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Research Article

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Identification of *Campylobacter jejuni* from Pig Feces in Kupang City, East Nusa Tenggara, Indonesia

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ABSTRACT

Background: Campylobacteriosis is a foodborne zoonotic disease caused by *Campylobacter* spp., recognized as one of the leading causes of gastroenteritis in humans worldwide. The presence of *Campylobacter jejuni* in pigs represents a potential public health concern due to its zoonotic transmission through contaminated food products.

Aims: This study aimed to isolate and identify *Campylobacter jejuni* in pigs raised on pig farms in Kupang City using microbiological and biochemical confirmation methods.

Methods: A total of 10 fecal samples were collected from clinically healthy pigs, consisting of three samples from one commercial farm and seven samples from seven different household farms. Colonization rates were determined using the Most Probable Number (MPN) method, followed by microscopic identification based on Gram staining and Hippurate hydrolysis confirmation testing to distinguish *Campylobacter jejuni* from other *Campylobacter* species.

Results: The MPN test demonstrated that all samples showed *Campylobacter* sp. growth on mCCDA selective media, characterized by grayish-white colonies spreading across the media surface, with MPN indices ranging from 95 to >1898 MPN/g. Microscopic examination revealed spiral-shaped, Gram-negative bacteria consistent with *Campylobacter* spp. Hippurate hydrolysis confirmation tests produced dark purple discoloration in all suspected isolates, confirming positive identification of *Campylobacter jejuni*. The findings indicated a 100% prevalence (10/10) of *Campylobacter jejuni* in the pig fecal samples examined.

Conclusion: The study confirmed the presence of *Campylobacter jejuni* in pigs raised on farms in Kupang City, highlighting the potential zoonotic risk associated with pig farming. These findings emphasize the importance of surveillance and control measures to prevent the spread of campylobacteriosis and protect public health.

INTRODUCTION

Campylobacter is a Gram-negative, microaerophilic bacterium with curved, spiral, or S-shaped rod morphology (Samanta & Bandyopadhyay, 2020). These bacteria are pathogenic to humans and a variety of farm animals, including pigs, poultry, and cattle (Kreling et al., 2020). *Campylobacter* is a zoonotic bacterium that is the main cause of gastroenteritis in humans around the world with the most pathogenic species being *C. jejuni*. The route of transmission via the fecal-oral route, primarily through consumption of foods of animal origin such as the consumption of raw meat, unpasteurized milk, and contaminated water (Tikhomirova et al., 2024). In addition, workers in slaughterhouses as well as individuals who have direct contact with feces or fluids of infected animals are also at high risk of exposure to *C.*

jejuni (Tangkonda et al., 2021). *Campylobacter* infection in humans, otherwise known as Campylobacteriosis, is generally *self-limiting* with the main symptoms being diarrhea, fever, nausea, abdominal pain, and vomiting within 3-5 days of exposure (Myintzaw et al., 2023). However, in certain cases, serious complications can occur, such as guillain-barré syndrome (GBS), miller fisher syndrome (MFS), reactive arthritis (REA), irritable bowel syndrome, meningitis, brain abscess, as well as lung infections (Facciolà et al., 2017) . These infections are more common in vulnerable age groups such as children and the elderly, as well as individuals with weakened immune systems, such as people with HIV/AIDS (Fischer et al., 2024).

East Nusa Tenggara Province (NTT) is one of the regions with the highest pig population in Indonesia, with a total of 2,132,124 pigs (Central Statistics Agency, 2024). Pig farming in NTT is growing rapidly due to the high demand for pork, both on a commercial and conventional scale. Apart from being a source of food, the existence of pigs in NTT also has a close relationship with local culture and customs (Tukan et al., 2023). One example of a cultural practice related to pork consumption is *lawar*, which is a traditional food made from raw pork with a mixture of spices. The consumption of this raw meat has the potential to increase the risk of transmission of *C. jejuni* to humans.

Until now, there has been no research on the prevalence of *Campylobacter* in pigs in Indonesia. However, some studies from other countries suggest that pigs can be a major reservoir of these bacteria. Research in Vietnam reported the prevalence of *C. jejuni* in pig feces at 53.7% (Carrique-Mas et al., 2014). Another study in Campania, Southern Italy found that *Campylobacter* sp. could be isolated from 50% of wild boar fecal samples (Kerkhof et al., 2022).

Based on this background, this study aims to isolate and identify of *Campylobacter jejuni* from the feces of pigs raised in Kupang City, East Nusa Tenggara Province, Indonesia. The results of this study are expected serve as a reference for efforts to prevent and control Campylobacteriosis in humans and animals, as well as to contribute to the development of more effective risk mitigation strategies in the livestock and public health sectors.

MATERIALS AND METHODS

Ten fecal samples were collected from eight pig farms in Maulafa District, Kupang City, Indonesia, selected purposively based on production system (one commercial and seven household farms). Three clinically healthy pigs were randomly sampled from a commercial farm in Penfui Village (population 30 pigs) and assigned codes FB1P, FB2P, and FB3P. From seven household farms in Naimata Village, one clinically healthy pig was sampled per farm and assigned codes FB4N through FB10N. Each sample represented a single pig. Approximately 10 g of fecal samples was collected immediately after spontaneous defecation without animal restraint or handling. The inner portion of the fecal mass, which had not contacted the pen floor or urine, was aseptically obtained using a sterile spatula and transferred into a sterile fecal container. All samples were immediately placed in a cool box maintained at 4-8°C and transported to the laboratory. Samples were processed within 4-6 hours after collection to preserve bacterial viability prior to isolation procedures.

The research materials included fresh pig feces samples, lysis horse blood, modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA) media (HiMedia M887I-500G), CCDA selective supplement (HiMedia FD135), Preston *Campylobacter* selective supplement (Oxoid SR0204E), nutrient broth (Oxoid CM0067), microaerophilic gas pack CampyGen (Oxoid), buffered peptone water (BPW), Hippurate Disks, and 3.5% Ninhydrin reagent.

Bacterial Isolation

Five grams of each sample was weighed and then homogenized in 45 ml BPW, inoculated using the three tube MPN method with three serial dilutions (5 mL, 1 mL, and 0.1 mL). The sample suspension was inoculated into Preston broth made from Nutrient Broth plus horse blood lysis and preston *Campylobacter* selective supplement and then incubated under microaerophilic conditions (5% oxygen, 10% carbon dioxide, and 85% nitrogen) using CampyGen gas packs at 42°C for 48 hours. After incubation, one loopful of the suspension was taken and cultured on mCCDA plates supplemented with CCDA selective supplement media using the T streak method and re-incubated under microaerophilic conditions at 42°C for 48 hours (ISO 10272-1, 2017; Neyaz et al., 2024).

Bacterial Identification

Macroscopic identification was carried out by colony observation based on the shape of the colony, the color of the colony, the edge of the colony, and the surface of the colony (elevation). The characteristics of *Campylobacter* sp. colonies on the mCCDA selective media appear grayish-white, flat, slightly wet, and spreading. Microscopic observation of bacterial cells through Gram staining. The characteristics of

Campylobacter bacterial cells are spiral and Gram-negative.

Hippurate Hydrolysis Test

This test was carried out by suspending one suspected bacterial colony in 400 µL of sterile distilled water to which hippurate media (Hippurate disc) was added, then incubated at 37°C for 2 hours, then 200 µl of 3.5% ninhydrin solution was added. After that, re-incubate at 37°C for 10 minutes. A positive reaction is indicated by a dark purple or blue color change. Meanwhile, A negative reaction is indicated by a clear or gray color (WOAH Terrestrial Manual, 2024)

RESULTS AND DISCUSSION

Results of *Campylobacter* sp. Colonization Rate with MPN Method

The colonization rate of *Campylobacter* sp. in pig feces was analyzed using the three-tube MPN method with three dilution series. This method was modified by incorporating an MPN plating technique, in which samples were inoculated into mCCDA selective media using the T streak method (ISO 10272-1, 2017). Colony growth was observed and calculated based on the MPN 333 table as per the Thomas Formula. The results of observation and testing of pig feces samples are presented in Table 1. *Campylobacter* was detected in all fecal samples (n=10).

The MPN index values obtained varied between samples, ranging from 95 MPN/g to ≥1898 MPN/g. The highest MPN index (≥1898 MPN/g) was found in young pigs: FB2P (3 weeks), FB8N (2 months), FB9N (3 months), and FB10N (4 months), indicating high colonization in this age group. In contrast, the lowest MPN index values (95 MPN/g) were found in FB4N (18 months) and FB5N (19 months), which are older age groups. This pattern suggests that *Campylobacter* is more commonly found in younger pigs.

Results of Macroscopic and Microscopic Identification of *Campylobacter* sp.

Macroscopically, *Campylobacter* sp. colonies growing on mCCDA selective media appear to spread on the surface of the media, with flat colony edges. The colony appears moist, relatively small in size, and has a grayish-white color. The shape of the colony tends to be slightly elongated or oval (Figure 1a).

Microscopic examination was performed using Gram staining of isolates taken directly from a single colony. The staining results showed that the bacteria appeared pink under the microscope, which indicates that the bacterium was Gram-negative. Morphologically, bacteria have a spiral or curved shape, resembling a comma or the letter S (*comma-shaped* or *S-shaped*), with a small and slender cell size (Figure 1b).

These microscopic observations showed the characteristics of *Campylobacter* sp., which is a curved rod-shaped, Gram-negative bacterium with a length of 0.5-5 µm and a width of 0.2-0.8 µm (Al-Khreshieh et al., 2023). Macroscopic and microscopic identification showed that all sample isolates had *Campylobacter* characteristics.

Table 1. Results of observation and bacterial characteristics of pig fecal isolates

Code	Age	MPN Index (MPN/g)	Macroscopic		Microscopic		Hippurate hydrolysis test	
			Color	Shape	Gram Stain	Shape	Color	Species
FB1P	11 months	271	Gray	Round	Gram -	Spiral	Purple	*C. jejuni*
FB2P	3 weeks	≥1898	Gray	Round	Gram -	Spiral	Purple	*C. jejuni*
FB3P	12 months	190	Gray	Round	Gram -	Spiral	Purple	*C. jejuni*
FB4N	18 months	95	Gray	Round	Gram -	Spiral	Purple	*C. jejuni*
FB5N	19 months	95	Gray	Round	Gram -	Spiral	Purple	*C. jejuni*
FB6N	12 months	190	Gray	Round	Gram -	Spiral	Purple	*C. jejuni*
FB7N	8 months	271	Gray	Round	Gram -	Spiral	Purple	*C. jejuni*
FB8N	2 months	≥1898	Gray	Round	Gram -	Spiral	Purple	*C. jejuni*
FB9N	3 months	≥1898	Gray	Round	Gram -	Spiral	Purple	*C. jejuni*
FB10N	4 months	≥1898	Gray	Round	Gram -	Spiral	Purple	*C. jejuni*

Hippurate Hydrolysis Test Results

The test results showed a dark purple color change in all sample isolates showing a positive reaction indicating that the tested isolate was *C. jejuni* (Figure 2). The hippurate hydrolysis test relies on the ability of the enzyme hippurate hydrolase produced by *C. jejuni* to hydrolyze sodium hippurate becomes benzoic acid and glycine. The glycine produced in this reaction was detected by adding ninhydrin reagent. When glycine was present, the reagent reacted and produced a deep purple color, indicating a positive result.

The appearance of this purple coloration confirmed the presence of hippurate hydrolase activity, which is characteristic of *C. jejuni* (WOAH Terrestrial Manual, 2024). The results of this test are consistent with the microscopic identification and morphology of previous colonies, and strengthen the suspicion that all isolates are *C. jejuni.*

The results of this study showed that *C. jejuni* was detected in all sample isolates from pig feces, with a prevalence of 100%. This study showed a higher prevalence rate compared to previous studies. Research by Young et al. (2000) in Texas, United States, reported a prevalence of *C. jejuni* of 76.3% in adult female pigs aged 6 months and in pregnant pigs, the prevalence of *Campylobacter* reached 100% comprising 89% *C. jejuni* and 11% *C. coli.* In piglets within 24 hours of birth, the prevalence of *Campylobacter* was 57.8% and increased to 100% at weaning age with *C. coli* being more dominant at birth (68.3%) and *C. jejuni* 31.7%. In piglets weaned at the age of 14 days, the prevalence of *C. jejuni* was 82%, while *C. coli* was 18%. Research by Carrique-Mas et al. (2014) in Vietnam reported the prevalence of *C. jejuni* as much as 53.7% in pig farms with high production rates. Another study in Grenada reported a *Campylobacter* sp. prevalence of 95.6% of 180 young pig feces samples with *C. jejuni* dominant as much as 65% and C. coli 54% (Matthew-Belmar et al., 2015). In addition, another study reported that *C. jejuni* was found at higher rates in pigs raised outdoors. These results differ from reports from Italy and Estonia, where all *Campylobacter* isolates from pig fecal samples were identified as *C. coli* (Di Donato et al., 2020; Tedersoo et al., 2023). In addition, studies in the literature also reported that *C. coli* is the most dominant species found in pigs.

The high prevalence rate of *C. jejuni* can be caused by various factors that affect each other, such as host age, sanitary conditions, and management systems aspects of bacterial metabolism, and geographical environmental characteristics. Young pigs tend to have a higher rate of *C. jejuni* colonization than adult pigs due to an immune system that has not been optimally developed. With age, the immune system becomes more mature and protective, thus being able to suppress the rate of colonization of these bacteria (Han et al., 2016; WOAH Terrestrial Manual, 2024). Previous studies reported that 56.6% of piglets were colonized by

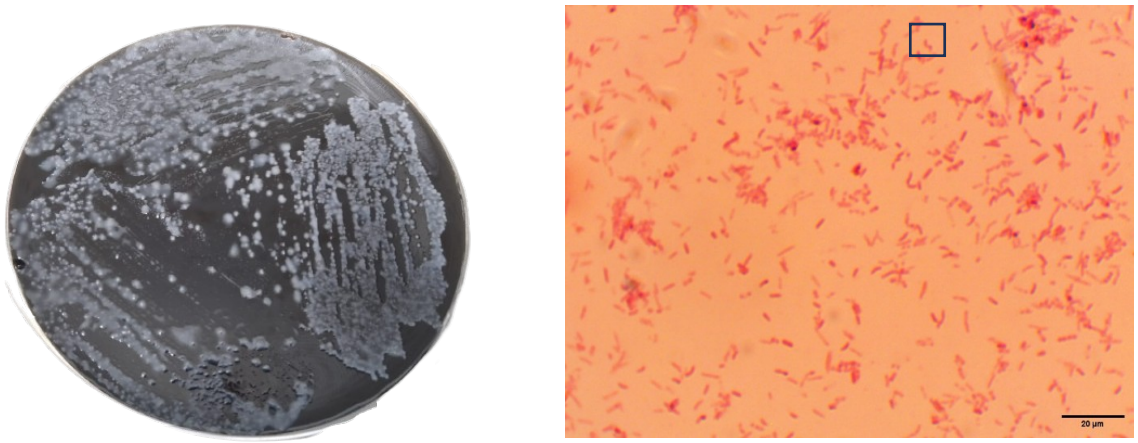

Figure 1. (a) *Campylobacter* sp. colonies on mCCDA selective media appear grayish-white and spread; (b) The cell morphology *of Campylobacter* sp. is spiral (in a box) and Gram-negative at 1000x magnification

C. jejuni by the fourth week postnatal. Colonization patterns that resembled those of their mothers indicate the possibility of transmission vertically from mother to offspring (Alter et al., 2005).

In addition, *C. jejuni* exhibits adaptive metabolic capabilities by utilizing various amino acids such as serine, aspartate, glutamate, proline, asparagine, and glutamine as energy sources for its growth (Hofreuter, 2014). Among these amino acids, serine is the most dominant source of energy and is metabolized through the enzyme L-serine dehydratase (SdaA) and the transporter serine (SdaC) (Hofreuter, 2014; Velayudhan et al., 2004). High concentrations of serine were found in the anterior part of the digestive tract, especially in the ileum with levels of up to 1.67 mmol/kg, much higher than in the caecum which is only about 0.14 mmol/kg (Rath et al., 2022).

This is in line with the distribution pattern of *C. jejuni* which generally inhabits the anterior part of the intestine, such as the duodenum, jejunum, and especially the ileum, while *C. coli* is more commonly found in the posterior parts, such as the cecum and colon. Recent studies have shown that *C. jejuni* is able to metabolize L-fucose, which not only supports growth but also promotes biofilm formation and strengthens adhesion and invasion capabilities to intestinal epithelial cells. This ability further strengthens colonization, especially in piglets that have not yet developed an optimal immune system (Middendorf et al., 2024).

This condition is also exacerbated by a poor cage sanitation system. In the pig farming system in Naimata, cages are rarely cleaned allowing the accumulation of feces and feed residues that are an ideal medium for the growth and spread of *C. jejuni*. Meanwhile, in Penfui, although cleaning is performed regularly, the lack of disinfectant use allows bacterial persistence. *C. jejuni* can form biofilms that survive in poor environmental conditions even after cleaning and disinfection, persisting in the cage environment for 21 days or more (García-Sánchez et al., 2017), additionally, it can survive in water at low temperatures for up to 4 months especially in environments with low oxygen levels and high organic matter (Oberheim et al., 2020).

The sustainability of *C. jejuni* colonies in the environment is also supported by the mixed maintenance system applied in these eight farms in two areas. Open cages allow for interaction between pigs and poultry, wild birds, and rodents tend to have higher levels of contamination. Chickens raised in the same area as pigs can contaminate feed and drinking water with feces containing *C. jejuni*, thus increasing the risk of cross-infection (Muralikrishna et al., 2018). Wild birds are considered the main reservoirs of *C. jejuni* due to their high mobility and ability to contaminate the environment with contaminated feces (Mulder et al., 2020).

In addition, the tropical climate of Kupang City, characterized by high ambient temperatures, may contribute to the high prevalence of *C. jejuni* in pigs.

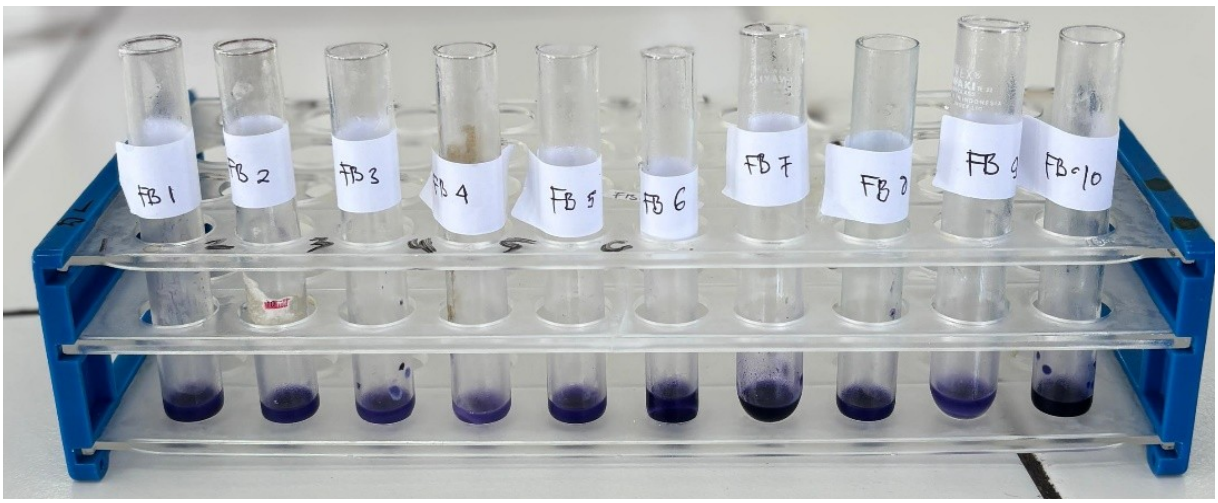

Figure 2. Results of the Hippurate hydrolysis test show a deep purple discoloration in all isolates, indicating a positive result for *C. jejuni*

CONCLUSION

In this study, *C. jejuni* was detected in 100% (10/10) of pig fecal samples. However, the small sample size and non-randomized sampling design limit generalizability. Larger, randomized studies are needed to determine the true prevalence of *C. jejuni* in this region.

AUTHORS CONTRIBUTION

A.R.T. and M.U.E.S. conducted sample collection, microbiological examinations, biochemical identification tests, and data analysis, and prepared the initial manuscript draft. E.T. contributed to the study conceptualization, supervision of the research, interpretation of the findings, and critical revision of the manuscript as the corresponding author. F.R.L. assisted in laboratory procedures, data validation, and manuscript editing. All authors read and approved the final version of the manuscript.

"The author declares that there is no conflict of interest with the parties involved in this research".

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