Detection of CSFV RNA was carried out using TaqMan[®] rRT-PCR assay targeting the 5' untranslated region (UTR) of the viral genome (GenBank Accession No. NC_000294.1). The assay employed the primers 5'-CCCTGGGTGGTCTAAG-3' (Forward) and 5'-CATGCCCTCGTCCAC-3' (Reverse), together with a fluorogenic probe 5'-FAM-CCTGAGTACAGGACAGTCGTCAGTAGTT-TAMRA-3'⁽²⁴⁾. The positive control reference material used was a standardized and certified CSFV provided by Disease Investigation Center. This positive control was derived from the PK-15 (ATCC CCL-33) cell line, which is permanently infected with CSFV. Amplification was performed in a real-time thermocycler under the following conditions: 45°C for 10 min, 95°C for 10 min, followed by 45 cycles at 95°C for 15 s and 60°C for 46 s.

Data analysis

The prevalence was calculated based on the number of serum samples that tested positive for the antibodies divided by the total number of serum samples tested. Seroprevalence data were analyzed and presented descriptively in tabular form to illustrate the distribution of CSFV antibodies across different regions. The seroprevalence in each areas were analyzed, then the seroprevalence was associated with age and breed. Data of the prevalence, odds ratio (OR) with 95% CI, and chi-square (χ^2) with a two tailed P value of 0.05 were analyzed using Open-Source Epidemiologic Statistics for Public Health (OpenEpi) version 3.01.

For the pathological study, pigs confirmed positive for CSFV by rRT-PCR were subjected to gross pathological examination followed by histopathological evaluation. Histological analysis of organ samples was conducted using a light microscope (Olympus, Tokyo, Japan). The findings, both gross and microscopic pathology, were described qualitatively in a descriptive manner.

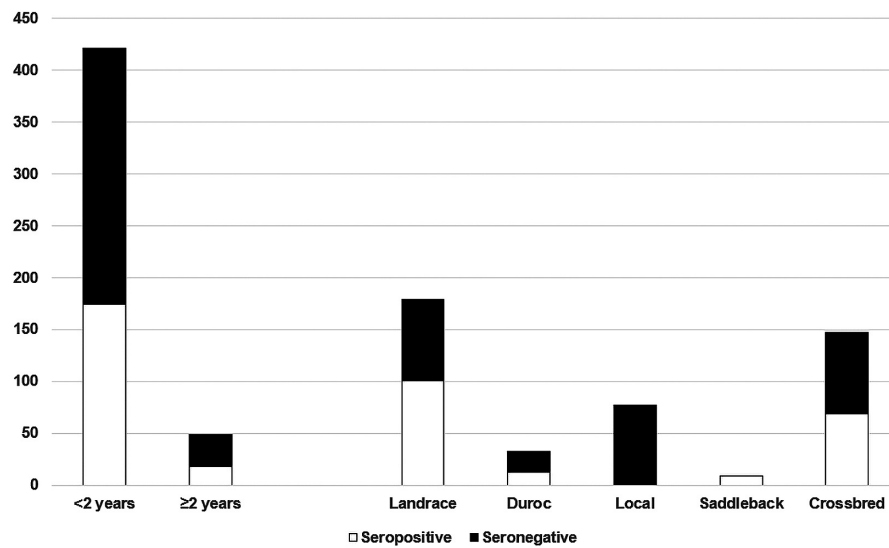


Fig. 2. Classical swine fever virus (CSFV) seroprevalence based on pig breed and age groups.

Table 1. Seroprevalence of CSFV antibodies in pigs across regions in Bali Province, Indonesia

Region	Number of samples	Number of positive (%)	95% CI
<i>Urban areas</i>			
Denpasar city	20	13 (65%)	35.0–40.8
Badung regency	56	27 (48.2%)	34.7–62.0
Gianyar regency	60	29 (48.3%)	35.2–61.6
<i>Rural areas</i>			
Jembrana regency	31	11 (35.5%)	19.2–54.6
Buleleng regency	100	17 (17%)	10.2–25.8
Tabanan regency	61	23 (37.7%)	25.6–51.0
Bangli regency	45	20 (44.4%)	29.6–60.0
Klungkung regency	26	17 (65.4%)	34.6–44.3
Karangasem regency	71	35 (49.3%)	37.2–61.4
Total	470	192 (40.8%)	36.4–45.4

Note. CI = Confidence Interval

Results

Out of the 470 serum samples collected, 192 tested seropositive for CSFV, resulting in an overall seroprevalence of 40.8%. Based on geographic distribution, the highest seropositivity rates were observed in the Klungkung regency, then followed by Denpasar city, Karangasem regency, Gianyar regency, Badung regency, Bangli regency, Tabanan regency, Jembrana regency, and Buleleng regency (Table 1).

In terms of age, younger pigs (< 2 years) showed a higher percentage of seropositivity against CSFV (174/421, 95% CI: 36.6–46.2)

compared to older pigs (> 2 years, 18/49, 95% CI: 23.4–51.7). Based on pig breed, the highest seropositivity was recorded in Saddleback (9/9, 95% CI: 66.4–100), followed by Landrace (101/179, 95% CI: 48.8–63.8), crossbred (69/147, 95% CI: 38.7–55.3), and Duroc pigs (12/32, 95% CI: 21.1–56.3), while no seropositive cases were found in local pig breeds (0/77) (Fig. 2).

In comparison, pigs from urban areas were 1.76 times more likely to be seropositive (OR: 1.76, 95% CI: 1.18–2.64, $P < 0.05$) compared to the pigs from rural area. A significant difference was observed in the seroprevalence against CSFV between pigs sampled from urban compared to

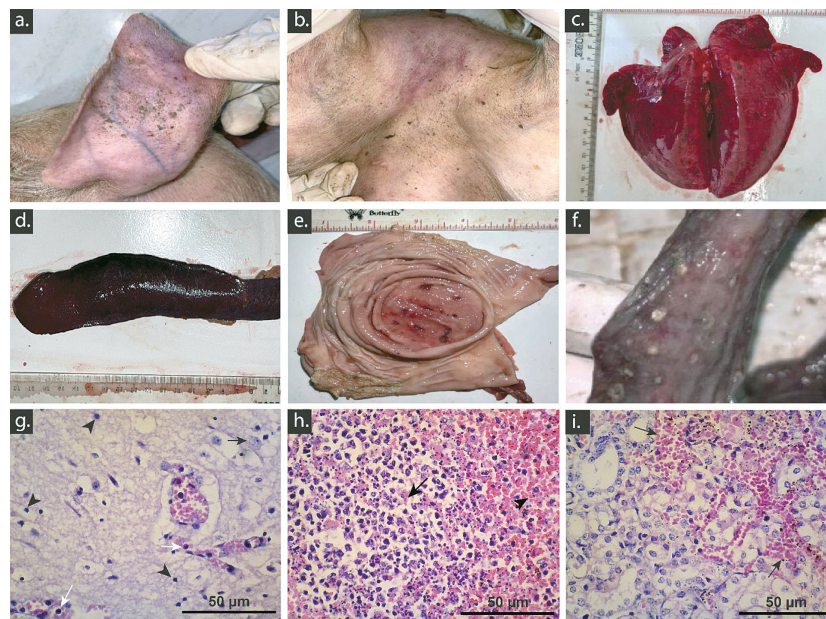


Fig. 3. Gross and histopathological lesions in pigs showing clinical signs of classical swine fever (CSF) and confirmed positive for classical swine fever virus (CSFV) by rRT-PCR. (a) Subcutaneous ecchymotic hemorrhage in the ears. (b) Subcutaneous ecchymotic hemorrhage in the medial aspect of the caudal extremities. (c) Hemorrhagic pneumonia (d) Splenomegaly marked with blunt edges, with a dark area indicative of infarction. (e) Multifocal ulceration and hemorrhage in the gastric mucosa (f) Button ulcers on the surface of the colon. (g) Mild inflammation in small blood vessels (white arrow), proliferation of glial cells (arrowhead), and microgliosis (black arrow) in brain. (h) Necrotic lymphoid follicle (arrow) and perifollicular hemorrhage (arrowhead) observed in spleen. (i) Intertubular hemorrhage in the kidney medulla (arrow). (Hematoxylin & Eosin, 400 $\times$).

rural areas ($P < 0.05$). The pigs with age less than two years were 1.21 times more likely to be seropositive (OR: 1.21, 95% CI: 0.65–2.23, $P > 0.05$) compared to pigs with age equal to or more than two years. Meanwhile, the crossbred pigs were 1.27 times more likely to be seropositive (OR: 1.27, 95% CI: 0.85–1.88, $P > 0.05$) compared to the purebred pigs. No significant difference was noted in the seroprevalence against CSFV between either age or breed ($P > 0.05$).

Out of 15 cases, six pigs exhibiting clinical signs of CSF without vaccination history against CSFV were confirmed positive for CSFV by rRT-PCR. The pigs age between 2–8 months, retrieved from Badung, Gianyar, Tabanan, and Karangasem regency. Listed from the most frequent, clinical signs were anorexia, high fever ($> 40^{\circ}\text{C}$), subcutaneous ecchymotic hemorrhage in ear (Fig. 3a), medial aspect of caudal extremities (Fig. 3b), abdominal area, and snout, diarrhea,

and neurological symptoms^{15,18,20} (Table 2). All confirmed cases underwent necropsy and were examined for both gross and histopathological changes. Gross and histopathological images were presented in Figure 3.

Gross pathological findings revealed lesions in the brain, trachea, lungs, kidney, spleen, and intestinal tract. These organs were swollen and dark-red colored, indicating inflammation and hemorrhage. The brain appeared swollen and congested, while the tracheal mucosa showed evidence of hemorrhage. The lungs also appeared swollen, reddish in color, indicate hemorrhagic pneumonia (Fig. 3c). Hemorrhage in renal medulla was observed. Spleen was enlarged, along with darker area, indicative of infarction (Fig. 3d). Hemorrhagic and ulcer were observed on the mucosal surface of stomach (Fig. 3e). On the mucosal surface of colon, several ulcerations were found (Fig. 3f). Esophagus and liver appeared

Table 2. Clinical signs and pigs age that were confirmed positive for CSFV by rRT-PCR

No.	Origin	Age (month)	Clinical signs
1.	Badung regency	3	Anorexia, high fever, subcutaneous ecchymotic hemorrhage in the ear, abdominal area, and snout, arthritis
2.	Gianyar regency	8	Anorexia, lethargy, yellowish diarrhea, show neurological signs
3.	Tabanan regency	2	Anorexia, lethargy, high fever, subcutaneous ecchymotic hemorrhage in medial aspect of caudal extremities
4.	Tabanan regency	2.5	Anorexia, lethargy, high fever, unable to stand, subcutaneous ecchymotic hemorrhage in ear and medial aspect of caudal extremities, petechial hemorrhage in the abdominal area
5.	Karangasem regency	4	Anorexia, diarrhea, subcutaneous ecchymotic hemorrhage in abdominal area
6.	Karangasem regency	6	Anorexia, high fever, subcutaneous ecchymotic hemorrhage in ear and media aspect of caudal extremities

unremarkable.

Histopathological examination of the brain revealed congestion, glial cells proliferation, microgliosis, and mild inflammation in the small blood vessels (Fig. 3g). In the trachea, mucosal erosion was observed, and the submucosal tissue infiltrated by inflammatory cells along with hemorrhage. The alveoli filled with eosinophilic exudate, and the septa was thickened, infiltrated by alveolar macrophages and also appears congested. The infarct area of the spleen showed extensive white pulp necrosis, along with perfollicular hemorrhage (Fig. 3h). Depletion of white pulp also observed. Intertubular hemorrhage was observed in the kidney, particularly in the medulla part (Fig. 3i). The stomach appeared having epithelial desquamation, mucosal erosion and also ulceration in some part, along with mononuclear inflammatory cells infiltration. Several necrotic debris were observed in the colonic lumina. The villus appeared collapse, and the desquamation of enterocytes were observed. Lamina propria was dominantly infiltrated by lymphocytes and also macrophages. Esophagus appeared unremarkable, while liver, sinusoids and several blood vessels were congested.

Discussion

The CSFV seroprevalence in this study was 40.8%. Vaccination status of the sampled pigs was not recorded, and the c-ELISA cannot differentiate antibodies from natural infection or vaccination. However, as previously mentioned, in Bali the

vaccination program is implemented only upon farmer request and requires reporting through the iSIKHNAS, where reports remain minimal. This indicates that vaccination coverage is very limited, and the high seroprevalence observed here is therefore most likely attributable to natural CSFV infection rather than vaccination.

Another contributing factor may be the level of virulence of the circulating virus strain, since highly virulent strains can stimulate a stronger immune response and result in higher antibody titers which may be more potentially detected by the diagnostic kits³³⁾. Additionally, Laksmi et al. reported 313 clinical CSF cases in 2012 across eight regencies and one city in Bali, with 66 resulting in death, further highlighting the presence of the disease in the region¹⁶⁾. Nevertheless, until today, to the best author's knowledge, there is no report of molecular study of current circulating strain of CSFV in Bali.

Klungkung regency had the highest seroprevalence recorded in this study, which may be attributed to its status as one of the major pig farming areas in Bali and where the pig population has continued to grow annually. Most farmers in this region still rely on semi-intensive or traditional management systems, which often lack the infrastructure needed to separate adult pigs from young pigs. This condition increases the likelihood of transmission from sow to offspring. Furthermore, young pigs are more vulnerable to CSFV infection due to their still-developing immune systems¹⁸⁾. In this study, pigs from urban areas were 1.76 times more likely to be seropositive compared to those from rural areas.

Although farmers in urban areas in Bali are generally more aware of biosecurity measures¹⁴, the higher seropositivity observed might be related to the higher density of pigs, more frequent introduction of animals, and stronger connection to commercial trading chains in urban settings. These factors could facilitate viral transmission despite better hygiene practices. However, this needs to be further investigated in future studies.

Based on age group, the seroprevalence was higher in younger pigs under two years compared to older pig equal to or over two years of age. However, there is no significant difference between them. Sawford et al. found higher seroprevalence rates in older pigs, likely due to longer periods of exposure and accumulated immune responses over time^{25,26}. The higher rate in younger pigs in this study may be related to maternally derived antibodies in piglets, or it is also possibly caused by recent field exposure as their immune system is still developing.

Based on breed, Saddleback pigs had the highest seroprevalence rate at 100% (9/9). The crossbred 1.27 times more likely to be seropositive to the virus than purebred. However, there is no significant difference of the seroprevalence against CSFV between crossbred and purebred. A study confirms that there is no clear evidence that pig breed plays a role in susceptibility to CSFV infection. Petrov et al. reported that various breeds, including Landrace, hybrids, and wild boars, were equally susceptible to infection²². Although clinical manifestations varied, the virus was detected in similar quantities across breeds, and most infected pigs developed acute disease.

Pathological findings of CSF depend on the course of the viral infection and the virulence. In acute infections, pathological findings include generalized lymphadenomegaly and petechial hemorrhage on serosal and mucosal surfaces of organs such as the lungs, kidneys, intestines, and bladder. Lesions in the lymphoreticular system, including splenic infarction and non-suppurative encephalitis, may also occur^{2,9}. In chronic cases, typical lesions include thymic atrophy, lymphoid depletion, intestinal necrosis, and ulceration². In this study, several lesions

were observed, including hemorrhage and multiorgan inflammation affecting the trachea, lungs, stomach, kidney, intestines, and spleen, with infiltrated inflammatory cells predominantly composed of the mononuclear cells. Glial cells proliferation and microgliosis were also detected in the brain, along with mild inflammation in the small blood vessels, suggesting injury response likely associated with the infection.

The limitations of this study include the lack of detailed records on individual pig status, as previously mentioned, such as vaccination history and information on specific farm conditions, such as management systems, biosecurity practices, and other relevant factors. In conclusion, in spite of the vaccination status against CSFV, this study revealed a relatively high seroprevalence of CSFV in pigs in Bali. A significant difference was observed in the seroprevalence against CSFV between pigs in urban and rural areas. However, no significant difference was found between seropositivity either their age or breed. We also successfully described the pathological changes of CSF in pigs that were positive for CSFV by rRT-PCR. Further research is needed to investigate the putative risk factors associated with CSFV seropositivity. Potential risk factors variables to explore in future studies include geographical location, farmer's knowledge about the disease, hygiene and biosecurity practices, the presence of other livestock species, and the molecular characteristics of circulating virus strains. Given the absence of reliable vaccination records, we recommend that local authorities strengthen the monitoring and documentation of CSFV vaccination through iSIKHNAS or other ways. Such efforts would enable more accurate evaluation of vaccination coverage and facilitate future epidemiological studies on disease prevalence and control effectiveness.

Conflict of Interest

All authors declared that there is no conflict of interest exists.

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References

- 1) Blaksell SD, Khounsy S, Van Aken D, Gleeson LJ, Westbury HA. Comparative susceptibility of indigenous and improved pig breeds to Classical swine fever virus infection: Practical and epidemiological implications in a subsistence-based, developing country setting. *Trop Anim Health Prod* 38, 467–477, 2006. doi: 10.1007/s11250-006-4434-0.
- 2) Blome S, Staubach C, Henke J, Carlson J, Beer M. Classical swine fever – an updated review. *Viruses* 9, 86, 2017. doi: 10.3390/v9040086.
- 3) Brown VR, Bevins SN. A review of classical swine fever virus and routes of introduction into the United States and the potential for virus establishment. *Front Vet Sci* 5, 31, 2018. doi: 10.3389/fvets.2018.00031.
- 4) Central Statistics of Bali Province. 2024. Livestock population by regency/city and type of livestock in Bali Province. <https://bali.bps.go.id/id/statistics-table/3/UzJWaVUxZHdWVGxwU1hSd1UxTXZlbnRITjA1Q2R6MDkjMw==/populasi-ternak-menurut-kabupaten-kota-dan-jenis-ternak-di-provinsi-bali-ekor---2021.html?year=2024> [accessed on May 2, 2025].
- 5) Coronado L, Bohorquez JA, Munoz-Gonzalez S, Perez LJ, Rosel R, Fonseca O, Delgado L, Perera CL, Frias MT, Ganges L. Investigation of chronic and persistent classical swine fever infections under field conditions and their impact on vaccine efficacy. *BMC Vet Res* 15, 247, 2019. doi: 10.1186/s12917-019-1982-x.
- 6) Donahue BC, Petrowski HM, Melkonian K, Ward GB, Mayr GA, Metwally S. Analysis of clinical samples for early detection of classical swine fever during infection with low, moderate, and highly virulent strains in relation to the onset of clinical signs. *J Virol Methods* 179, 108–115, 2012. doi: 10.1016/j.jviromet.2011.10.008.
- 7) Everett H, Crooke H, Gurralla R, Dwarka R, Kim J, Botha B, Lubisi A, Pardini A, Gers S, Vosloo W, Drew T. Experimental infection of common warthogs (*Phacochoerus africanus*) and bushpigs (*Potamochoerus larvatus*) with classical swine fever virus. I: Susceptibility and transmission. *Transbound Emerg Dis* 58, 128–134, 2011. doi: 10.1111/j.1865-1682.2011.01202.x.
- 8) Ganges L, Crooke HR, Bohorquez JA, Postel A, Sakoda Y, Becher P, Ruggli N. Classical swine fever virus: the past, present, and future. *Virus Res* 289, 198151, 2020. doi: 10.1016/j.virusres.2020.198151.
- 9) Gómez-Villamandos JC, Garcia de Leaniz I, Nunez A, Salguero FJ, Ruiz-Villamor E, Romero-Trevejo JL, Sanchez-Cordon PJ. Neuropathologic study of experimental classical swine fever. *Vet Pathol* 43, 530–540, 2006. doi: 10.1354/vp.43-4-530.
- 10) Gong W, Li J, Wang Z, Sun J, Mi S, Lu Z, Cao J, Dou Z, Sun Y, Wang P, Yuan K, Zhang L, Zhou X, He S, Tu C. Virulence evaluation of classical swine fever virus subgenotype 2.1 and 2.2 isolates circulating in China. *Vet Microbiol* 232, 114–120, 2019. doi: 10.1016/j.vetmic.2019.04.001.
- 11) Hartini R, Fitria Y, Miswati Y. Monitoring dan diagnosa Classical Swine Fever (CSF) di Provinsi Riau tahun 2023. *Buletin Informasi*

- Kesehatan Hewan 26, 75–80, 2024.
- 12) Kardena IM, Adi AAAM, Astawa INM, O'Dea M, Robertson I, Sahibzada S, Bruce M. Individual and herd-level seroprevalence in association with potential risk factors of Japanese encephalitis in pigs collected from urban, periurban, and rural areas of Bali, Indonesia. *Vet Med Int* 2023, 9682657, 2023. doi: 10.1155/2023/9682657.
 - 13) Kardena IM, Adi AAAM, Astawa NM, O'Dea M, Laurence M, Sahibzada S, Bruce M. Japanese encephalitis in Bali, Indonesia: ecological and socio-cultural perspectives. *Int J Vet Sci Med* 9, 31–43, 2021. doi: 10.1080/23144599.2021.1975879.
 - 14) Kardena IM, Dharmayudha AAGO, Gunawan IWNF, Jayanti PD, Astawa INM, Adi AAAM, Suarsana IN, Suartha IN, Dargantes AP. Seroprevalence of swine hepatitis E virus and the farmers' potential risk of infection in the Province of Bali, Indonesia. *Vet World* 17, 1810–1820, 2024. doi: 10.14202/vetworld.2024.1810-1820.
 - 15) Kumar H, Mahajan V, Sharma S, Singh AR, Arora AK, Banga HS, Verma S, Kaur K, Kaur P, Meenakshi K, Sandhu KS. Concurrent pasteurellosis and classical swine fever in Indian pigs. *Journal of Swine Health and Production* 15, 279–283. 2007.
 - 16) Laksmi LKN, Hartawan DHW, Puspitasari E, Suryadinata LMF, Sutami N, Purnatha N. Correlation analysis of vaccination and hog cholera clinical case at Bali Province in 2009–2013. *Buletin Veteriner BBVet Denpasar* 16, 1–8, 2014.
 - 17) Marami LM, Gebremedhin EZ, Sarba EJ, Tola GK, Endalew SS, Melkamsew AT, Presti VDM, Vitale M. Seroprevalence and associated risk factors of canine *Leptospira* and *Brucella* species infection in West Shewa Zone, Central Ethiopia. *Vet Med (Auckl)* 12, 33–42, 2021. doi: 10.2147/vmrr.s297155.
 - 18) Moennig V, Floegel-Niesmann G, Greiser-Wilke I. Clinical signs and epidemiology of classical swine fever: a review of new knowledge. *Vet J.* 165, 11–20, 2003. doi: 10.1016/s1090-0233(02)00112-0.
 - 19) Moudgil P, Prakash A, Jindal N, Lather D, Gupta R. Pathology and molecular characterization of classical swine fever virus from piggery units in Haryana. *Indian Journal Animal Sciences* 91, 443–448, 2021. doi: 10.56093/ijans.v91i6.115441.
 - 20) Palanivel KM, Sathivelan SM, Gopinathan A, Sriram SK, Kumarasamy P. Incidence of Mortality among swine due to Classical Swine Fever – postmortem findings. *Indian Journal of Animal Research* 46, 86–88, 2012.
 - 21) Patil SS, Suresh KP, Hemadri D, Hiremath J, Sridevi R, Krishnamoorthy P, Bhatia S, Roy P. Spatial seroprevalence of classical swine fever in India. *Trop Anim Health Prod* 53, 389, 2021. doi: 10.1007/s11250-021-02829-6.
 - 22) Petrov A, Blohm U, Beer M, Pietschmann J, Blome S. Comparative analyses of host responses upon infection with moderately virulent classical swine fever virus in domestic pigs and wild boar. *Virol J* 11, 134, 2014. doi: 10.1186/1743-422x-11-134.
 - 23) Rell F, Apada AMS, Yusuf B, Suharto RH, Jamaluddin AW. Viral infections posing significant threats to swine populations in Indonesia and globally: a review. *Buletin Veteriner Udayana* 17, 183–192, 2025. doi: 10.24843/bulvet.2025.v17.i01.p19.
 - 24) Risatti GR, Callahan JD, Nelson WM, Borca MV. Rapid detection of classical swine fever virus by a portable real-time reverse transcriptase PCR assay. *J Clin Microbiol* 41, 500–505, 2003. doi: 10.1128/JCM.41.1.500-505.2003.
 - 25) Sawford K, do Karmo A, do Conceicao F, Geong M, Tenaya IWM, Hartawan DHW, Toribio JALML. An investigation of classical swine fever virus seroprevalence and risk factors in pigs in Timor-Leste. *Prev Vet Med* 122, 99–106, 2015. doi: 10.1016/j.prevetmed.2015.09.012.
 - 26) Sawford K, Geong M, Bulu PM, Drayton E, Mahardika GNK, Leslie EEC, Robertson I, Putra AAAG. An investigation of classical swine fever virus seroprevalence and risk factors in pigs in East Nusa Tenggara, eastern Indonesia. *Prev Vet Med* 119, 190–202, 2015.

- doi: 10.1016/j.prevetmed.2015.02.002.
- 27) Shimizu Y, Hayama Y, Murato Y, Sawai K, Yamaguchi E, Yamamoto T. Epidemiology of classical swine fever in Japan – a descriptive analysis of the outbreaks in 2018–2019. *Front Vet Sci* 7, 573480, 2020. doi: 10.3389/fvets.2020.573480.
 - 28) Simmonds P, Becher P, Bukh J, Gould EA, Meyers G, Monath T, Muerhoff S, Pletnev A, Rico-Hesse R, Smith DB, Stapleton JT, ICTV Report Consortium. ICTV virus taxonomy profile: Flaviviridae. *J Gen Virol* 98, 2–3, 2017. doi: 10.1099/jgv.0.000672.
 - 29) Singh VK, Rajak KK, Kumar A, Yadav SK. Classical swine fever in India: current status and future perspective. *Trop Anim Health Prod* 50, 1181–1191, 2018. doi: 10.1007/s11250-018-1608-5.
 - 30) Sudo K, Yamada M, Ochiai M, Iwamoto S, Sakaki H, Kushida C, Yamashita M, Yamamoto A, Nagasaka T, Oidemizu M, Saito A, Yamamoto K. The formation process of button ulcers in pigs experimentally infected with a subgenotype 2.1 isolate of classical swine fever virus. *J Vet Med Sci* 83, 990–993, 2021. doi: 10.1292/jvms.20-0654.
 - 31) Tarradas J, Torre MED, Rosell R, Perez LJ, Pujols J, Munoz M, Munoz I, Munoz S, Abad X, Domingo M, Fraile L, Ganges L. The impact of CSFV on the immune response to control infection. *Virus Res* 185, 82–91, 2014. Doi: 10.1016/j.virusres.2014.03.004.
 - 32) Tenaya IWMT, Diarmita IK. Situation overview and surveillance results of hog cholera in area responsibility of Balai Besar Veteriner Denpasar in 2009–2012. *Buletin Veteriner BBVet Denpasar* 15, 1–9, 2013.
 - 33) Wang J, Sun Y, Meng XY, Li LF, Li Y, Luo Y, Wang W, Yu S, Yin C, Li S, Qiu HJ. Comprehensive evaluation of the host responses to infection with differentially virulent classical swine fever virus strains in pigs. *Virus Res* 255, 68–76, 2018. doi: 10.1016/j.virusres.2018.06.012.
 - 34) World Organisation for Animal Health. 2022. Terrestrial Animal Health Code: Chapter 15.2. Infection with classical swine fever virus. https://www.woah.org/fileadmin/Home/eng/Health_standards/tahc/current/chapitre_csf.pdf [accessed on May 2, 2025].
 - 35) Zhao P, Wang C, Xia Y, Hu Y. Seroprevalence investigation of classical swine fever virus before, during, and after African swine fever virus outbreak in some provinces of China from 2017 to 2021. *Viral Immunol* 35, 33–40, 2022. doi: 10.1089/vim.2021.0109.
 - 36) Zhou B. Classical swine fever in China – an update minireview. *Front Vet Sci* 6, 187, 2019. doi: 10.3389/fvets.2019.00187.