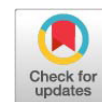


Research Article



Identification of Zoonotic Intestinal Parasites in Madura Cattle in Bangkalan, Madura, Indonesia

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Abstract | Zoonotic intestinal parasites in cattle remain a concern due to their impact on livestock productivity and potential public health implications. This study aimed to determine the prevalence and diversity of zoonotic intestinal parasites in Madura cattle across eight sub-districts in Bangkalan Regency, Indonesia, and to evaluate their distribution according to age, sex, and geographic location. A cross-sectional survey was conducted from March to May 2024 involving 400 fecal samples collected using simple random sampling. Samples were examined using direct smear, sedimentation, and modified Fulleborn flotation techniques. Data were analyzed descriptively and inferentially using Chi-square tests. The overall prevalence of zoonotic intestinal parasites was 29% (116/400; 95% CI: 24.6–33.4%). Five parasite taxa were identified: *Toxocara vitulorum*, *Fasciola gigantica*, *Blastocystis hominis*, *Balantidium coli*, and *Entamoeba* spp. *Blastocystis hominis* was the most frequently detected species (19.5%). Prevalence differed significantly among sub-districts ($p < 0.001$), with the highest rate in Kwanyar (50%) and the lowest in Kokop (16%). Age group was significantly associated with infection ($p < 0.001$), with cattle aged 6 months to 2 years showing the highest prevalence (42.4%). No significant association was found between sex and infection status ($p = 0.839$). Mixed infections were observed in several animals. These findings indicate that zoonotic intestinal parasites remain endemic in Madura cattle and are influenced by geographic and age-related factors. Strengthened routine monitoring, improved sanitation, and targeted control strategies are recommended to reduce infection pressure and potential zoonotic risks.

Keywords | Zoonotic intestinal parasites, Madura cattle, Gastrointestinal helminths, Protozoa, Prevalence, Bangkalan regency

Received | November 04, 2025; **Accepted** | August 28, 2026; **Published** | September 15, 2026

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Citation | Hastutiek P, Lastuti NDR, Mufasirin, Suwanti LT, Ya'cob Z (2026). Identification of zoonotic intestinal parasites in madura cattle in bangkalan, madura, indonesia. Adv. Anim. Vet. Sci., 14(10):2234-2241.

DOI | <https://dx.doi.org/10.17582/journal.aavs/2026/14.10.2234.2241>

ISSN (Online) | 2307-8316



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INTRODUCTION

Madura cattle are a native Indonesian breed primarily raised in East Java, especially on Madura Island. This breed has considerable development potential owing to its genetic adaptability to hot climates and harsh environmental conditions. Madura cattle are resistant to tick infestations, can thrive on low-quality feed, and have lower

feed requirements compared to imported breeds. They are also easy to manage, exhibit high reproductive capacity, and demonstrate strong disease resistance (Nurgartiningih, 2011). As a stabilized crossbreed of Zebu and Banteng (*Bos javanicus*), Madura cattle represent an important genetic resource for Indonesian livestock production. Beef cattle are a primary source of animal protein for communities on Madura Island. According to the Central Statistics Agency

of Bangkalan Regency (2023), the total cattle population across 18 sub-districts reached 63,347 head and continues to increase annually.

As an important agricultural commodity in rural areas, cattle farming requires sound management to ensure optimal growth and productivity. However, small-scale livestock production systems frequently face challenges, including reproductive disorders and chronic parasitic infections. Gastrointestinal parasitism is of particular concern because it can impair growth performance and productivity, resulting in substantial economic losses. These losses include reduced body weight gain, decreased work performance, and lower quality of meat and by-products. Infected cattle may experience weight gain reductions exceeding 40% compared to healthy animals (Sudradjat, 1991). Moreover, several gastrointestinal parasites have zoonotic potential, posing risks not only to animal health but also to public health.

Protozoa and helminths inhabiting the digestive tract are major causes of gastrointestinal disorders in cattle. Protozoan infections disrupt nutrient absorption and damage the intestinal epithelium, thereby reducing digestive efficiency and enzyme production (Zalizar, 2017). Clinical manifestations commonly include diarrhea, weight loss, dehydration, and poor growth performance. Various gas-trointestinal protozoa have been reported in cattle, including *Eimeria* sp., *Balantidium* sp., *Giardia* sp., *Entamoeba* sp., *Cryptosporidium* sp., and *Blastocystis* sp. (Susana et al., 2019). In Bangkalan, Hastutiek et al. (2019) reported a 71.4% prevalence of gastrointestinal protozoa in beef cat-tle. Suwanti et al. (2020) detected *Blastocystis* spp. in 100% of Madura cattle sampled from Socah and Kamal sub-districts using wet mounts and in vitro culture. Another study examining 183 fecal samples using the sugar flotation method found that 32.8% were positive for protozoan and nematode infections (Hastutiek et al., 2022). Additionally, examinations of feces from Madura cattle slaughtered in Surabaya revealed that approximately 60% were infected with helminths and protozoa, including *Fasciola* sp., *Balantidium* sp., and *Entamoeba* spp. (unpublished data). Madura cattle were also reported to have a higher nematode infection rate (20.8%) compared to Balinese (8.3%), PO (4.2%), and Limousin cattle (0%) (Haajidah et al., 2020).

Although previous studies have documented gastrointestinal protozoa in Bangkalan, most investigations focused primarily on protozoan infections in lowland areas and did not comprehensively assess helminths or specifically address zoonotic intestinal parasites. Furthermore, earlier surveys did not systematically compare ecological settings such as coastal and highland sub-districts, nor did they evaluate the diversity of parasites with established zoonotic

potential. Consequently, baseline epidemiological data integrating both protozoan and helminth zoonotic parasites across different agro-ecological zones in Bangkalan remain limited.

The novelty of the present study lies in its comprehensive assessment of both zoonotic protozoa and helminths in farmed Madura cattle across coastal and highland sub-districts in Bangkalan Regency. This study provides the first integrated mapping of zoonotic intestinal parasite prevalence and diversity in key livestock-trading areas of Bangkalan, thereby identifying geographic and age-related patterns of infection that may inform targeted surveillance and control strategies. By focusing specifically on parasites with zoonotic relevance, this research contributes new epidemiological evidence to support One Health-oriented parasite control, biosecurity enhancement, and risk-based monitoring in Madura Island.

Therefore, this study aimed to identify and determine the prevalence and diversity of zoonotic intestinal parasites in Madura cattle across eight sub-districts in Bangkalan Regency, including both coastal and highland areas, and to evaluate their distribution according to sex, age group, and geographic location.

MATERIALS AND METHODS

ETHICAL CONSIDERATIONS

This study was designed as a cross-sectional field-based observational survey involving non-invasive fecal sample collection and structured interviews with cattle owners. No experimental manipulation or invasive procedures were performed on the animals. Fresh fecal samples were collected directly from the rectum using standard hygienic techniques to minimize handling time and stress.

Prior informed consent was obtained from all cattle owners before sample collection and interviews were conducted. The study protocol followed institutional guidelines for field-based animal health surveys and complied with accepted standards for animal welfare. Because the study involved routine diagnostic sampling without experimental intervention, it did not require formal ethical approval for animal experimentation under institutional regulations. All procedures were carried out with the permission of livestock owners and in accordance with good veterinary practice.

STUDY AREA

Bangkalan Regency is located on Madura Island, Indonesia, and covers an area of approximately 1,260 km², consisting of 18 sub-districts. Madura cattle are a stabilized crossbreed of Zebu and Banteng (*Bos javanicus*) originating from

Madura Island. They are typically reddish-brown with characteristic white markings on the legs and rump. Adult bulls generally weigh between 250 and 300 kg (Popescu and Smith, 1988).

The study was conducted in eight sub-districts with relatively large cattle populations, including two coastal sub-districts (Kwanyar and Modung) and six highland sub-districts (Kokop, Konang, Tanah Merah, Galis, Blega, and Geger). Each sub-district was represented by one selected village. Kwanyar and Modung are located in lowland areas at approximately 25 meters above sea level, whereas the remaining sub-districts are situated in highland areas ranging from approximately 25 to 200 meters above sea level (Figure 1).

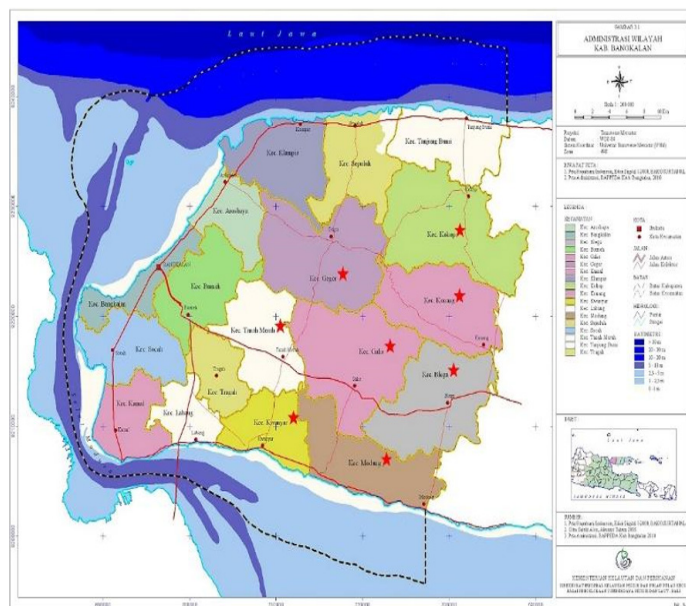


Figure 1: Research area (★).

SAMPLE COLLECTION AND FECAL EXAMINATION

In the eight selected sub-districts, cattle populations ranged from approximately 400 to 850 animals per sub-district. Most farmers owned between one and three cattle. To obtain a total sample size of 400 animals, approximately 50 cattle were sampled per sub-district.

When only one animal was present on a farm, that animal was included in the study. When two or more animals were present, individuals were selected using a simple random sampling procedure. Each animal was assigned a temporary identification number based on its stall position or ear tag, and selection was performed using a random number method to minimize selection bias. This approach ensured that each eligible animal had an equal probability of being included.

Sampling was conducted between March and May 2024. Animals were categorized into three age groups: less than

6 months, 6 months to 2 years, and more than 2 years. Both bulls and cows were included.

In addition to fecal sampling, structured interviews and questionnaires were administered to farmers to collect information on farmer characteristics (age, gender, education level, and farming experience) and herd management practices (number of animals, management system, previous treatments, pen structure and materials, environmental conditions, and sanitation practices).

Fresh fecal samples were obtained directly from the rectum, placed into labeled polyethylene zip-lock bags, and preserved in 2.5% potassium dichromate solution. Samples were transported in an ice box to the laboratory for examination.

Laboratory analysis was performed using direct smear (native), sedimentation, and modified Fulleborn flotation techniques (Mumpuni *et al.*, 2007; Villeneuve *et al.*, 2015). Helminth eggs and protozoan cysts or oocysts were identified based on morphological characteristics using standard identification keys (Soulsby, 1986). No staining or molecular diagnostic techniques were applied. Therefore, parasite identification was limited to morphologically distinguishable taxa and could not determine molecular subtypes or confirm zoonotic genotypes.

An animal was considered positive if helminth eggs or protozoan cysts/oocysts were detected by any of the examination methods. Prevalence was calculated as: $\text{Prevalence (\%)} = (\text{Number of positive samples} / \text{Total number of samples examined}) \times 100$.

DATA ANALYSIS

Data were analyzed descriptively and inferentially. Prevalence was expressed as percentages with 95% confidence intervals (95% CI). Differences in parasite prevalence according to sex, age group, and sub-district were evaluated using the Chi-square (χ^2) test. When expected cell frequencies were less than five, Fisher's exact test was applied. A p-value of less than 0.05 was considered statistically significant. All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 26.0 (IBM Corp., Armonk, NY, USA).

RESULTS

OVERALL PREVALENCE OF ZOONOTIC INTESTINAL PARASITES

Out of 400 fecal samples examined from Madura cattle across eight sub-districts in Bangkalan Regency, 116 samples were positive for zoonotic intestinal parasites, resulting in an overall prevalence of 29% (95% CI: 24.6–

33.4%) (Table 1). The research area and distribution of sampling locations are presented in Figure 1.

Table 1: Prevalence of zoonotic intestinal parasites in Madura cattle by sub-district.

Sub-district	Number of samples	Positive (n)	Prevalence (%)
Konang	50	9	18
Kokop	50	8	16
Blega	50	20	40
Galis	50	18	36
Geger	50	12	24
Tanah Merah	50	15	30
Modung	50	9	18
Kwanyar	50	25	50
Total	400	116	29.00

The highest prevalence was recorded in Kwanyar (25/50; 50%), whereas the lowest prevalence was observed in Kokop (8/50; 16%) (Table 1). The difference in prevalence among sub-districts was statistically significant ($\chi^2 = 24.48$; $df = 7$; $p < 0.001$), as summarized in Table 2.

Table 2: Summary of chi-square analysis for factors associated with zoonotic intestinal parasite infection in madura cattle.

Variable	Pearson Chi-Square (χ^2)	Df	Asymp. Sig. (2-sided)	Interpretation
Sub-district	24.48	7	< 0.001	Significant
Sex	0.04	1	0.839	Not significant
Age group	28.79	2	< 0.001	Significant
N of valid cases	400			

DISTRIBUTION OF PARASITE SPECIES

Five zoonotic gastrointestinal parasite species were identified, consisting of two helminths (*Toxocara vitulorum* and *Fasciola gigantica*) and three protozoa (*Blastocystis hominis*, *Balantidium coli*, and *Entamoeba* spp.) (Table 3). The microscopic morphology of the identified parasites is shown in Figure 2.

Blastocystis hominis was the most frequently detected species, identified in 78 of 400 samples (19.5%). This was followed by *Balantidium coli* (15/400; 3.75%), *T. vitulorum* (6/400; 1.5%), *F. gigantica* (5/400; 1.25%), and *Entamoeba* spp. (4/400; 1.0%) (Table 3).

Single-species infections were more common than mixed infections. Co-infection with *B. hominis* and *T. vitulorum* was observed in eight samples. One animal from Blega sub-district harbored four parasite species simultaneously (*B. hominis*, *B. coli*, *F. gigantica*, and *T.*

vitulorum) (Table 3).

Table 3: Distribution of zoonotic intestinal parasite species detected in Madura cattle (n = 400).

Parasite species	Positive samples (n)	Prevalence (%)
<i>Blastocystis hominis</i>	78	19.5
<i>Balantidium coli</i>	15	3.75
<i>Toxocara vitulorum</i>	6	1.5
<i>Fasciola gigantica</i>	5	1.25
<i>Entamoeba</i> spp.	4	1.0
Mixed infection (<i>B. hominis</i> + <i>T. vitulorum</i>)	8	2.0
Four-species infection	1	0.25

Regarding distribution by sub-district, only one parasite species was detected in Konang, Galis, and Modung. Two parasite species were identified in Kokop, Geger, Tanah Merah, and Kwanyar, while Blega showed the highest diversity, with four different parasite species detected (Table 3).

PREVALENCE BASED ON SEX

Among 90 bulls examined, 25 were positive (27.77%), whereas 91 of 310 cows were infected (29.35%) (Table 4). Statistical analysis revealed no significant association between sex and infection status ($\chi^2 = 0.04$; $df = 1$; $p = 0.839$), as shown in Table 5.

Table 4: Prevalence of zoonotic intestinal parasites based on sex.

Sex	Number of samples	Positive (n)	Prevalence (%)
Bulls	90	25	27.77
Cows	310	91	29.35
Total	400	116	29.00

Table 5: Chi-square analysis of association between sex and zoonotic intestinal parasite infection.

Variable	χ^2	df	p-value	Interpretation
Sex	0.04	1	0.839	Not significant

Chi-square test: $\chi^2 = 0.04$; $df = 1$; $p = 0.839$.

At the sub-district level, the highest prevalence among bulls was observed in Kwanyar (7/9; 77.78%), while the lowest prevalence occurred in Konang, Kokop, and Modung (one positive case each). Among cows, the highest prevalence was also recorded in Kwanyar (18/41; 43.9%), whereas the lowest was found in Kokop (7/40; 17.5%) (Table 4).

PREVALENCE BASED ON AGE GROUP

Age group was significantly associated with parasite infection ($\chi^2 = 28.79$; $df = 2$; $p < 0.001$), as presented in Table 7. Cattle aged 6 months to 2 years showed the highest overall prevalence (42.4%), followed by cattle aged <6 months (35.8%), whereas cattle aged >2 years had the lowest prevalence (15.87%) (Table 6).

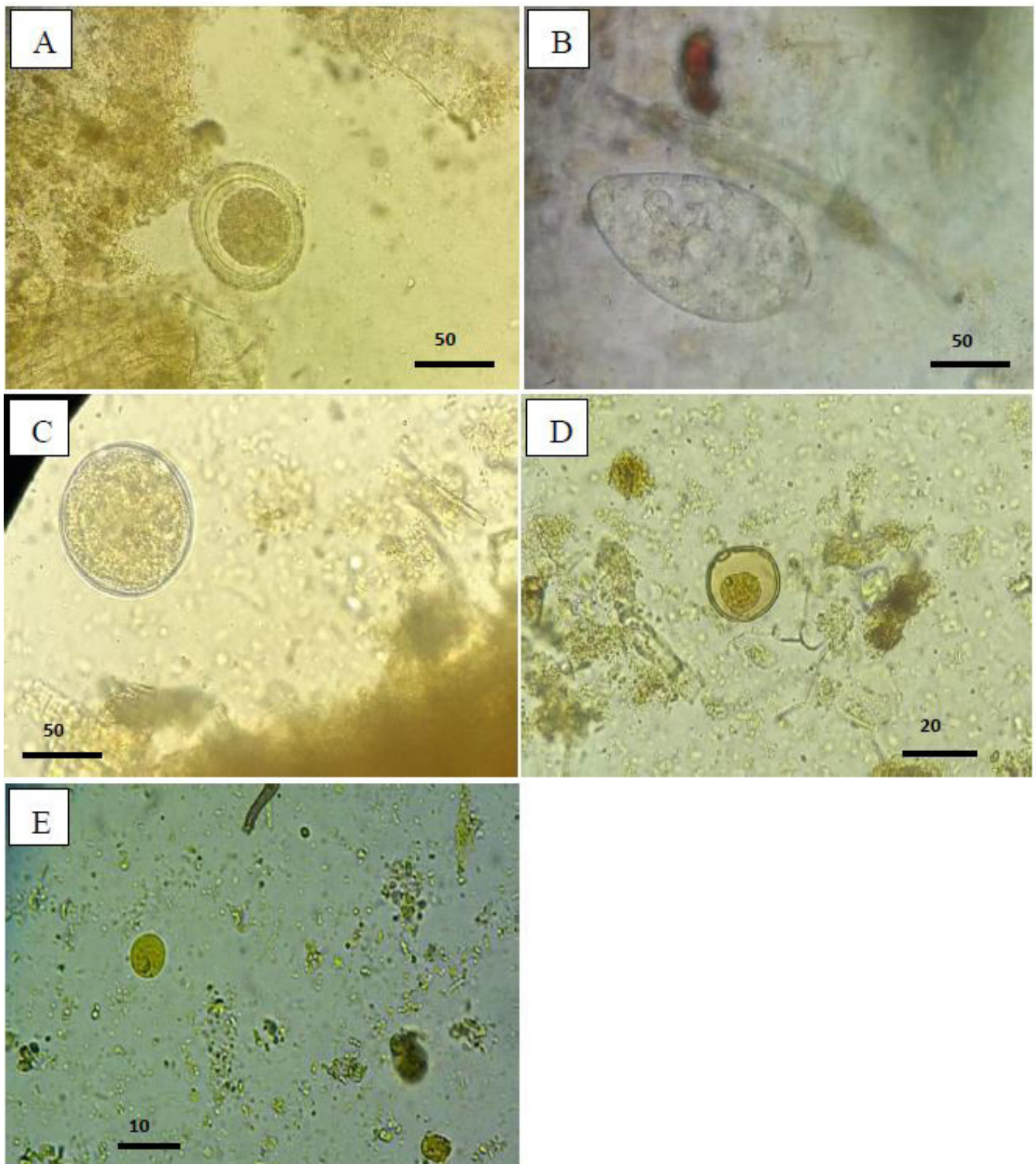


Figure 2: A. *Toxocara vitulorum*, B. *Fasciola gigantica*, C. *Balantidium coli*, D. *Blastocystis hominis* and E. *Entamoeba* spp.

Cattle younger than 6 months showed an overall infection prevalence of 35.8%. Although *T. vitulorum* is biologically relevant in this age group due to potential prenatal and lactogenic transmission, the percentage reported above reflects total zoonotic intestinal parasite infection rather than species-specific prevalence (Table 6).

ENVIRONMENTAL AND MANAGEMENT OBSERVATIONS

Fecal sampling was conducted in areas with varying altitudes. Modung, Blega, and part of Kwanyar are located in lowland areas (<25 meters above sea level), whereas Konang, Kokop, Galis, Geger, and part of Tanah Merah are situated in highland areas (25–200 meters above sea level).

The geographical distribution of these areas is illustrated in Figure 1.

Table 6: Prevalence of zoonotic intestinal parasites based on age group.

Age group	Number of samples	Positive (n)	Prevalence (%)
< 6 months	53	19	35.8
6 months–2 years	158	67	42.4
> 2 years	189	30	15.87
Total	400	116	29.00

Table 7: Chi-square analysis of association between age group and zoonotic intestinal parasite infection.

Variable	χ^2	df	p-value	Interpretation
Age group	28.79	2	< 0.001	Significant

Blega, Galis, Tanah Merah, and Kwanyar function as major livestock gathering and temporary holding points for inter-island trade and religious slaughter during Idul Adha. These sub-districts showed relatively higher infection prevalence compared to others (Table 1).

Field observations indicated suboptimal pen sanitation, limited drainage systems, and improper fecal disposal practices. These conditions were commonly observed across study sites and may contribute to environmental contamination and sustained transmission.

DISCUSSION

This study demonstrated that zoonotic intestinal parasite infections remain present in Madura cattle in Bangkalan Regency, with an overall prevalence of 29%. Although lower than several previously reported studies in Indonesia, this finding confirms the continued circulation of gastrointestinal parasites with zoonotic potential in traditional cattle production systems.

The overall prevalence observed in this study was lower than the 71.4% protozoan infection rate previously reported in coastal areas of Bangkalan (Hastutiek *et al.*, 2019). Differences in prevalence may reflect variations in diagnostic techniques, ecological settings, seasonal factors, and herd management practices. In contrast, the prevalence in the present study was comparable to findings in Jombang, where gastrointestinal parasites were detected in 35% of beef cattle (Ifqiyah *et al.*, 2021), but lower than reports from dairy cattle in Grati, where protozoan infections reached 85% (Margaretha *et al.*, 2024). These variations highlight the influence of environmental and management conditions on parasite transmission dynamics.

Significant differences in prevalence were observed among sub-districts, with Kwanyar showing the highest infection rate (50%) and Kokop the lowest (16%). Several high-prevalence sub-districts, including Blega, Galis, Tanah Merah, and Kwanyar, function as livestock gathering and temporary holding points for inter-island trade and religious slaughter. Increased animal movement and congregation in these areas may facilitate parasite transmission through environmental contamination and close animal contact. However, because movement data were not quantitatively analyzed in this study, this interpretation should be considered epidemiologically plausible rather than causally confirmed.

Five zoonotic parasite taxa were identified: *Toxocara vitulorum*, *Fasciola gigantica*, *Blastocystis hominis*, *Balantidium coli*, and *Entamoeba* spp. Among these, *B. hominis* was the most frequently detected species. The predominance of *Blastocystis* spp. in livestock has also been reported in other regions, and high prevalence has been associated with gastrointestinal disturbance and potential host pathology (Stensvold *et al.*, 2007; Yoshikawa *et al.*, 2009). Reports from Sumbawa indicated protozoan infections in Bali cattle reaching 62%, including *Blastocystis* spp., *Balantidium* spp., and *Entamoeba* spp. (Hamdani *et al.*, 2021), supporting the widespread distribution of protozoan parasites in tropical production systems.

The detection of *Entamoeba* spp. warrants cautious interpretation. Morphological examination does not allow differentiation among species or confirmation of zoonotic genotypes. Although *Entamoeba coli* is generally considered non-pathogenic in humans, its detection—when present—may indicate fecal contamination and suboptimal hygiene conditions (Haidar and De Jesus, 2023). Because molecular characterization was not performed in this study, the specific zoonotic relevance of the detected *Entamoeba* spp. cannot be conclusively determined.

Mixed infections were observed in several animals, including one case harboring four parasite species simultaneously. Polyparasitism may negatively affect nutrient absorption and immune function, potentially reducing growth performance. Although clinical outcomes were not assessed in this study, mixed infections are generally associated with cumulative physiological burdens in livestock.

No significant association was found between sex and infection status, suggesting comparable exposure risk among bulls and cows under similar management systems. This contrasts with findings from India, where higher infection rates were reported in females (Singh *et al.*, 2017). Differences between studies may reflect variations in

herd composition, physiological status, and management conditions.

Age was significantly associated with infection, with the highest prevalence observed in cattle aged 6 months to 2 years. This age-related pattern is consistent with previous findings indicating increased susceptibility in young animals due to incomplete immune development and wider grazing exposure (Singh *et al.*, 2017). Cattle younger than six months showed substantial overall infection prevalence; this age group is epidemiologically important for *T. vitulorum*, which can be transmitted transplacentally or through colostrum. However, species-specific prevalence was not analyzed separately within each age category.

Environmental and management conditions likely contribute to parasite persistence. Traditional extensive grazing systems expose cattle to contaminated fields and communal grazing areas. Parasite eggs and oocysts can survive for extended periods in moist environments, particularly during rainy seasons (Soulsby, 1986; Marskole *et al.*, 2016). Previous studies in similar settings have documented soil contamination with helminth eggs and protozoan oocysts around cattle pens and grazing areas (Mahartina *et al.*, 2020; Putri *et al.*, 2021).

Field observations in this study indicated suboptimal pen sanitation, limited drainage systems, and improper fecal disposal. Such conditions may facilitate environmental contamination and reinfection cycles. In addition, flies such as *Musca domestica* may act as mechanical vectors of protozoan cysts and oocysts, potentially contributing to parasite dissemination (Szostakowska *et al.*, 2004). Similar environmental contamination associated with inadequate sanitation has been reported in livestock farms in Korea (Ismail *et al.*, 2010).

Although the parasites identified in this study have recognized zoonotic potential, this research did not assess human infection in Bangkalan. Therefore, zoonotic risk should be interpreted in the context of established transmission pathways and environmental exposure rather than confirmed human case data from the study area. Further integrated One Health investigations are needed to evaluate potential cross-species transmission.

Overall, these findings indicate that zoonotic intestinal parasites remain endemic in Madura cattle and are influenced by geographic distribution and age-related susceptibility. Strengthened biosecurity, routine fecal monitoring, improved sanitation, and targeted control measures in livestock aggregation areas may contribute to reducing infection prevalence and limiting potential zoonotic transmission.

CONCLUSION

Zoonotic intestinal parasites remain prevalent in Madura cattle in Bangkalan Regency, with an overall infection rate of 29%. Five parasite taxa were identified, namely *Toxocara vitulorum*, *Fasciola gigantica*, *Blastocystis hominis*, *Balantidium coli*, and *Entamoeba* spp., with *B. hominis* being the most frequently detected. Infection prevalence differed significantly among sub-districts and age groups but was not associated with sex. Cattle aged 6 months to 2 years showed the highest susceptibility. These findings indicate that zoonotic intestinal parasites are maintained within traditional cattle production systems in Bangkalan. Strengthened routine parasitological monitoring, improved sanitation and waste management, enhanced biosecurity practices, and targeted control strategies in high-prevalence and livestock-aggregation areas are recommended to reduce infection pressure and mitigate potential zoonotic risks while improving cattle health and productivity.

ACKNOWLEDGEMENTS

The authors would like to express their sincere gratitude to the Dean of the Faculty of Veterinary Medicine, Universitas Airlangga, for providing financial support for this research through the Faculty's RKAT fund under Rector's Decree of Universitas Airlangga No. 886/Unair/2024, dated 27 March 2024. The authors also gratefully acknowledge the support of the Research Group of One Health, Faculty of Veterinary Medicine, Universitas Airlangga. We also thank the Animal Husbandry Office of Bangkalan Regency for granting permission and providing facilities for obtaining fecal samples from Madurese beef cattle raised in smallholder farms.

NOVELTY STATEMENT

To the best of our knowledge, this study provides new information on the identification of five gastrointestinal helminth and protozoan parasites with potential zoonotic significance in fecal samples from Madurese beef cattle raised in smallholder farms. The occurrence of these potentially zoonotic gastrointestinal parasites in Madurese cattle has important implications for both veterinary and public health. The findings provide baseline information that may support relevant veterinary and public health institutions in developing appropriate parasite surveillance and control strategies to reduce the potential risk of zoonotic transmission.

AUTHOR'S CONTRIBUTION

PH designed the study and supervised the research. NDRL

& LTS conducted laboratory examinations. M & ZY contributed to data analysis and manuscript preparation. All authors reviewed and approved the final manuscript.

GENERATIVE AI AND AI ASSISTED TECHNOLOGY STATEMENT

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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