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Incidence and Risk Factors of *Toxocara vitulorum* Infection in Beef Cattle of Yogyakarta, Indonesia

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ABSTRACT

Toxocara vitulorum (*T. vitulorum*), an Ascarid nematode, infects the small intestine of cattle and buffalo, particularly in newborn calves. The present study aimed to identify the occurrence of *T. vitulorum* collected from cattle in Yogyakarta, Indonesia, and to examine the surface structure of its eggs by scanning electron microscopy (SEM). The present study did not observe asymptomatic clinical signs of toxocariasis, including diarrhoea and weight loss. Fecal samples were collected from 247 cattle of various breeds, consisting of 65 males and 182 females across three regions including Bantul (78 cattle), Sleman (63 cattle), and Kulon Progo (106 cattle). Qualitative and quantitative methods, including flotation and modified McMaster methods, were respectively employed to analyze nematode egg counts. SEM was utilized to characterize the surface morphology of *T. vitulorum* nematodes. A total of 9 cattle were found to excrete *T. vitulorum* eggs in their feces (3.64%). The average fecal egg count was 2.861 eggs per gram (EPG), with positive cases observed exclusively in female cattle. The risk factors influencing toxocariasis in this study were breeds and frequency of cleaning the stall. A higher odd ratio of *T. vitulorum* infection was found in mixed Ongole breeds than in Limousin or Simmental breeds. Moreover, cattle housed in rarely cleaned stalls showed a higher odd ratio than those in regularly cleaned ones. Factors such as age, fecal consistency, and population density factor showed no significant association with toxocariasis. The SEM analysis of *T. vitulorum* eggs revealed an oval shape with distinct surface ornamentations, including interlocking ridges and depressions. The cage cleanliness and cattle breed were the most common risk factors associated with infected cattle.

Keywords: Ascarid, Cattle, Prevalence, Risk factor, Scanning Electron Microscopy, Toxocariasis

INTRODUCTION

Helminth infections in livestock cause suboptimal growth, weight loss, reduction in feed conversion rate, decreased endurance, decreased reproductive capacity, and decreased carcass quality (Hamid et al., 2023). Such substantial economic and health impacts have placed helminthiasis among the strategic diseases in Indonesia (Winarso et al., 2015). *Toxocara vitulorum* is a gastrointestinal helminth from the neoascaris group, and the adult stage of *T. vitulorum* is frequently observed in calves. It has also been found to infect buffaloes, cattle, and zebu, and is particularly prevalent in tropical and subtropical regions (Dewair and Bessat, 2020). *Toxocara vitulorum* causes economic losses, especially in cattle and buffaloes, due to its high mortality rate of up to 37.3%, as reported by Rast et al. (2014). Clinical manifestations include anemia, diarrhea, weight loss, anorexia, and small intestine obstruction (El Shanawany et al., 2019). *Toxocara vitulorum* has direct life cycles without an obligate intermediate host, but its transmission is more complex than simply ingesting infectious eggs (Bowman, 2020). While ingestion of eggs containing two-stage larvae is a common route of infection, the parasite can be transmitted through ingestion of larvae in the mother's milk (Urhan et al., 2023). A female worm can lay up to 200,000 eggs per day, with eggs exhibiting thick walls that allow them to withstand extreme environmental conditions, such as heat and drought for a prolonged period (Roberts, 1990; Delling et al., 2020). *Toxocara vitulorum* worm eggs can live in the environment for up to two years (Ziegler and Macpherson, 2019). The eggs will hatch into their first, second, and third larval stages in moist and warm environments. The larvae require a developmental period of 7-12 days at a temperature of 28-29°C (Sihombing and Mulyowati, 2018; Aboamer et al., 2019).

Toxocariasis is widespread across all regions of Indonesia (Purwandani et al., 2021). Nevertheless, its prevalence in the Yogyakarta region and the associated risk factors have not been reported to date. Yogyakarta is a province situated

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on the island of Java with the fourth largest cattle population (Statistics ICBo, 2022). Toxocariasis predominantly affects cattle or buffalo calves, with a reported frequency of 45% in young animals (Biswas et al., 2022). Calves aged 1-2 months exhibit higher mortality rates up to 50% if the disease is left untreated (Biswas et al., 2021). In contrast, older animals (over six months) demonstrate increased resistance to infection (Ziegler and Macpherson, 2019). The rainy season has the highest transmission rate because calves become infected with parasitic worms after ingesting worm eggs while grazing on expansive and contaminated pastures (Davila et al., 2010).

Toxocara vitulorum egg structure is protected by a thick wall, making it resistant to dry environments (Aboamer et al., 2019). In Indonesia, a tropical country with a humid climate and consistent year-round precipitation, toxocariasis remains particularly challenging to control and eradicate. This parasitic disease is commonly found in bovine and bubaline calves (Winarso et al., 2015). Third-stage larvae (L3) present in colostrum can potentially infect newborn calves. Infective eggs swallowed in the digestive tract will grow into L3 in animals older than 6 months of age (Ziegler and Macpherson, 2019).

The respiratory organ can be affected by the migration of L3, potentially leading to pneumonia. Other symptoms that may occur include difficulty passing stool, fluid loss, reduction in body mass, and swelling beneath the jaw (Davila et al., 2010). Visceral larvae migrants in adult cattle induced by *T. vitulorum* are often asymptomatic (Davila et al., 2010). *Toxocara vitulorum* larval migration in calves can induce liver and lung damage (Ziegler and Macpherson, 2019). Furthermore, the presence of adult worms in the small intestine can cause diarrhea, weight loss, and, in severe cases, mortality, which predominantly affects young animals. A significant proportion of calves up to 5 months of age are susceptible to toxocariasis when poor maternal hygiene facilitates the transmission of *T. vitulorum* through colostrum. Several drugs, including piperazine, pyrantel, febantel, and oxfendazole, are effective against *T. vitulorum* in its adult stage (Ziegler and Macpherson, 2019). Pyrantel and levamisole are both efficacious anthelmintic agents for eliminating *T. vitulorum* third-stage intestinal larvae (Ziegler and Macpherson, 2019; Afshar et al., 2023).

Several factors, including geography, season, age, gender, bodily condition, fecal consistency, nutritional status, husbandry techniques, etc., might impact the prevalence of toxocariasis. Numerous factors have been recognized as potential contributors to *T. vitulorum* infection across diverse geographical regions worldwide, including age, gender, body condition, season, and fecal consistency (Woodbury et al., 2012; Biswas et al., 2021). *Toxocara vitulorum* infection is associated with these factors; hence, studying the correlation between its prevalence and the associated factors is essential for developing strategies to mitigate the economic losses caused by this parasitic infection. Despite its significance, detailed morphological studies of *T. vitulorum* eggs using scanning electron microscopy (SEM) remain limited. SEM can reveal intricate details of the eggshell surface, including any ridges, pores, and curved structures. It has much higher magnifications than light microscopy, enabling the visualization of unique structures. This study aimed to provide valuable information on *T. vitulorum* prevalence, factors influencing infection, and detailed morphological characteristics of the parasite eggs.

MATERIALS AND METHODS

Ethical approval

This research was conceptualized and executed in compliance with animal welfare regulations promulgated by the Research Ethics Committee, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Indonesia.

Materials

The tools used in this study included a scanning electron microscope (JSM-6510LA, Japan), microtubes, a vacuum critical drier, a gold coater (JEOL JEC-3000FC, Japan) object glasses, cover glasses, double object glasses, a magnetic stirrer, test tube racks, centrifuge tubes, a centrifuge, a microscope, pipettes, and syringes. Stool samples were processed using saturated sugar solution (prepared by mixing sugar from Gulaku, Indonesia, with aquadest until a specific gravity of 1.3 was reached), saturated NaCl solution (prepared by mixing salt from Refina, Indonesia, with aquadest, and aquadest. A questionnaire was used to collect research variables such as cattle breed, age, sex, stool consistency, management, and environmental variables. The authors designed the questionnaire consisting of a Likert scale and multiple-choice questions. Moreover, personal interviews with farmers were conducted to fill out the questionnaires while visiting the farm to collect the feces. To ensure validity, the questionnaire underwent expert review and content validation by authors from the Department of Parasitology.

Sampling method and sample size

In this investigation, one sample per animal was collected with a total of 247 stool samples (65 males and 182 females) from different breeds of cattle (Table 1). Three different regencies (Sleman, Bantul, and Kulon Progo) in

Yogyakarta were selected randomly from a total of 5 regencies in Yogyakarta. Two villages were chosen randomly from each regency and one cattle stall was randomly chosen. Fecal samples were collected from all cattle in the selected stalls. The feces were collected by palpation of the rectum, stored in plastic bags, and kept in a refrigerator (4°C) before the examination of samples maximally within a week. The samples were evaluated at the Laboratory of Parasitology of the Faculty of Veterinary Medicine, Universitas Gadjah Mada. Individual risk variables such as age, calf gender, cattle breed, and stool consistency were investigated. Cage management variables include cage cleaning frequency and cage density. The features of feed and water supplies were the next consideration. Sampling was distributed across six villages within the three selected districts, using a random sampling method at the sub-district, village, and breeder levels.

Table 1. The qualitative examination with the floating method and quantitative with the McMaster method on 247 samples from different breed cattle in Yogyakarta, Indonesia during May 2022 until July 2022

District	Number of samples	Results	
		Flotation method	EPG range
Sleman	78	2.56 % (2/78)	50-9800
Bantul	63	1.58 % (1/63)	50
Kulon Progo	106	5.66 % (6/106)	50-9150
Total	247	3.64 % (9/247)	50-9800

EPG: Eggs per grams

Sample examination technique

The floating technique was used to evaluate stool samples, as described by Deplazes et al. (2016). The reference of this study is based on Zajac et al. (2021). For this technique, 3 grams of feces were placed in a mortar, mixed with sufficient water, and stirred until homogenous. The homogenous solution was then placed into a centrifuge tube (OneMed, Indonesia), filling it halfway. The centrifuge tube was then spun for 5 minutes at 3000 rpm. After discarding the clear liquid above the precipitate, saturated NaCl solution was added until ¾ parts of the tubes were mixed well. The centrifuge tube was then spun for 5 minutes at 3000 rpm. Furthermore, the centrifuge tube was subsequently placed in a rack, and saturated NaCl was added dropwise until the liquid surface formed a convex meniscus. The setup was left undisturbed for 3 minutes. After 3 minutes, an object glass slide was gently placed onto the convex surface, then promptly inverted for examination under a light microscope (Olympus, Japan) at 10×10 magnification.

For quantitative analysis, the modified McMaster technique was used. In this method, 3 grams of feces were mixed with water in a 1:14 ml of water ratio and homogenized using a magnetic stirrer. A 0.3 ml of the fecal solution was collected and deposited in the McMaster chamber, pre-filled with 1.2 g/cm3 saturated sugar. The mixture was allowed to settle for 3 minutes after homogenization with a needle. Observations were performed by counting all of the eggs in the chamber and multiplying the total number of eggs by 50 to get the total number of eggs per gram of feces.

Scanning electron microscopy

The eggs were purified and fixed in glutaraldehyde for a minimum of 35 minutes and up to three days, followed by three 5-minute double distilled water rinses. The samples were then dehydrated by suspension for 10 minutes in 30%, 50%, 70%, 80%, 90%, and three 100% ethanol treatments before being dried in a CO2 critical point drier, coated with gold, and analyzed by scanning electron microscopy (SEM; JEOL JSM-6510LA, Japan). The SEM analysis focused on comparing the morphological features of *T. vitulorum* eggs with those of other *Toxocara* species and measuring their surface characteristics.

Statistical analysis

The prevalence of infection was determined using data from the flotation technique. The collected prevalence rate statistics were examined using the following methods including Prevalence (%) = (Number of positive samples/Total number of samples) × 100. Descriptive measurements were applied for quantitative data analysis. Univariate analysis was used to assess the effect of individual risk factors on *Toxocara* infection based on a single variable, and the strength of the connection was evaluated using the Odds Ratio (OR). Multivariate logistic regression analysis was used to identify potential risk factors associated with *Toxocara* infection based on multiple independent variables. A $p < 0.05$ was considered statistically significant. Descriptive analysis methods were used to examine the SEM results. All statistical analyses were performed using IBM SPSS version 26 (IBM Corporation, USA).

RESULTS AND DISCUSSION

Prevalence and risk factor analysis of parasites are fundamental to understanding the epidemiology of parasitic diseases and developing effective control strategies. *Toxocara vitulorum* is difficult to manage since adult female ascarids reproduce rapidly, producing a significant number of eggs per day. Adult female ascarids are highly prolific, capable of laying a substantial quantity of approximately 110,000 worm eggs daily (Roberts, 1990; Delling et al., 2020). The eggs can remain viable for many years with protective eggshells which provide resistance against adverse climatic conditions (Venjakob et al., 2017). As a result, grazing lands can act as a permanent source of infection. Additionally, larvae migrate in the tissues, remaining dormant or hypoSE-biotic and continuing their maturation at times of stress or decreased immunity (Biswas et al., 2021).

In this study, the prevalence and risk factors of *T. vitulorum* infection in beef cattle from three districts in Yogyakarta were assessed. The overall prevalence of *T. vitulorum* infection was relatively low in the studied areas. In Sleman, only 2.56% of the samples tested positive for the infection, while in Kulon Progo, it was slightly higher at 5.66%. Out of 87 samples from Bantul, only one sample (1.58%) was positive for ascarid infection. Previous reports on *T. vitulorum* infection in Yogyakarta demonstrated a significant difference between lower-lying areas and higher altitudes. The incidence rates were found to be 12% and 19.3%, respectively (Suwito and Santoso, 2021). These findings suggest that altitude may play a role in the variation of infection rates: lower temperatures at higher altitudes may hinder parasite development (Ndamukong-Nyanga et al., 2015) Differences in soil composition, humidity, and ultra-violet (UV) radiation levels at various altitudes may impact parasite survival and infectivity in the environment (Alum et al., 2014). Notably, the McMaster method, which measures the number of eggs per gram of feces (EPG), revealed that Sleman had the highest load of *Toxocara* infection among the tested calves, with an average of 4,925 EPG, Figure 1 presents the results of fecal examination from cattle in Sleman. Kulon Progo followed with an average of 2,961 EPG. The mean EPG for *T. vitulorum* infection among calves was 2,861 EPG. Based on the results of the present study, the overall prevalence of *T. vitulorum* infection among calves was determined to be 3.64% (Table 1). According to Biswas et al. (2021), the severity of infection is categorized as light (50-500 EPG), moderate (500-1,000 EPG), and heavy (> 1,000 EPG).

In this study, cattle aged two to four years exhibited a higher prevalence of *T. vitulorum* infection compared to younger calves. This poses a greater risk of transmission to calves since cattle of that age are beginning to produce offspring, while *T. vitulorum* is mostly transmitted through larvae from lactating mothers. Furthermore, eggs can survive in the environment for up to two years, facilitating transmission to other cattle within the herd. It is worth noting that infection in adult cattle is typically self-limiting, as frequent exposure to *T. vitulorum* induces self-cure infection (Biswas et al., 2021). Other studies have indicated that adult worms may be present in mature cattle due to immunosuppression resulting from pregnancy, infection with other pathogens, or stress-related factors (Dorny et al., 2015; Urhan et al., 2023).

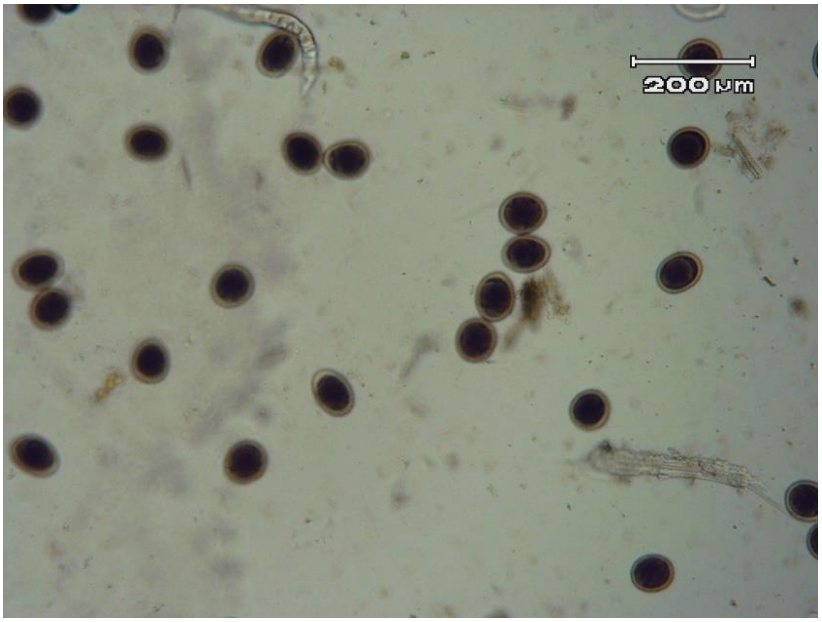

Figure 1. The qualitative examination of the feces revealed the presence of a large number of *T. vitulorum* eggs from a 22-month-old cattle in Sleman, Yogyakarta. Magnification 100x

Analysis of risk factors included variables such as sex (male and female), age (< 1y, 1-2y, 2-4y, > 4y), fecal condition (normal, watery, diarrhea), breed (Ongole cross, Simental, Limousine, Simental x Ongole, Limousine x Ongole), frequency of cage cleaning (daily and weekly), and population in the cage (< 5 heads and > 5 heads, Table 2). Results showed that cleaning frequency groups across all geographic locations had a significant impact on *Toxocara* infection (Table 2). This study showed that the risk of toxocariasis infection was 10.67 times higher in calves housed in stalls that were cleaned rarely compared to those that were cleaned daily. Farmers need to maintain clean pens to reduce the risk of parasitic diseases in their cattle (Aboamer et al., 2019).

The breed of cattle was discovered to be an important risk factor, with the most at-risk races (p = 0.04) being the Simmental and Ongole crosses (OR=3.69), as well as the Ongole-cross breed (OR=2.62) compared with a Limousine-Ongole cross. Bahbahani et al.'s (2018) research has demonstrated that *Bos indicus* breeds, such as Ongole, frequently exhibit enhanced resistance to certain parasites in comparison to *Bos taurus* breeds such as Simmental and Limousine. This heightened resistance is attributed to specific immune-related genes that have undergone positive selection in tropical environments where these breeds are predominantly raised (Wang et al., 2016). Although differences in infection prevalence were observed across age groups, the association between age and infection (p = 0.57, Table 2) was not statistically significant. Similarly, no significant relationship was found between infection and fecal consistency (p = 0.87) or cage population density (p = 0.57). Comparisons with other studies provide additional context. For instance, in Central Ethiopia, *T. vitulorum* was found sporadically, with a prevalence of 2.2% (Terfa et al., 2023). The study of Bărburaș et al. (2022) demonstrated that *T. vitulorum* eggs were found in buffalo calves of various age groups, with the prevalence of infection ranging from 11% to 23% and eggs per gram values varying with age. Moreover, the presence of adult buffaloes in the same barn was identified as a risk factor for *T. vitulorum* infection in the buffalo calves. In a fatal case of toxocariasis in a yak calf aged 28-56 days in Tyrol, Austria, morphology and sequence analysis confirmed the worms as *T. vitulorum* (Schoener et al., 2020).

Table 2. The risk factors associated with 247 samples collected from different cattle breeds in the study location during May to July 2022

Variables	Number of sample	Number of positive (Percentage)	OR	p-value
Gender				
Male	65	0 (0%)	Ref	3.34 (0.07)
Female	182	9 (4.94%)	a	
Age				
<1 years	31	1 (3.22%)	1.70	2.003 (0.57)
1-2 years	63	2 (3.17%)	2.98	
2-4 years	119	6 (5.04%)	1.71	
>4 years	34	0 (0%)	Ref	
Stool condition				
Normal	204	8 (3.92%)	7.93	0.27 (0.87)
Watery	42	1 (2.38%)	2.09	
Diarrhea	1	0 (0%)	Ref	
Breed*				
Ongole cross	53	5 (9.43%)	2.62	9.77 (0.05)
Simental	87	0 (0%)	1.28	
Limousine	11	0 (0%)	1.00	
Simental x Ongole	22	2 (9.09%)	3.68	
Limousine x Ongole	69	2 (2.89%)	Ref	
Population install				
< five cattle	134	4 (26.86%)	Ref	0.36 (0.55)
> five cattle	113	5 (21.23%)	1.54	
Cleaning frequency*				
Everyday	137	1 (21.89%)	Ref	7.44 (0.006)
Not everyday	110	8 (27.27%)	10.67	

Ref: Reference category. a: The response outcome is 0. OR: Odds ratio

Despite the availability of effective treatment options, *T. vitulorum* infection in calves remains a persistent challenge. A single dose of pyrantel administered at 14-21 days of age has proven effective in controlling the parasite (Rast et al., 2013; Delling et al., 2020). Rast et al. (2014) revealed poor reproduction, high calf morbidity, and mortality, coupled with limited farmer knowledge and effective control of endemic toxocariasis, hinder optimal large ruminant production in mixed smallholder farming systems in Southeast Asia. The substantial net benefit per calf attainable through a single pyrantel treatment should encourage smallholder farmers to adopt this intervention, particularly as the demand for livestock products escalates in the region, necessitating a shift towards more production-oriented farming practices.

Toxocara vitulorum lays eggs that range in form from oblong to spherical. Ripples decorate the egg's exterior and may have smaller circular or polygonal depressions that are left behind as these ridges interdigitate (Figures 2 and 3).

The present study uncovered that *T. vitulorum* eggs had characteristic ridges on the surface, similar to those found on the eggshells of the ascaridid nematodes *Ascaris lumbricoides*, *A. suum*, and *T. canis* (Ubelaker and Allison, 1975; Taira and Fujita, 1991). High-resolution imaging obtained via SEM in this study provided detailed three-dimensional visualizations of *T. vitulorum* egg surface structures. Notably, the ridge of *T. vitulorum* was found to exhibit relatively sharp profiles, neither smooth nor flat. In comparison, the ridge of *T. canis* eggs demonstrated a more uniformly connected structure (Bojanich et al., 2018). The eggs of *T. vitulorum* are rounded to oval in shape, each measuring 68-95 µm in diameter. The dimensions of *Toxocara* species eggs exhibit remarkable variations. *T. canis* ova possesses major and minor axes ranging from 71.6 to 91.2 µm and 63.4 to 79.0 µm, respectively. In contrast, *T. cati* eggs display measurements of 63.7-88.1 µm for the major axis and 53.3-73.3 µm for the minor axis. *Toxocara malaysiensis* ova, however, demonstrates dimensions of 60-68 µm by 68-76 µm for their respective axes (Kim et al., 2020). The *T. vitulorum surface* of the egg is ornamented with prominent ridges that are distinguished from other ascarid eggs. The presence of specific morphological differences between the eggshells of *T. canis* and *T. vitulorum* was reported basically in nature (Ashour et al., 1996).

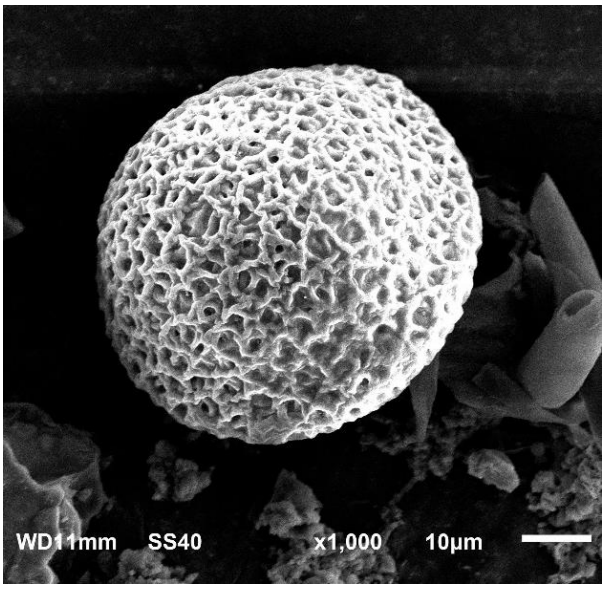

Figure 2. The surface of *T. vitulorum*'s egg isolated from Simental mix Ongole Cattle breed in Sleman, Indonesia. Magnification 1,000x

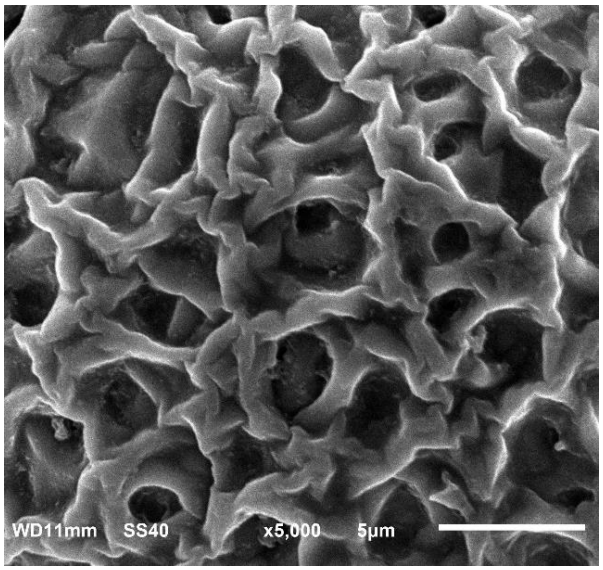

Figure 3. *T. vitulorum* isolated from Simental mix Ongole Cattle breed in Sleman, showing detailed characteristic ridges and depressions. Magnification 5,000x

CONCLUSION

The present study provided valuable insights into the prevalence and intensity of *Toxocara vitulorum* infection in cattle across three districts, revealing an overall prevalence rate of 3.64%. Among the evaluated risk factors, cage cleanliness, and cattle breed were found to be statistically associated with infection prevalence. Scanning electron microscopy revealed distinct morphological features of *T. vitulorum* eggs, including their oval shape and characteristic surface ornamentations with interlocking ridges and depressions. These findings contribute to enhancing diagnostic accuracy and facilitate the development of effective treatment or egg-elimination strategies in the environment. Future studies should focus on validating these results across diverse geographical regions and environmental conditions.

DECLARATIONS

Availability of the data and materials

All data supporting the findings of this study are available within the manuscript.

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Authors' contributions

Vika Ichsania Ninditya and Fitrine Ekawasti collected the data and drafted the manuscript, Wisnu Nurcahyo designed the study and finalized the manuscript, Joko Prastowo analyzed the data, and Irkham Widiyono reviewed and finalized the manuscript. All authors reviewed and confirmed the final manuscript.

Competing interests

The authors have not declared any conflict of interest.

Ethical considerations

All authors have verified the ethical considerations, including plagiarism, misconduct, fabricated or false data, consent to publish double publication and/or submission, and redundancy.

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