Journal of Parasite Science
Vol. 9, No. 1, March 2025, Pages 25 - 31

JOURNAL OF PARASITE SCIENCE
2656-5331 (online) | 2599-0993 (print)
https:/e-journal.unair.ac.id/JoPS

Original Research

OPEN ACCESS

Detection of Protozoan Intestinal Zoonotic in Madura Cattle in Bangkalan Madura District

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ABSTRACT

The study aimed to detect the protozoan intestinal zoonotic that can potentially be a strategic disease in Madura cattle. The prevalence of the disease was linked to the sex and age of cattle in Bangkalan Regency. Cattle feces samples were collected 400 samples from eight sub-districts in Bangkalan District. Feces were examined natively, sedimentation, and floating. The species of protozoa was determined based on its morphology. The prevalence was expressed in percent, the study results were presented in figures and tables. The prevalence of protozoan intestinal zoonoses was 27.75%. The highest rate was found in Kwanyar (50%). Four species, namely *Blastocystis hominis*, *Balantidium coli*, *Entamoeba coli*, and *Giardia bovis*. In a single infection, *B. hominis* as the most protozoa was found to infect 85 cattle in eight sub-districts, *B. coli* was found in 16 cattle in four sub-districts and *E. coli* in 5 cattle in Kwanyar. A total of 107 samples were found to be infected with a single protozoan species, while 4 samples contained two species. The infection rate in bulls was 26.66%, which was slightly lower than in cows at 28.06%. Protozoan infections were more common in cattle aged 6 months to 2 years (42.4%) and those under 6 months (35.8%) compared to cattle over 2 years old (16.4%). This study confirmed the presence of intestinal zoonotic protozoa in cattle in Bangkalan District. Further research is needed to assess the prevalence of protozoa, identify risk factors, and analyze their geographic distribution, for developing effective prevention and control strategies.

ARTICLE INFO

Article history

Received: February, 4th 2025
Revised: March, 12th 2025
Accepted: March, 12th 2025
Published: March, 15th 2025

Keywords

Detection,
Protozoa,
Madura cattle,
Zoonotic.

INTRODUCTION

Madura cattle are a native Indonesian breed primarily raised in East Java, particularly on Madura Island. These cattle have strong potential for development due to their genetic adaptability to hot climates and challenging environments. They exhibit resistance to tick infestations, can thrive on low-quality feed, and require less food compared to imported breeds. Additionally, Madura cattle are easy to manage, have a high reproductive capacity, and show strong resistance to various diseases (Nurgiartiningsih, 2011). In 2016, the beef cattle population in Bangkalan District reached 200,279 heads and was projected to grow annually, supported by the artificial insemination program that crossbreeds Madura cattle with Limousin cattle (MADRASIN) (Bangkalan District Animal Husbandry Office, 2016).

Beef cattle, as a key commodity in rural farming, require proper management to ensure optimal development. However, smallholder livestock businesses often face challenges related to reproductive disorders and chronic parasitic diseases, particularly protozoan infections. Gastrointestinal diseases, in particular, demand special attention as they can hinder livestock growth

and development, leading to economic losses. These losses stem from reduced productivity, decreased work capacity, lower body weight, and diminished quality of meat, hides, and internal organs. Additionally, young animals may experience stunted growth, and certain infections pose a risk of zoonotic transmission to humans. Infected cattle may experience delayed weight gain, with reductions exceeding 40% compared to healthy cattle (Sudradjat, 1991).

Based on the results of the examination of fecal samples from Madura cattle slaughtered at the Surabaya Slaughterhouse (RPH), it showed that they were infected with protozoa *Eimeria* sp., *Balantidium* sp., and *Entamoeba* sp, (unpublished). However, until now, there has never been a study on protozoan diseases that infect the gastrointestinal tract in Madura cattle on farms in Bangkalan District. For this reason, it is necessary to research gastrointestinal protozoa mapping to monitor gastrointestinal protozoan diseases of Madura cattle on cattle farms in Bangkalan District, Madura Island.

MATERIALS AND METHODS

Ethics Test

This research is in the form of survey research with a sample in the form of feces collected without providing intervention to cattle as subjects, so an ethical test is not necessary. Fecal samples are taken as soon as the cow excretes feces so that it does not harm the cow or the researcher, but it requires the approval of the breeder.

Research Areas

The research was conducted in 2 sub-districts in coastal areas and 6 sub-districts in highland areas (each district was taken as one village) in Bangkalan District with a large livestock population. Feces sampling was conducted at different locations at different sea-level altitudes. Kwanyar and Modung are located in the lowlands with an altitude below approximately 25 m above sea level, while Kokop, Konang, Tanah Merah, Galis, Blega, and Geger are located in the highlands with an altitude above approximately 25-200 m above sea level.

Sample Collection and Analysis of Fecal Examination

The research began by conducting a survey of locations with large livestock populations and representing the collection area. A total of 400 fecal samples were collected from 8 districts in Bangkalan (50 fecal samples were taken from each district). Fecal samples were taken from bulls and cows and were randomly divided into less than 6 months old, more than 6 months to 2 years old, and more than 2 years old. Sample collection was carried out from April to May 2024. At the time of sampling, a questionnaire was also filled out by conducting interviews with farmers about 1). breeder (name, age, gender, education, breeding experience), 2). cattle (type and number, sex, age, type of maintenance, treatment that has been given, other livestock raised, type/material of cage, environmental conditions/livestock maintenance.

Fresh feces are taken in moderation, put in a plastic bag, and added 2.5% potassium bichromate. Each plastic bag is labeled or marked with a sample number adjusted to the sample data collection and stored in a container filled with ice. The stool sample that has been obtained is examined with (1). A simple (native) method, done by taking a small amount of feces and then adding one or two drops of water is flattened, covered with a glass cover, and examined under a 100x magnification microscope (10x objective). (2) The simple sedimentation method is carried out by making a suspension of 1 part of feces with 10 parts of water, after being filtered the suspension is left for 45 minutes, this process is repeated until the supernatant is clear then removed leaving a little, then the sediment is taken with a pipette placed on a glass object and covered with a glass cover, examined under a microscope with a magnification of 100x (objective 10x) (3). the *modified Fulleborn* flotation method is carried out by making a fecal suspension with a ratio of 1 part feces to 10 parts water, filter and filtrate put in a centrifuge tube, centrifuge for 2-5 minutes at a speed of 1500 RPM, repeat until the supernatant is clear, the solvent is removed, replace with saturated NaCl up to 1 cm from the mouth of the tube, be sipped again in the same way, then place the tube on the tube rack slowly drip saturated NaCl until the liquid looks convex at the mouth of the tube, place the glass cover slowly on the centrifugal tube left for 1-2 minutes, then the glass cover is taken and placed on the glass object, examined under a microscope (Mumpuni *et al*., 2007; Villeneuve *et al*., 2007). Determining the presence of protozoa that infect the livestock is done using an identification key (Soulsby, 1986). The results of the examination are positive if protozoa are found in one of the methods, and the cow is declared infected with protozoa. The prevalence of protozoan infections is expressed in percent with the formula:

$$\text{Prevalence} = \frac{\text{Positive Result}}{\text{Number of Samples}} \times 100\%$$

Data Analysis

The data obtained from this descriptive research are presented in the form of figures and tables.

RESULTS AND DISCUSSION

Based on these results, it was found that there were 111 of 400 samples (27.75%) positively infected by protozoan intestinal zoonotic. The highest infections were found in Kwanyar 50% (25/50), and the lowest in Kokop and Konang 16% (8/50).

Research results showed that in 111 positive samples, there were 107 samples infected by one kind of protozoa and 4 samples by two kinds of protozoa. The single species protozoa infections were at Konang, Kokop, Galis, Tanah Merah, Modung, and Kwanyar. District with protozoa

infection of two species at Blega and Geger (Table 1).

Table 1. The prevalence of protozoa intestinal zoonotic in Madura cattle in each sub-district in Bangkalan District

Sub-District	Number of samples	Infection-positive samples Protozoa		Number of positive samples (%)
		One	Two	
Konang	50	8	-	8 (16)
Kokop	50	8	-	8 (16)
Blega	50	13	3	16 (32)
Galis	50	18	-	18 (36)
Geger	50	10	1	11 (22)
Tanah Merah	50	16	-	16 (32)
Modung	50	9	-	9 (18)
Kwanyar	50	25	-	25 (50)
Total	**400**	**107**	**4**	**111 (27.75)**

The results showed that there were four species of protozoa intestinal zoonotic infecting Madura cattle, namely *Blastocystis hominis*, *Balantidium coli*, *Entamoeba coli*, and *Giardia bovis* (Figs. 1-4).

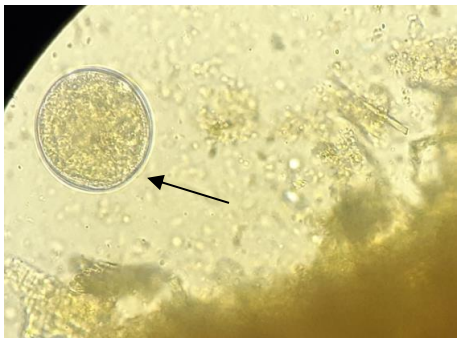

Figure 1. *Balantidium coli* (400x)

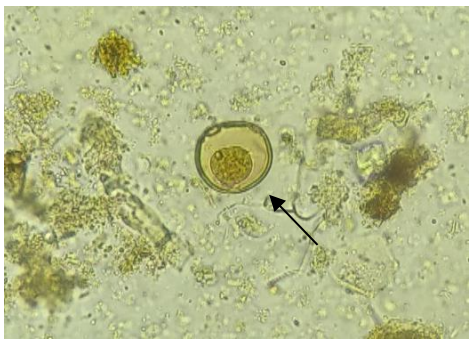

Figure 2. *Blastocystis hominis* (400x)

Figure 3. *Giardia bovis* (400x)

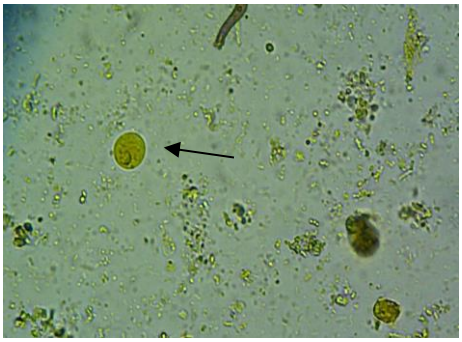

Figure 4. *Entamoeba coli* (400x)

Some types of protozoa intestinal zoonotic in Madura cattle from eight districts in Bangkalan District are listed in Table 2. In a single infection, *Blastocystis hominis* as the most protozoa were found to infect 85 samples in eight sub-districts, followed by *Balantidium coli* found in 16 samples in four sub-districts and *Entamoeba coli* in 5 samples in only one sub-district (Kwanyar) and *Giardia bovis* in 1 sample in one sub-district also (Blega). Infection of two types of protozoa which were *B. coli* and *E. coli*; *G. lamblia* and *B. hominis* was found in one sub-district (Blega); *B. hominis* and *E. coli* (Geger).

Based on sex, the prevalence of protozoa intestinal zoonotic infection in bulls 26.66% (24/90) was lower than in cows 28.06% (87/310). The comparison of each district showed that the highest protozoa infection in bulls in Kwanyar was 7 (77.78%) out of 9 fecal samples examined and the lowest in Modung and Kokop was one cow each. In cows in Blega, the most were 13 (38.46%) from 39 samples, and the lowest in Kokop was 7 (17.5%) from 40 samples (Table 3).

The prevalence of protozoan intestinal zoonotic infection in cattle < 6 months of age was 35.8% (20/53), with the highest case found in Blega (55.6%), and the lowest found in Modung (11.1%). Furthermore, the prevalence of protozoa intestinal zoonotic infection in cattle aged from less than 6 months to 2 years is 42.4% (60/158). The highest prevalence was found in Galis and Tanah Merah at 57.14% each, and the lowest was found in Konang and Kokop (16%) each. For cattle more than 2 years, the prevalence of protozoa intestinal zoonotic infection was 16.4% (31/189). The highest prevalence was found in Kwanyar was 40% (6/15), and the lowest found in Modung 8% (2/25) (Table 4).

Table 2. The prevalence of protozoa intestinal zoonotic in Madura cattle in Bangkalan District

Sub-District	Number of samples	Samples positively infected with protozoa				Number of positive samples (%)
		One type of protozoa species	Total	Two types of protozoa species	Total	
Konang	50	*Blastocystis hominis*	8	-	-	8
Kokop	50	*Blastocystis hominis*	8	-	-	8
Blega	50	*Blastocystis hominis*	10	*B. coli* *E. coli*	1	16
		B. coli	2	*G. bovis* *B. hominis*	2	
		G. bovis	1	-	-	
Galis	50	*Blastocystis hominis*	18	-	-	18
Geger	50	*Blastocystis hominis*	8	*B. hominis* *B. coli*	1	11
		B. coli	2	-	-	
Tanah Merah	50	*Blastocystis hominis*	16	-	-	16
Modung	50	*B. coli*	8	-	-	9
		Blastocystis hominis	1	-	-	
Kwanyar	50	*Blastocystis hominis*	16	-	-	25
		B. coli	4	-	-	
		E. coli	5	-	-	
Total	**400**		**107**			**111**

Table 3. The prevalence of Protozoa Zoonotic Intestinal of Madura Cattle in Bangkalan District based on sex

Sub-District	Bulls		Cow		Total of Samples	
	Number of samples	Positive Samples (%)	Number of samples	Positive Samples (%)	Number of samples	Positive samples (%)
Konang	9	2 (22.22)	41	6 (14.63)	50	8 (16)
Kokop	10	1 (10)	40	7 (17.5)	50	8 (16)
Blega	11	3 (54.54)	39	13 (38.46)	50	16 (32)
Galis	18	5 (27.27)	32	13 (40.62)	50	18 (36)
Geger	13	2 (15.38)	37	9 (24.32)	50	11 (22)
Tanah Merah	12	3 (25)	38	13 (34.21)	50	16 (32)
Modung	8	1 (12.5)	42	8 (19.04)	50	9 (18)
Kwanyar	9	7 (77.78)	41	18 (43.9)	50	25 (50)
Total	**90**	**24 (26.66)**	**310**	**87 (28.06)**	**400**	**111(27.75)**

Table 4. The prevalence of protozoa zoonotic intestinal of Madura cattle in Bangkalan District based on age

Sub-District	Age of samples						Sample Positive
	< 6 months	Positive sample (%)	>6 month - 2 years	Positive sample (%)	> 2 years	Positive sample (%)	
Konang	5	1 (20)	25	4 (16)	20	3 (15)	8
Kokop	7	2 (28.57)	20	4 (16)	23	2 (8.7)	8
Blega	9	5 (55.6)	15	7 (46.67)	26	4 (15.38)	16
Galis	5	2 (40)	21	10 (57.14)	24	6 (25)	18
Geger	3	1 (33.4)	18	6 (33.33)	29	4 (13.8)	11
Tanah Merah	9	4(44.44)	14	8 (57.14)	27	4 (14.8)	16
Modung	9	1 (11.1)	16	6 (37.5)	25	2 (8)	9
Kwanyar	6	4 (44.44)	29	15 (51.7)	15	6 (40)	25
Total	**53**	**20 (35.8)**	**158**	**60 (42.4)**	**189**	**31 (16.4)**	**111**

Feces sampling was conducted at different locations at different sea-level altitudes. Modung, Blega, and part of Kwanyar are located in the lowlands with an altitude below 25 meters above sea level, while Konang, Kokop, Galis, Geger, and parts of Tanah Merah are located in the highlands with an altitude above 25-200 meters above sea level. Kwanyar and Modung are located in coastal areas, while Blega, Galis, Tanah Merah, and Kwanyar are livestock traffic areas, meeting points for cattle from several locations, temporary shelters for cattle that will be sold outside the island or preparations ahead of Eid al-Adha.

Based on this study, the results were obtained that out of 400 samples tested, as many as 111 samples (27.75%) were positively infected with protozoa intestinal zoonotic. The highest prevalence of protozoan infections was found in Kwanyar, which was 50% (25/50) of positive samples, followed by Galis was 36% (18/50), Blega and Tanah Merah were 32% respectively (16/50), Geger was 22% (11/50), Modung was 18% (9/50), Konang and Kokop were 16% respectively (8/50) (Table 1). The high prevalence observed in this study aligns with the findings of Volpato *et al*. (2017), who reported a significant occurrence of intestinal protozoan infections in dairy calves in Brazil.

Similarly, the results of this study indicate a relatively high prevalence of protozoan infections in Madura cattle across eight sub-districts. Therefore, regular monitoring through fecal examinations is essential to ensure effective control of infections, ultimately improving livestock health and productivity.

The results showed that of the 111 positive samples, there were 107 (96.4%) samples infected with one species of protozoan and 4 (3.6%) samples infected with two species of protozoa. The highest single-species protozoan infection was found in Kwanyar at 50% (25/50), while the lowest was found in Kokop and Konang at 16% (8/50) each sub-districts with protozoan infections of two species in Blega and Geger.

The study identified four species of zoonotic intestinal protozoa infecting the digestive tract of Madura cattle: *B. coli, B. hominis*, *E. coli*, and *G. bovis* (Figures 1–4). However, *Isospora* and *Cryptosporidium* were not detected in this study, differing from previous research on Madura cattle (Hastutiek *et al*., 2019). Among the identified protozoa, *B. hominis* was the most prevalent, occurring as both single and mixed infections across eight sub-districts. A high prevalence of *Blastocystis* spp. infection has been associated with fatal outcomes in livestock (Stensvold *et al*., 2007; Yoshikawa *et al*., 2009). In France, the prevalence of gastrointestinal protozoan infections in dogs was reported at 42.2%, primarily caused by *Blastocystis* spp. and *Cryptosporidium* spp., both of which pose infection risks to their hosts (Osman *et al*., 2015). Additionally, *E. bovis* infection was found in 36% of cows, particularly those suffering from diarrhea (Al-Shabbani et al., 2016). The prevalence of *Blastocystis* spp., *Giardia* spp., and *Entamoeba* spp. has been reported at 14.6%, 12.45%, and 7.45%, respectively (Badparva, Fallahi, and Aarab-Mazar, 2015).

The prevalence of protozoan infections varied by sex, with bulls showing a lower infection rate of 26.66% (24/90) compared to cows at 28.06% (87/310). A detailed comparison of infection prevalence by sex across different sub-district is presented in Table 3. Age also played a significant role in infection rates, with the highest prevalence observed in cattle aged between 6 months and 2 years, reaching 42.4% (60/158). Among the sub-districts, Kwanyar recorded the highest prevalence at 51.7%, while Konang and Kokop had the lowest at 16% each (Table 4). A study in India reported that 83.08% of cattle were infected with endoparasites, with cows exhibiting a higher infection rate (85.97%) than bulls (69.23%). The study also found that adult cows over six months old had an infection rate of 85.97%, influenced by immune factors, calving, and lactation, which contributed to weakness and malnutrition.

In this study, Madura cattle aged 6 months to 2 years exhibited a higher prevalence of protozoan infections compared to those under 6 months old. This difference is likely due to their grazing behavior, as older cattle roam in wider areas where protozoan contamination is more prevalent. In contrast, younger calves have a lower risk of infection since they are less exposed to contaminated environments and primarily rely on suckling for nutrition.

The results of this study are the same as the study conducted in India on small ruminants; the prevalence of adult cattle with an age of mor than 6 months (85.97%) is higher than that of less than 6 months (61.17%) (Singh *et al*., 2017). Zoonotic parasites can be transmitted in four ways, among which the parasite species found in this study can be transmitted 1) Direct parasites from livestock to humans are *Giardia*, *Blastocystis*, *Entamoeba*, and *Balantidium* 2) *meta-zoonotic* parasites are transmitted from livestock to humans through invertebrates as intermediate hosts.

Forage given to cattle that are caged and grazed comes from the garden and around the rice fields. Madura cattle are mostly grazed in rice fields, gardens, and fields, which are very susceptible to zoonotic intestinal protozoan infections. If among the cattle grazed some are positively infected, then the land becomes a good place for developing various types of parasites. The development of parasites in livestock can be caused by various factors, including humidity and vegetation. Forage given to Madura cattle has gone through the wilting process but still shows a fairly high prevalence of infection and most types of zoonotic intestinal parasites enter the body through the mouth from contaminated feed and water. The contamination of the material may also have a role *for M. domestica* flies in the transmission of gastrointestinal protozoa, this finding emphasizes the need to enforce the fly's control measures in farms and landfills (Szostakowska *et al*., 2004).

The high prevalence of intestinal zoonotic parasitic infections in Madura cattle is likely

influenced by the rainy season, as the increased humidity creates an ideal environment for parasite development. Sampling conducted during this period, when humidity levels are high and temperatures are lower, aligns with conditions that favor parasite growth. Several factors contribute to parasitic infections in livestock, including geographical location, environmental conditions, housing quality, sanitation, stocking density, temperature, humidity, and vegetation (Levine, 1990). Poor sanitation practices among farmers, such as disposing of feces near the enclosures, can create a moist environment that increases the risk of reinfection. Additionally, traditional housing structures that lack proper drainage for feces and urine further facilitate the proliferation of protozoa.

Data shows that cows are infected with intestinal zoonotic parasites by one, two, and three types of parasites and even one cow can be infected with two types of worms and two types of protozoan. This occurs due to the weak body's resistance to these infections. Single or mixed infections by parasites in cattle make it difficult to know the specific effects caused, usually in the form of combined or mixed effects of existing parasites (Levine, 1990).

Madura cattle, whether kept in enclosures or allowed to graze freely, are susceptible to protozoan infections due to contamination of the grass they consume. If one animal becomes infected, the surrounding environment can serve as a breeding ground for various protozoan species. Although the forage provided to Madura cattle undergoes a preparation process, the prevalence of protozoan infections in their gastrointestinal tract remains high, likely due to contamination of both their feed and water sources.

The increased humidity during the rainy season is believed to contribute to the high prevalence of protozoan infections in the gastrointestinal tract of Madura cattle. The combination of high humidity and lower temperatures creates an ideal environment for protozoa to thrive. Various factors influence parasitic infections in livestock, including geographical location, environmental conditions, cage quality, sanitation and hygiene, stocking density, temperature, humidity, and vegetation (Soulsby, 1986; Marskole *et al*., 2016). This study also revealed inadequate cage management and sanitation practices, as feces removed from the enclosures were often disposed of nearby, leading to increased moisture levels that heighten the risk of reinfection. Additionally, the traditional design of cattle enclosures lacks proper drainage systems for waste disposal. The presence of infections caused by one or multiple protozoan species in this study may be linked to weakened immune systems, making the cattle more susceptible to infection (Thompson and Smith, 2016; Marskole *et al*., 2016). Poor ventilation further exacerbates the issue by reducing sunlight exposure, resulting in damp conditions that promote parasitic growth. Similar cases have been reported in Korea, where pigs and cows were found to be infected with *B. coli* and *Entamoeba* spp., with cysts and oocysts contaminating the environment due to poor sanitation (Ismail *et al*., 2010). Additionally, survey data indicated that most farmers were over 50 years old and had limited formal education, with many having never attended school or only reaching junior high school. This lack of education contributed to difficulties in understanding new information, ideas, and technologies that could enhance cattle health and management.

The high prevalence of protozoan infections observed in this study suggests that gastrointestinal infections in Madura cattle are persistent and require effective treatment and control measures.

CONCLUSION

Detection of protozoa intestinal zoonotic in Madura cattle as a strategic disease. The prevalence of protozoan intestinal zoonoses 27.75%. Four species of protozoa infect the colony, consisting of *B. hominis, B. coli, E. coli,* and *G. bovis.* 107 cows were infected with one species of protozoa, and 4 were infected with two species of protozoa. The prevalence of protozoan intestinal zoonotic infection in female cows is 28.06% higher than that of bulls by 26.66%. The prevalence of protozoan infection was highest in cattle aged more than 6 months to 2 years at 42.4%, followed by cattle less than 6 months old at 35.8% and cattle more than 2 years old at 16.4%.

ACKNOWLEDGEMENT

The authors sincerely thank the Faculty of Veterinary Medicine, Universitas Airlangga, for their support in this study. Our appreciation also goes to Ir. Abdul Razak, MM; Akhmad Azizun Hamid, DVM., SAP., MM.Pub; Ali Makki, DVM; Siti Sumirah, DVM from Bangkalan District Animal Husbandry Office. This article was partially supported by the RKAT funding from Faculty of Veterinary Medicine, Universitas Airlangga, Indonesia, in the 2018 fiscal year, under grant number 886/UN3/2018.

AUTHORS' CONTRIBUTIONS

AG, PH, and MF were responsible for collecting fecal samples. PH and MF conducted the microscopic analysis. PH and AG wrote the initial draft and revised the manuscript. All authors reviewed and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

FUNDING INFORMATION

Provided by the Excellent Fundamental Research (PDU) funding from Universitas Airlangga, Indonesia.

ETHICAL APPROVAL

This study involved the laboratory analysis of untreated cattle feces. The samples were collected following standard procedures, directly from the

rectum without causing any disturbance to the animals, and the collection process was supervised by a qualified veterinarian.

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