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Prevalence of Gastrointestinal Protozoa Infection in Piglets on Pig Breeding Farms in Gianyar Regency, Bali

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Abstract. Gastrointestinal protozoan infections, including those caused by *Eimeria* sp., *Cystoisospora* sp., *Entamoeba* sp., and *Balantidium* sp., can result in significant livestock losses. This study aimed to ascertain the prevalence of gastrointestinal protozoan infections and compare the prevalence rates between suckling and weaned piglets on pig breeding farms in Gianyar Regency. A total of 216 piglet fecal samples were analyzed, comprising 87 samples from the suckling phase and 129 from the weaned phase. Gastrointestinal protozoa were identified through morphological examination, utilizing the sedimentation method for *Balantidium* sp. and *Entamoeba* sp., and the saturated salt flotation method for Coccidia examination. Differences in the prevalence of protozoan infections between suckling and weaned piglets were assessed using the Chi-Square Test with IBM SPSS Statistics. The findings indicated that the prevalence of gastrointestinal protozoan infections in piglets on breeding farms in the Gianyar Regency was 51.85% (112/216). The identified protozoa included *Balantidium* sp. (37.96%), *Entamoeba* sp. (25.92%), and Coccidia (7.87%). The prevalence of gastrointestinal protozoan infections in suckling piglets was 35.63% (31/87), whereas that in weaned piglets was 62.79% (81/129), demonstrating a significant difference (p=0,0001). There were diverse infections, with some piglets harboring more than one protozoan type. The prevalence rate of gastrointestinal protozoan infections was deemed high, with weaned piglets exhibiting greater susceptibility to infection than suckling piglets.

Keywords: *Balantidium* sp.; Coccidia; *Entamoeba* sp.; suckling piglet; weaning piglet

I. INTRODUCTION

Pig farming in Bali plays a significant role in the community. Beyond consumption, many Balinese individuals engage in pig ownership because of sociocultural factors, traditional customs, and economic enhancement [1]. As a livestock commodity, pigs offer the advantage of producing numerous offspring (polytocous) and exhibiting high adaptability [2]. The quality of pig breeding is critically influenced by the care and management of piglets, as the early phase of a pig's life is crucial and affects its long-term development [3]. A deficiency in public knowledge regarding effective livestock maintenance results in suboptimal livestock development [4].

The selection of optimal pig breeds constitutes a fundamental step in ensuring the success of a farming enterprise [5]. Gianyar, recognized as one of the largest pig-producing regions in Bali, reported a pig population of 85,579 heads according to data from the BPS in 2022 [6]. The pig breeding operations in Gianyar are intended for distribution throughout Bali in subsequent stages. The piglet production process encompasses care from the suckling to the weaning phase. Effective selection, care, and management of piglets significantly impacts both the number of animals born and their quality in subsequent production stages [7]. Piglets are devoid of antibody protection, rendering them susceptible to various factors that may compromise their health and welfare [8]. Preventative measures against disease are deemed more effective in maintaining piglet health than in treating already sick animals [9].

Gastrointestinal protozoan infections have been shown to adversely affect piglet health [10]. Notable protozoa that can infect pigs include *Entamoeba* sp., *Balantidium* sp., and *Isospora* sp. [11]. A study by Agustina et al [10] involving 250 piglet fecal samples from farms in Bali Province reported a 91.6% prevalence of protozoan infections. The protozoa identified were Amoeba (82.6%), *Balantidium* sp. (61.2%), and *Eimeria* sp. (54%). Another study by Widisuputri et al [12], reported a 100% prevalence of gastrointestinal protozoan infections in pigs in Bali, with the following protozoa identified: *Entamoeba* sp. (99%); *Balantidium* sp. (79%), *Isospora suis* (6.8%), *Eimeria* sp. (78%), and *Blastocystis* sp. (69%), using samples from pig groups aged < 6 months and > 6 months. Recent research by Pinatih et al [13], indicated a 94.8% prevalence of gastrointestinal protozoan infections in pigs in Bali based on 117 samples. The protozoa identified were *Eimeria* sp. (90.5%), *Entamoeba* sp. (26.4%), *Isospora suis* (6.8%), and *Balantidium* sp. (5.1%), with risk factors including sex, feed, and housing management.

Gastrointestinal parasitic infections impose a significant economic burden on pig farmers [14]. These parasites can lead to substantial financial losses, often unnoticed by farmers, owing to the absence of overt clinical symptoms [15]. Such losses result in reduced production, which is attributable to inhibited livestock growth and increased operational costs [16]. Gastrointestinal protozoan infections, including those caused by *Eimeria* sp., *Cystoisospora* sp., *Entamoeba* sp., and *Balantidium* sp., can be fatal to piglets [17], [18].

According to the research conducted by Agustina et al. [10], Widisuputri et al [12], and Pinatih et al [13] The prevalence of protozoan infections in pigs is > 90%. This can adversely affect pig health, leading to reduced production and potential economic loss. However, these prior studies have not specifically examined the differences in the prevalence of gastrointestinal protozoan infections between weaning- and suckling-phase piglets. Consequently, this study is crucial for determining differences in the prevalence of gastrointestinal protozoan infections between suckling and weaning-phase piglets, which can serve as a foundation for selecting management and therapeutic strategies on pig breeding farms in the Gianyar Regency.

II. METHODS

A. Research Location and Duration

The sampling sites for this investigation were distributed across seven districts within Gianyar Regency, Bali Province (Figure 1), namely Blahbatuh, Sukawati, Gianyar, Ubud, Payangan, Tegallalang, and Tampaksiring. The samples were analyzed at the Parasitology Laboratory of the Faculty of Veterinary Medicine, Udayana University, Denpasar. The research was conducted from December 2024 to February 2025.

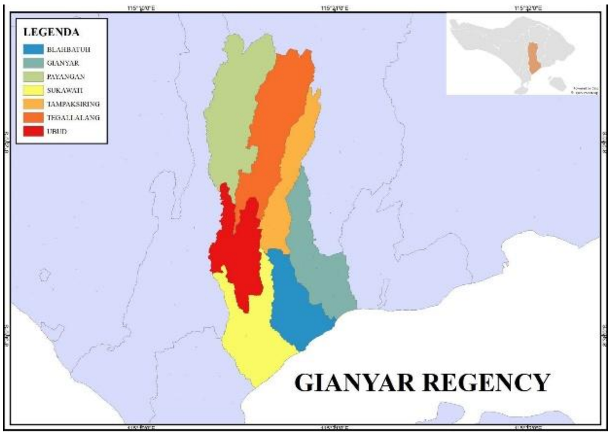

Figure 1. Map of the Research Location in Gianyar Regency.

B. Research Design

This observational study employed a cross-sectional design to ascertain the prevalence of gastrointestinal protozoan infections in piglets at breeding farms within Gianyar Regency. The sampling methodology used was purposive sampling, with the stipulation that the sampling locations must be within the Gianyar Regency. The samples were collected from piglet feces during both the suckling and weaning phases.

Sample Collection

The study used 216 piglet fecal samples, comprising 87 from the suckling phase and 129 from the weaning phase. These samples were collected from 17 farms situated in Gianyar Regency. The fecal samples were freshly collected using gloves and subsequently placed into sample bags containing 10% formalin for analysis via the sedimentation method and into separate sample bags containing 2.5% potassium dichromate for analysis via the flotation method. To facilitate the examination process, the samples were coded and labeled with numbers, the regional origin, the farmer's name, and the collection time. Samples preserved in 10% formalin were available for immediate examination, whereas those preserved in potassium dichromate required approximately seven days before analysis could commence.

C. Sample Examination

Fecal samples preserved in 10% formalin were analyzed by sedimentation, while those in 2.5% potassium dichromate were analyzed by flotation. Morphological identification was conducted by previous studies [19], [20], [21].

The sedimentation method was conducted as follows: approximately 3 g of fecal matter was placed into a glass beaker and combined with 30 ml of distilled water. The mixture was stirred until homogeneous, then filtered. The resulting filtrate was transferred to a centrifuge tube, brought to a final volume of 10 ml, and centrifuged at

1,500 rpm for 5 min. After centrifugation, the supernatant was discarded, leaving only the sediment. A small portion of the sediment was then placed on a microscope slide, mixed with distilled water, and covered with a cover glass. The sample was examined under a microscope at magnifications of 100x and 400x [22].

The flotation method was conducted as follows: approximately 3 g of fecal matter was placed into a beaker, combined with 10 ml of distilled water, stirred until a homogeneous mixture was achieved, and subsequently filtered. The resulting filtrate was transferred to a centrifuge tube to a final volume of 10 ml and centrifuged at 1,500 rpm for 5 min. Following centrifugation, the tube was removed, and the supernatant was discarded, retaining only the sediment. The sediment was then combined with 10 ml of saturated NaCl solution and centrifuged again at 1,500 rpm for 5 min. The centrifuge tube was positioned upright in a test tube rack. Using a Pasteur pipette, an additional saturated NaCl solution was added until a convex meniscus was formed at the surface. After allowing the flotation process to proceed for 5 min, a cover glass was placed on the surface. The cover glass was subsequently lifted and placed on a microscope slide for examination.

The samples were observed under a microscope at magnifications of 100x and 400x [22]. Protozoan morphological identification was performed utilizing the references provided in [21]. To distinguish between cysts of *Balantidium* sp. and *Entamoeba* sp., identification was conducted based on nuclear morphology and measurements obtained using the ImageJ application (https://imagej.net/ij/). Photographs of the prepared samples were uploaded, and the cyst diameters were measured. The diameter of *Balantidium* sp. cysts ranges from 40 to 60 µm, whereas that of *Entamoeba* sp. cysts ranges from 4 to 17 µm [20].

D. Data Analysis

The data analysis in this study employed descriptive methods, with results presented in figures and tables to ascertain the prevalence and types of gastrointestinal protozoan infections based on examinations of piglet fecal samples. The prevalence of gastrointestinal protozoan infection was calculated using the prevalence formula: dividing the number of protozoa-positive fecal samples by the total number of samples examined, then multiplying by 100%. The difference in the prevalence of gastrointestinal protozoan infections between suckling and weaning phase piglets was assessed using the Chi-Square Test, conducted with the IBM SPSS Statistics software. A significant difference in prevalence was indicated by a p-value <0.05 [23].

III. RESULTS AND DISCUSSION

This study analyzed 216 piglet fecal samples, comprising 87 from the suckling phase and 129 from the weaning phase. These samples were obtained from 17 farms across seven districts in Gianyar Regency. The findings indicated a prevalence of gastrointestinal protozoan infection in piglets at breeding farms in the Gianyar Regency of 51.85% (112/216), with a prevalence of 35.63% (31/87) in suckling piglets, and 62.79% (81/129) in weaning piglets. Morphological identification revealed the presence of *Balantidium* sp., Coccidia, and *Entamoeba* sp. in piglets at breeding farms in Gianyar Regency, with prevalence rates of 37.96% (82/216), 25.92% (56/216), and 7.87% (17/216), respectively (Fig.2). Further analysis of Coccidia oocyst-positive samples showed that the positive samples comprised unsporulated oocysts (7.4%), *Eimeria* sp. (15.27%), and *Cystoisospora* sp. (6.48%) (Table 1).

The prevalence of gastrointestinal protozoan infection among piglets on breeding farms in the Gianyar Regency was 51.85%. This outcome was influenced by the condition of the pens on these farms, where husbandry management practices were either semi-intensive or intensive. Typically, these farms maintain more than seven pigs and clean the pens one to two times daily using brooms, shovels, and water sprays sourced from boreholes, regional drinking water companies (PDAM), or springs. The floors were constructed using cement or iron, and the introduction of new pigs was rare. Improved barn management practices may reduce the prevalence of infection.

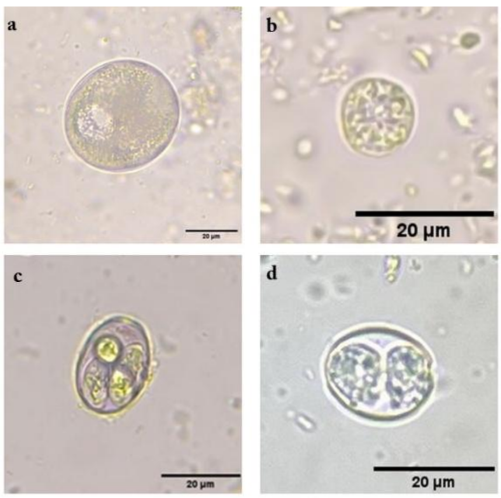

Figure 2. Identification of Gastrointestinal Protozoa in Piglets at Breeding Farms, (a) *Balantidium* sp. showing a visible macronucleus, (b) *Entamoeba* sp. observed with multiple nuclei, (c) *Eimeria* sp. displaying four sporocysts, and (d) *Cystoisospora* sp. containing two sporocysts (Scale=20 µm).

The diversity of gastrointestinal protozoan infection in piglets was most prevalent in single infection (35%) compared to double infection (14%) and triple infection (3%). The diversity of gastrointestinal protozoan infections among single infections was dominated by Balantidium sp. (21.75%), and double infections were dominated by a combination of Balantidium sp. and Coccidia (11.57%) (Table 2).

TABLE 1.
PREVALENCE OF GASTROINTESTINAL PROTOZOA INFECTIONS IN PIGLETS AT BREEDING FARMS IN GIANYAR REGENCY

Infection	Suckling Pigs		Weaning Pigs		Overall Prevalence		p-value
	n	Prev (%)	n	Prev (%)	n	Prev (%)	
Protozoa	31	35.63	81	62.79	112	51.85	0.0001
Balantidium sp.	15	16.09	62	52.71	77	37.96	0.0001
Entamoeba sp.	5	5.74	17	9.3	22	7.87	0.341
Coccidia	19	19.54	44	30.23	63	25.92	0.079
Unsporulated oocyst	3	3.44	13	10.07	19	7.4	0.068
Eimeria sp.	11	12.64	22	17.05	33	15.23	0.377
Cystoisospora sp.	5	5.74	9	6.97	14	6.48	0.719

TABLE 2
DIVERSITY OF GASTROINTESTINAL PROTOZOA INFECTIONS IN PIGLETS AT BREEDING FARMS IN GIANYAR REGENCY

Protozoa	Suckling Pigs		Weaning Pigs		Overall Prevalence (%)
	n	Prev (%)	n	Prev (%)	
Balantidium sp.	11	12.64	36	27.9	21.75
Entamoeba sp.	3	3.44	2	1.55	2.31
Coccidia	12	13.79	11	12.64	10.64
Balantidium sp. & *Entamoeba* sp.	0	0.00	4	3.1	1.85
Balantidium sp.+*Coccidia*	3	3.44	22	17.05	11.57
Entamoeba sp.+*Coccidia*	2	2.29	36	0.00	0.92
Balantidium sp.+*Entamoeba* sp.+*Coccidia*	0	0.00	2	4.65	2.77

The prevalence observed in this study was lower than that reported in previous Bali studies by Agustina et al. [10] à 91,6 %, Widisuputri et al. [12] at 100%, and Pinatih et al. [13] at 94,8%. This discrepancy may be attributed to the different inspection methods and pig pen management systems employed. Agustina et al. [10] utilized the Ritchie method, whereas Widisuputri et al. [12] and Pinatih et al. [13] employed the floating method with saturated sucrose. These methodological differences may account for the variation in the results. Furthermore, the housing management of the samples in the study by Pinatih et al. [13] involved traditional and semi-intensive systems, whereas in the Gianyar Regency, semi-intensive and intensive systems were used. Traditional housing systems can increase the susceptibility of pigs to parasitic infections [24]

The prevalence of infection among piglets during the weaning phase was markedly higher than that during the suckling phase (p=0.0001). The weaning process introduces environmental and psychosocial stressors that adversely affect gastrointestinal health, thereby increasing the susceptibility of piglets to diarrhea and infection [25], [26], [27]. Protozoan transmission occurs through coprophagia, which involves the transfer of oocytes from the feces present in the environment [28]. During the weaning phase, the farmer directly feeds the piglets, which increases their risk of exposure to fecal contamination, particularly if manure accumulates around the barn.

The prevalence of *Balantidium* sp. infection in piglets during the weaning phase (27.9%) was significantly higher (p=0.0001) than that during the suckling phase (12.64%) (Table 1). This increased prevalence is attributed to weaning stress, which disrupts the balance between gut microbiota and metabolism, thereby heightening the susceptibility to *Balantidium coli* infection [29]. Weaned piglets are particularly vulnerable, with a substantial presence of trophozoites in their feces [18]. Transmission occurs through the ingestion of food or water contaminated with cysts [30], including via coprophagia, as cysts are present in the feces. During this phase, piglets begin to consume food and water independently, thus increasing their risk of infection. The present study determined a *Balantidium* sp. prevalence of 37.96% in piglets on farms located in Gianyar Regency. This prevalence is notably lower than that reported in previous studies conducted in Bali by Widisuputri et al. [12] and Agustina et al. [10], who documented prevalence rates of 83% and 61.2%, respectively. The observed discrepancy may be attributed to enhanced cage sanitation and improved water source quality, such as boreholes, PDAMs, or springs that may be susceptible to sewage contamination. Furthermore, variations in detection methodologies may influence the results [10], [12].

The prevalence of *Entamoeba* sp. infection in suckling (5.74%) and weaning (9.3%) piglets did not differ significantly (p=0.341). This prevalence was also lower than that of other protozoa (7.87%) (Table 1), potentially attributable to environmental factors that are critical in parasitic infections [19]. In Gianyar Regency, the pens were generally clean, featuring cement or iron floors, and the bedding was dry and well-maintained. This prevalence was considerably lower than that reported by Agustina et al. (82.4 %) [10]. This discrepancy may be due to differences in the examination methods and sampling locations. This study employed Ritchie's method with sodium acetate-acetic acid-formalin (SAF) solution, which may offer greater efficacy [31]. Additionally, samples were obtained from traditional markets, where piglet contact is high, thereby increasing the risk of infection. This is supported by the findings of Jasman et al. [32] for Jambi City, who documented a prevalence of 40% in traditional markets and 25% in modern markets.

The prevalence of Coccidia infection among suckling (19.54%) and weaning (30.23%) piglets was not exhibit a statistically significantly different (p=0.079), with an overall prevalence of 25.92% (Table 1). This relatively low prevalence may be attributed to effective cage management practices in the Gianyar Regency, such as regular cleaning and the use of cement or iron for cage bedding. The identified Coccidia species included unsporulated oocysts (7.4%), *Eimeria* sp. (15.27%), and *Cystoisospora* sp. (6.48%). The presence of unsporulated oocysts may have resulted from low oxygen levels, inappropriate storage temperatures, or insufficient sporulation time in the study samples. Optimal sporulation is achieved at temperatures ranging from 16 to 39°C with adequate oxygen availability [19]. The prevalence of coccidia observed in our study was lower than that reported by Pratiwi et al. [33] in highland Bali, where it was 45.8% among pigs younger than six months. This discrepancy may be attributed to differences in the study location and farm scale. Our research involved sample collection from large-scale farms (housing more than seven pigs per cage) with enhanced biosecurity measures, whereas the study by Pratiwi et al. focused on smaller farms (housing seven or fewer pigs). These findings are corroborated by the research of Bulu et al. [34] in Kupang District.

The diversity of gastrointestinal protozoan infections in piglets was highest in single infections (35%), compared to double infections (14%) and triple infections (3%). Single infections were predominantly caused by *Balantidium* sp. (21.75%), whereas dual infections were primarily characterized by a combination of *Balantidium* sp. and Coccidia (11.57%) (Table 2). This observation contrasts with the findings of Widisuputri et al. [12] in Bali, which indicated that all samples were infected with *Entamoeba* sp. (100%), with multiple protozoan infections occurring in 6% of cases, and three-(18%) and four-type (16%) infections involving combinations of *Entamoeba* sp., *Balantidium* sp., *Blastocystis* sp., and *Eimeria* sp, respectively. A study conducted in Thailand by Thanasuwan et al. [35] documented the prevalence of single (25.31%), double (38.21%), triple (14.81%), and quadruple or quintuple infections (6.79%), although the study did not specify the predominant parasite species. In contrast, a study in Ukraine by Bohach et al. [23] identified single infections (57.4-73.6%) predominantly caused by *Cystoisospora suis* oocysts, as well as multiple infections (22.7-32.1%) and triple infections (9.6-14.9%) in piglets aged 2-4 months. The study also noted that the diversity of infections was influenced by farm size.

As reported by Kochanowski et al. [36], single infections are prevalent in medium to large farms, whereas triple infections are more frequently observed in small farms. Variations in findings across studies may be attributed to differences in examination methods, geographical location, climate, parasite species, and husbandry systems. Matsubayashi et al. [37] highlighted those factors such as age, sex, immunity, sanitation, population density, geographical distribution, and husbandry management also influence the diversity of infections. Understanding the prevalence and diversity of parasites is crucial for comprehending their distribution, ecological dynamics, and interactions with animals [38], [39]. Protozoan infections can adversely affect livestock by reducing productivity, inhibiting growth, increasing medical expenses, and causing mortality [14], [16], [18].

CONCLUSION

Based on the findings of the aforementioned study, the prevalence of gastrointestinal protozoan infection among piglets at breeding farms in Gianyar Regency was notably high at 51.85% (112/216). The gastrointestinal protozoa identified in piglets at these farms included Balantidium sp. (37.96%, 82/216), Entamoeba sp. (7.87%, 17/216), and Coccidia (25.92%, 56/216), indicating a diverse infection profile. The difference in prevalence between suckling piglets (35.63%, 31/87) and weaned piglets (62.79%, 81/129) was statistically significant (p=0.0001), with weaned piglets exhibiting a higher susceptibility to gastrointestinal protozoan infection than suckling piglets.

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