Septicemic colibacillosis with concurrent intestinal parasitic infection in piglets

Palagan Senopati Sewoyo[1*], I Putu Cahyadi Putra[2], I Nengah Kerta Besung[3], I Komang Pridayasa[4], I Gede Wiraguna Arsadita[4], I Gusti Ngurah Kade Mahardika[5]

[1]Department of Veterinary Pathology, Faculty of Veterinary Medicine, Udayana University, Denpasar, 80234, Bali, Indonesia

[2]Department of Veterinary Parasitology, Faculty of Veterinary Medicine, Udayana University, Denpasar, 80234, Bali, Indonesia

[3]Department of Veterinary Bacteriology and Mycology, Faculty of Veterinary Medicine, Udayana University, Denpasar, 80234, Bali, Indonesia

[4]Veterinary Professional Education Program, Faculty of Veterinary Medicine, Udayana University, Denpasar, 80234, Bali, Indonesia

[5]Department of Veterinary Biomedical and Biomolecular, Faculty of Veterinary Medicine, Udayana University, Denpasar, 80234, Bali, Indonesia

ORCID author 1: https://orcid.org/0000-0002-8713-7725

ORCID author 2: https://orcid.org/0000-0001-7620-5334

ORCID author 3: https://orcid.org/0000-0002-5285-592X

ORCID author 4: https://orcid.org/0009-0003-0768-9592

ORCID author 5: https://orcid.org/0009-0001-8433-7852

ORCID author 6: https://orcid.org/0000-0001-5525-0793

***Corresponding author:**

Palagan Senopati Sewoyo, DVM, MS.

Department of Veterinary Pathology, Faculty of Veterinary Medicine, Udayana University, Denpasar, 80234, Bali, Indonesia.

Email: senopati.sewoyo@unud.ac.id

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ABSTRACT

Colibacillosis is an important cause of morbidity and mortality in pigs, particularly in young animals, and its severity may be exacerbated by concurrent enteric infections. This report describes two cases of septicemic colibacillosis in a 10-day-old suckling piglet and an 8-week-old weaning piglet from Bali, Indonesia. Clinical signs included diarrhea, dehydration, weakness, reduced activity, anorexia, emaciation, pale mucous membranes, and respiratory distress. Fecal examination revealed concurrent parasitic infections, consisting of *Entamoeba* spp. in the suckling piglet and mixed infection with *Entamoeba* spp., *Balantioides coli*, and *Trichuris suis* in the weaning piglet. Following death, complete necropsy, histopathological, and bacteriological examinations were performed. Gross pathological findings were characterized by multisystemic necrohemorrhagic and inflammatory lesions. Histopathological examination revealed lesions consistent with septicemia, including meningitis, bronchopneumonia, pericarditis, perihepatitis, nephritis, and enterocolitis of varying severity. In the suckling piglet, ulcerative colitis with *Entamoeba* spp. trophozoites was observed. In the weaning piglet, severe typhlocolitis accompanied by *B. coli* trophozoites was identified. *Escherichia coli* was consistently isolated from multiple organs in both piglets, confirming the diagnosis of septicemic colibacillosis.

Keywords: balantidiosis, coinfection, entamoebiasis, piglets, septicemia

1. Introduction

Pig breeding farms face several challenges, one of which is the management of newborn or neonatal piglets. Neonatal piglets are highly susceptible to various health problems, including diarrhea. Diarrhea in young pigs may be caused by several agents such as *Escherichia coli*, rotavirus, porcine epidemic diarrhea virus, porcine circovirus type 2 (PCV-2), transmissible gastroenteritis virus (TGEV), gastrointestinal helminth such as *Trichuris suis*, *Oesophagostomum dentatum*, and gastrointestinal protozoan parasites such as *Entamoeba* spp. and *Balantioides coli* [1, 2].

E. coli infection in pigs, also known as swine colibacillosis, causes a broad spectrum of diseases ranging from diarrhea to systemic illness [3, 4]. Transmission is thought to occur through infected pigs, contaminated housing, feed, water, and aerosols [3]. In systemic cases, the primary disease commonly affects piglets younger than four days old, whereas in older pigs it is generally considered a secondary disease associated with immunodeficiency triggered by other infectious diseases [4]. A study by Ippolito *et al.* [5] reported that primary systemic cases may also occur in post-weaning piglets. In its systemic form, the clinical signs may occur with or without diarrhea. Systemic forms are typically caused by extraintestinal pathogenic *E. coli* (ExPEC). These strains are not enteropathogenic; however, when ExPEC successfully reaches organs beyond the intestinal tract, it can induce severe and often fatal disease [6]. Infection with other enteric agents is suspected to serve as one of the entry routes for ExPEC into the bloodstream. Concurrent infection with other pathogens may further exacerbate the clinical condition. Based on the available literature, reports describing septicemic colibacillosis with concurrent intestinal parasitic infection in pigs remain limited, particularly those providing detailed pathological descriptions. In their study, Herman *et al.* [7] only described the characteristics of *E. coli* isolated from pigs affected by septicemic colibacillosis. Although pathological studies remain limited, molecular investigations of *E. coli* strains causing septicemia in pigs have been widely reported in China [8, 9, 10]. In Indonesia, particularly in Bali, where pigs represent one of the primary sources of animal protein and the pig farming industry is well developed, reports on this diseases remain limited. This case report presents detailed pathological findings from two piglets diagnosed with septicemic colibacillosis and concurrent intestinal parasitic infections,

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providing insights into the disease in a regional setting where information remains scarce.

2. Case Presentation

Case 1: A 10-day-old suckling female piglet was raised on a semi-intensive pig farm located in Apuh Village, Tegallalang District, Gianyar Regency, Bali, Indonesia. According to the owner, the piglet had experienced persistent yellowish-white diarrhea since three days of age, accompanied by reduced appetite, poor body condition, dehydration, weakness, and reduced activity. In addition to the affected piglet, eight piglets from different sows exhibited similar clinical signs, and five of them had died within the previous three days. None of the pigs on the farm had been vaccinated against any disease. The farm housed a total of 227 pigs, consisting of 163 suckling piglets, 55 sows, and nine boars, resulting in a morbidity rate of 4.9% (8/163), a mortality rate of 3.0% (5/163), and a case fatality rate (CFR) of 62.5% (5/8). At the time of the outbreak, the area was experiencing heavy rainfall, which increased the humidity levels within the housing facilities.

Case 2: An 8-week-old weaned Landrace pig originating from a smallholder farm in Perean Tengah Village, Baturiti District, Tabanan Regency, Bali, Indonesia, was presented for examination. According to the owner, the pig had been ill for approximately 15 days. Clinical signs included lethargy, physical weakness, depression, anorexia, severe dehydration, and emaciation. The farmer had administered enrofloxacin antibiotic and metabolic supplements for five days; however, the animal's condition continued to deteriorate and eventually resulted in death. The farm maintained a homogeneous population of weaned pigs without breeding sows or boars, with a total population of 127 animals. The pigs were housed in a closed-house system with concrete flooring, and biosecurity measures were routinely implemented through regular disinfection. The reported morbidity rate was 2.36% (3/127), the mortality rate was 1.57% (2/127), and the CFR reached 66.7% (2/3). The affected group had previously received vaccination against classical swine fever (CSF) at 21 days of age.

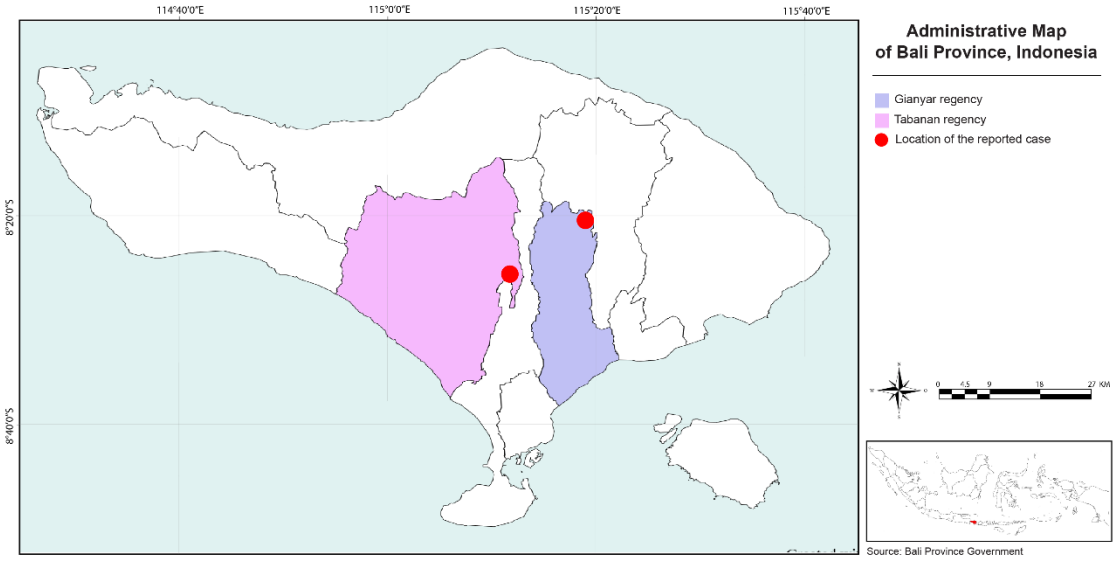


Figure 1. Geographic location of the reported case

Table 1. Results of fecal examination in two piglets with septicemic colibacillosis

Parasite	Stage	Case 1	Case 2
Entamoeba spp.	Cyst	Positive, 198,000 CPG	Positive, 213,500 CPG
Balantioides coli	Cyst, Trophozoite	Negative	Positive, 107,000 CPG
Trichuris suis	Egg	Negative	Positive, 500 EPG

Abbreviation: CPG=Cyst per gram; EPG=Egg per gram

Before death, fecal examinations were performed in both cases using direct smear, sedimentation, and quantitative Stoll's methods. In the first case, the fecal examination revealed infection with *Entamoeba* spp. In the second case, *T. suis* eggs, *B. coli* trophozoites and cysts, and *Entamoeba* spp. cysts were identified (Figure 2). Detailed fecal examination results are presented in Table 1. Postmortem pathological examination revealed lesions in multiple organs. Congestion was observed in the brain, while swelling was noted in the heart, liver, and kidneys. Catarrhal inflammation was present in the small intestine, accompanied by ulcerative hemorrhages in the colon and cecum. Gastrointestinal distension was also observed. In the second case, *T. suis* was detected

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macroscopically on the cecal mucosal surface, while button-like ulcers were present in the colon.

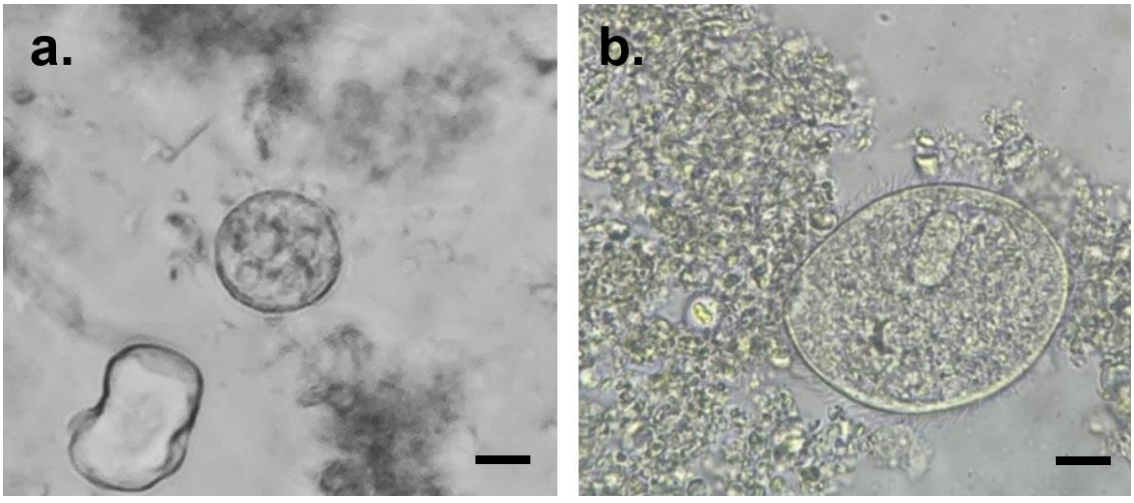


Figure 2. (a) *Entamoeba* spp. cyst and (b) *Balantioides coli* trophozoite, obtained using the sedimentation method from both cases (Scale bar = 10 µm).

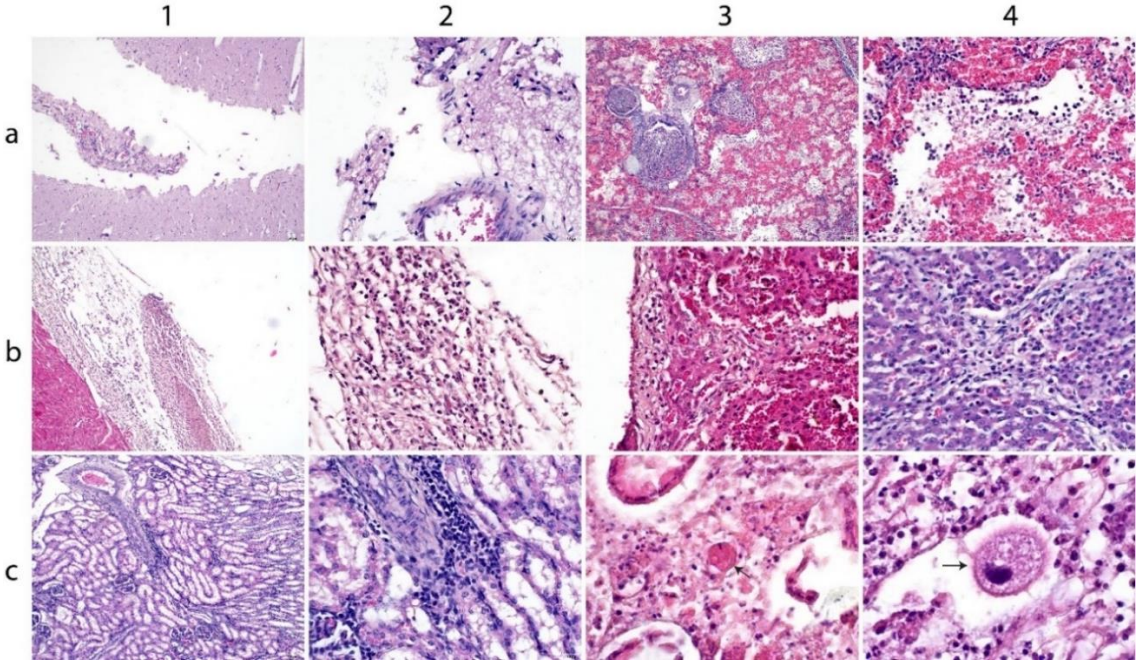


Figure 3. Photomicrographs of organs from piglets with septicemic colibacillosis. Both piglets exhibited multisystemic lesions consistent with septicemia, including (a1-2) meningitis, (a3-4) bronchopneumonia, (b1-2) pericarditis, (b3-4) perihepatitis and interlobular hepatitis, and (c1-2) interstitial nephritis. (c3) *Entamoeba* spp. trophozoites

(arrow) were observed infiltrating the lamina propria of the colon in Case 1. (c4) *Balantioides coli* trophozoites with kidney-shaped nucleus (arrow) invading the lamina propria were observed in the colon of Case 2. (Hematoxylin-Eosin).

Histopathological examination revealed meningitis, mild esophagitis, and mild tracheitis accompanied by epithelial desquamation, hemorrhagic bronchopneumonia, pericarditis, interlobular hepatitis in case 1, and perihepatitis in case 2, interstitial nephritis, and ulcerative colitis with the presence of *Entamoeba* spp. trophozoites in the first case and *B. coli* trophozoites in the second case (Figure 3). Inflammatory cell infiltration was predominantly composed of neutrophils and lymphocytes, with occasional macrophages. Bacteriological culture of the brain, heart, lungs, kidneys, and intestinal tissues, together with Gram staining, revealed bacterial characteristics consistent with *E. coli* in both cases. Given the sporadic outbreak of CSF in Bali, and to exclude the possibility of this disease, a real-time reverse transcription polymerase chain reaction (rRT-PCR) assay targeting classical swine fever virus (CSFV) was performed using primers 5′-CCCTGGGTGGTCTAAG-3′ (forward) and 5′-CATGCCCTCGTCCAC-3′ (reverse), together with a fluorogenic probe 5′-FAM-CCTGAGTACAGGACAGTCGTCAGTAGTTTAMRA-3′, under real-time thermocycler conditions as previously described by Kardena *et al.* [11]. The rRT-PCR results for CSFV were negative.

3. Discussion

The pathological and histopathological findings indicate a systemic disease process. This is supported by the isolation and identification of *E. coli* from multiple organs, consistent with a diagnosis of septicemic colibacillosis. The *E. coli* strains known to cause septicemia belong to ExPEC. Porcine ExPEC is considered a relatively emerging pathotype and exhibits virulence characteristics similar to avian and human ExPEC strains, both of which are capable of causing septicemia [12]. Due to its similarity to human strains, porcine ExPEC also carries zoonotic potential [6]. However, the mechanisms underlying the colonization of porcine ExPEC in the bloodstream of pigs remain unclear and require further investigation [13]. More specifically, porcine ExPEC

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of serogroup O11 is commonly associated with septicemia or meningitis in pigs, whereas serogroup O101 is more frequently detected in pigs with respiratory disease [10]. Antigens from these serogroups have also been reported to cause extraintestinal infections in cattle and humans [14]. Another *E. coli* pathotype commonly affecting piglets is enterotoxigenic *E. coli* (ETEC), which are associated with diarrhea [15]. A limitation of this case report is that molecular-level identification was not performed, and therefore the exact *E. coli* strain could not be confirmed. Nevertheless, based on the systemic nature of the lesions, the involvement of ExPEC is strongly suspected. The observed diarrhea may have been caused by *E. coli* itself and potentially exacerbated by concurrent enteric pathogens such as *Entamoeba* spp., *B. coli*, and *T. suis*. These enteric agents are known to increase intestinal permeability and cause mucosal damage, thereby potentially acting as a portal of entry for ExPEC into the systemic circulation.

As previously described, systemic *E. coli* infection in pigs is generally considered a primary condition in piglets younger than four days of age. In addition, it is often associated with other infectious diseases that induce immunodeficiency [4]. Based on the epidemiological profile showing relatively low morbidity and mortality rates, the likelihood of a primary viral enteric agent may be reduced, as viral outbreaks typically present with higher incidence rates. However, in this case report, no PCR testing was performed for common viral enteric pathogens in piglets; therefore, this cannot be definitively confirmed. Potential viral agents that may act as primary causes of the diarrhea include rotavirus, porcine epidemic diarrhea virus, PCV-2 and TGEV.

Entamoeba spp., particularly *E. histolytica*, *E. polecki*, and *E. suis*, are commonly found in pigs [16, 17]. Most of these species are generally considered non-pathogenic. However, *E. suis* and *E. polecki* have been reported to cause severe enteritis and even hemorrhagic colitis through invasion of the lamina propria [16]. It has been suggested that mixed infections are a major contributing factor to severe disease associated with *Entamoeba* spp. [17, 18, 19]. Transmission occurs via contaminated water or feed containing mature cysts, which excyst in the stomach and subsequently develop into invasive trophozoites in the intestine [20]. Komatsu *et al.* [1] reported that co-infection of *Entamoeba* spp. with other pathogens is associated with diarrhea, such as *Salmonella* spp. and *Lawsonia intracellularis*. These two agents have been suggested to play an

important role in facilitating invasion of *Entamoeba* into the intestinal mucosa.

B. coli is a commensal protozoan found in pigs, humans, and primates. It typically resides in the large intestine, particularly the ceco-colonic region [21]. However, this protozoan can become pathogenic when tissue damage is present due to other microorganisms. Severe infections, including colitis and mucosanguineous diarrhea, have been reported in immunocompromised animals [22]. Cho *et al.* [23] reported that co-infection with *Eimeria* spp., *Trichuris* spp., and *Strongyloides* spp. in sheep resulted in severe enteritis associated with *B. coli*. Gross pathological findings typically include white ulcerative nodules on the colonic mucosa in pigs infected with *B. coli*, resulting from mucosal invasion [24]. This damage is attributed to proteolytic enzymes such as hyaluronidase produced by the organism, leading to degradation of the extracellular matrix. Other lesions that may be observed include hemorrhage, peritonitis, and even intestinal perforation [25]. In the present case, button-like ulcerative lesions resembling those seen in chronic CSF and salmonellosis caused by *Salmonella typhimurium* were observed. However, PCR testing for CSF virus was negative, and bacterial culture did not yield *Salmonella* spp., suggesting that these lesions may were caused by invasion of *B. coli*.

The findings in this case report are particularly important in the regional context of Bali, Indonesia. This region is predominantly Hindu, where pork represents one of the main sources of animal protein. In addition to its role in human consumption, pigs also hold significant socio-cultural value within the local community, contributing to both economic activities and religious ceremonies [11]. In China, the pig industry is one of the major livestock sectors and a primary source of animal protein, and it has been extensively studied. Porcine ExPEC has been reported in pigs in China with a prevalence of 10.1% (315/3,127) [10]. Given the importance of the pig industry in this region, further studies on the distribution of porcine ExPEC is warranted due to its potential public health risks.

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Authors' Contribution

Writing original draft preparation: Palagan Senopati Sewoyo

Methodology: Palagan Senopati Sewoyo, I Putu Cahyadi Putra, I Nengah Kerta Besung

Analysis and interpretation of data: Palagan Senopati Sewoyo, I Putu Cahyadi Putra, I Komang Pridayasa, I Gede Wiraguna Arsadita

Investigation: Palagan Senopati Sewoyo, I Putu Cahyadi Putra, I Komang Pridayasa, I Gede Wiraguna Arsadita

Supervision and critical revision for important intellectual content: I Nengah Kerta Besung, I Gusti Ngurah Kade Mahardika

Ethics

Not applicable.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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