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Detection of *Theileria sp.* in Grazing Cattle at Tamangapa Landfill Makassar

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Abstract

This study aims to detect the presence or absence of *Theileria* sp. parasitic infection in cattle grazing at the Tamangapa landfill, Makassar. The study was conducted from January to February 2023. A total of 43 blood samples were collected from a population of 781 cattle. The blood smear samples were stained with Giemsa, examined microscopically, and analyzed descriptively. The results showed that 12 samples were positive and 31 samples were negative for *Theileria* sp. Microscopic examination confirmed the presence of rod- or stick-shaped parasites within erythrocyte cells. The prevalence of infection was higher in female (32.43%) than in male cattle (0%) and in cattle aged ≥2 years (39%) than in those younger than 2 years old (20%). The findings of this study confirm the presence of *Theileria* sp. parasitic infection in cattle at the Tamangapa landfill, with an overall prevalence of 27.91%. Further research is needed to identify the species of parasite involved and to investigate the risk factors for this disease.

Keywords: Detection, cow, landfill, Theleria sp., blood smear

Introduction

Cattle play a crucial role in the Indonesian economy, providing multiple benefits. They serve as a vital source of animal protein, provide milk, are employed for tasks such as cart-

pulling, and their manure serves as valuable fertilizer. Traditionally, cattle are raised through grazing on pasturelands. However, they are also released to graze in landfills. One of the landfill that is used as a place for grazing is the Tamangapa landfill in Makassar, South Sulawesi. According to Nur (2013), the Tamangapa landfill serves as the primary waste disposal site for Makassar residents. The humid conditions of Tamangapa landfill provide an optimal environment for ticks, which act as vectors for blood parasites. In addition, according to Muthiadin *et al.* (2018), cattle grazing in landfills predominantly feed on fermented organic waste, leading to adverse effects on their health, including parasite infestations in these damp environments.

One of the blood parasites that can infect cattle is *Theileria* sp., causing a condition known as theileriosis. *Theileria* is a genus of blood parasite commonly found and economically significant in ruminants within tropical and subtropical regions (Oosthuizen *et al.*, 2009). Approximately, one million cattle deaths per year and USD 300 million are lost annually because of the infection of this parasite (Ganaie *et al.*, 2019). The consequence of theileriosis on livestock include stunted growth, weight loss, reduced working capacity, and diminished reproductive potential (Dyahningrum *et al.*, 2019). Furthermore, the disease leads to death, reduced milk production, abortion, and the incurring of expenses for disease management (Gharbi and Darghouth, 2015). An outbreak of theileriosis can also occur, such as the one reported by Widiasih *et al.* (2019) in the Mukti Andini cattle herd, Cucukan Village, Prambanan District, Klaten Regency in January 2019. *Theileria sp.* is naturally transmitted by ticks that thrive in moist environments such as landfills.

Currently, there is no published data on theileriosis in the Tamangapa landfill. Access to information regarding theileriosis at Tamangapa landfill is essential for anticipating and effectively managing potential sudden outbreaks. Given this context, it is necessary to conduct a research on the detection of *Theileria* sp. in cattle grazing at the Tamangapa landfill, Makassar.

Research Methods

This is a descriptive study that provided an overview of *Theileria* sp. infection in cattle grazing at the Tamangapa landfill, Makassar. A total of 43 peripheral blood samples were collected from the vena auricularis of cattle grazing at the Tamangapa landfill from January to February 2023. The blood smear samples were then delivered to Maros Veterinary Center (Balai Besar Veteteriner Maros) for laboratory examination. The materials and equipments used in this study were cotton, tissue, 70% alcohol, absolute methanol, Giemsa solution with buffer solution (in a 1:4 ratio) with a pH of 6.5, immersion oil, an Olympus brand microscope model BX51M, dropper pipette, object glass, sterile needle with size 22 G, and digital camera.

The blood samples were smeared onto object glasses and labeled with appropriate sample numbers. They were fixed with absolute methanol for three minutes, allowed to dry, and then stained with Giemsa solution for 45 minutes. The samples were then rinsed with clean water and left to dry in an upright position. Microscopic examination was conducted at 100x magnification, utilizing immersion oil. A sample was considered positive if *Theileria* sp. was identified. The data from positive and negative samples were presented descriptively, supplemented with images, tables, and subsequent discussions. Conclusions were drawn regarding the presence or absence of *Theileria* sp. The prevalence was calculated by dividing the number of positive cases by the total number of samples, expressed as a percentage (%).

Results and Discussion

The examination of 43 samples showed that 12 of them were positive for *Theileria* sp. The result was based on the microscopic observation of blood parasite in the form of rods or sticks within erythrocytes, which are morphological characteristics of *Theileria* sp. (Figure 1). According to Taylor *et al.* (2016), Theileria sp. in erythrocytes typically exhibits a rod-shaped morphology, although it can also manifest as round, oval, or comma-shaped. Pratika and Rahmawati (2022) also reported similar findings, noting various morphologies of *Theileria* sp. within erythrocytes, including rods, rounds, ovals, and commas.

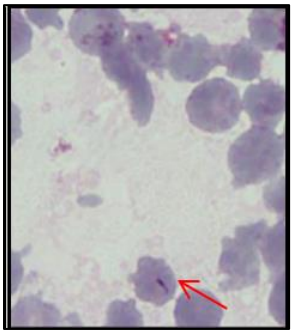

Figure 1. *Theileria* sp. within an erythrocyte (red arrow) stained with Giemsa from cattle grazing at Tamangapa landfill Makassar.

The study identified 12 positive cases of *Theileria* sp. infection in the blood samples, indicating a theileriosis prevalence of 27.91% (12 out of 43) among the total cattle studied at the Tamangapa landfill. A similar study was conducted by Putri (2022) in Maniangpajo District, Wajo Regency, South Sulawesi Province, who reported a theileriosis prevalence of 58.3%. Amuda (2018) found a 31% prevalence in Pohuwato Regency, while Nugraha *et al.* (2015) reported a 32.5% prevalence in Gorontalo Regency. Other studies have reported varying proportions of positive cases, such as 33% in Kutalimbaru District, Deli Serdang Regency (Prastia, 2021), 22.3% in Ujung Jaya District, Sumedang Regency, West Java (Anggraini, 2013), 60.53% in Cikalong District, Tasikmalaya (Ichsan, 2014), 0.5% in Moyo Hilir District, Sumbawa Regency, West Nusa Tenggara (Anggraini *et al.*, 2019), and 57.04% in Cipatujah, Tasikmalaya Regency (Wibowo, 2014). Furthermore, the Ministry of Agriculture (2014) reported theileriosis cases in various regions, including 30.8% in six regencies in South Kalimantan, 3.8% in 10 districts in North Sumatra, 0.4% in Aceh, 83.3% in Teluk Naga, 46.8% in Legok, 43% in Lebak, and 46.9% in Cileungsi. Anggraini *et al.* (2019) identified several factors contributing to *Theileria* sp. infection in cattle, including season and rainfall, stall conditions, environment, age, sex, and the sensitivity of diagnostic tests. Budiati (2002) also highlighted cattle genetics, age, stress levels, and management practices as contributing factors to blood parasitic infections caused by *Theileria* sp.

Table 1. Findings of theileriosis

No	Variable		Number of Samples	*Theileria* infection		Percentage of cases (%)
				+	-	
1.	Gender	Male	6	0	6	0% (0/6)
		Female	37	12	25	32,43% (12/37)
2.	Age	<2 Years	25	5	20	20% (5/25)
		≥2 Years	18	7	11	39% (7/18)
	All samples		43	12	31	27,91% (12/43)

According to Anggraini *et al.* (2019), high rainfall leads to high humidity. These environmental conditions support the proliferation of ticks and facilitate infection with the

blood parasite *Theileria* sp. Similar conditions were found in this study, as the sampling period ranged from the end of January to the middle of February, when rainfall was high enough so that humidity was also increased. High humidity provides an optimal environment for the development of ticks as vectors, increasing the likelihood of protozoan infections compared to drier seasons. However, this is different from the findings of Rustam (2021), who reported higher tick infestations in dry lands compared to wetland areas. It is worth noting that wetland areas were devoid of tick infestations in Rustam's study because the research was conducted during the dry season.

Anggraini *et al.* (2019) also highlight the significance of housing conditions in influencing livestock susceptibility to disease. Cattle sheds with abundant fecal deposits can serve as favorable breeding grounds for disease vectors. This aligns with the conditions observed in landfills, where cattle graze and obtain nutrition from fermented organic waste, leading to adverse effects on cattle health, especially susceptibility to parasite attacks in damp environments (Muthiadin *et al.*, 2018). In this study, the cattle shared similar environmental conditions, grazing and sourcing food from the Tamangapa landfill, known for its humid conditions and ample garbage piles, providing an optimal breeding ground for ticks, the vectors of *Theileria* sp. Additionally, the conditions of cattle pens in this study were also humid, characterized by large piles of feces mixed with mud at the pen bottoms. This condition presented a potential for tick proliferation as vectors of *Theileria* sp.

This study identified the highest occurrence of theileriosis in farmer pen 1, where 11 cattle were affected. This finding was supported by the presence of ticks in the cage, i.e. *Boophilus* sp., a hard-skinned tick species known to feed on blood and cause anemia in cattle. This finding aligned with Widiasih *et al.* (2019), who attributed theileriosis in Indonesia to *Theileria orientalis*, transmitted by *Boophilus microplus* ticks. Budiati (2002) further explained the life cycle of *Theileria* sp., occurring within the bodies of ticks, namely *Boophilus* sp., and their hosts. According to the Ministry of Agriculture (2014), theileriosis is naturally transmitted by ticks. Generally, *Theileria orientalis* is transmitted by *Boophilus microplus* and *Haemaphysalis bispinosa*. The risk factor contributing to the presence of ticks in farmer pen 1 was the high density of cattle within the pen. This pen housed up to 200 cattle, resulting in higher density compared to other pens. This facilitated the spread of ticks among cattle, primarily observed in farmer pen 1. This finding aligned with the statement from Rustam et al. (2018) that the population density could influence the spread of *Boophilus* sp. ticks.

Theileria sp. infection, when categorized by sex, can be seen in Table 1, where all positive samples belonged to female cows, resulting in a theileriosis prevalence of 32.43% (12 out of 37) among female and 0% among male cattle. This indicated a higher prevalence of *Theileria* sp. infections in female cows than in bulls. This discrepancy may have been caused by female cows experiencing stress more frequently than their male counterparts. Increased stress levels in cows can lower their resistance to disease, making them more susceptible to *Theileria* sp. infection. This is consistent with the findings of Anggraini *et al.* (2019), who reported a higher prevalence of blood parasite infections in female cattle. The higher prevalence in female cows may have been attributed to the stress associated with pregnancy, calving, and lactation, periods during which female cows were more vulnerable to disease. According to Rustam *et al.* (2018), hormonal factors such as prolactin and progesterone can increase female animals' susceptibility to parasite infestations. Female animalss are more prone to stress than males, and processes like pregnancy, calving, and lactation often lead to heightened stress levels. This stress factor induces hormonal changes and weakens the cow's immunity, making female cows more susceptible to parasites.

Additionally, Selim et al. (2022) found a higher prevalence of theileriosis in female cattle, which can be attributed to stressors such as pregnancy, childbirth, and milk production.

Theileria sp. infection, when categorized by age, can also be seen in Table 1, where the cattle were categorized into young (<2 years) and adult (≥ 2 years) cattle. The results showed that *Theileria* sp. infection in the <2 years category (20%) was lower than in the ≥ 2 years category (39%). The lower prevalence of *Theileria* sp. infection in young cattle can be attributed to the passive immunity they received from their mothers via colostrum. This passive immunity played a crucial role in strengthening their immune system, providing resistance against diseases, including parasitic infections. In contrast, adult animals began to lose this passive immunity, rendering them more susceptible to diseases. This is in accordance with Anggraini *et al.* (2019), who suggested that young livestock acquired antibodies from their mothers, enhancing their resistance to parasitic infections. Antibodies from infected parents could be transmitted to calves through colostrum, providing protection against infections. The passive immunity began to disappear when the cattle were more than one year old, so they tended to be more infected than young cattle that still had antibodies from their mothers. In addition, adult livestock are more likely to produce offspring, further increasing the risk of parasitic infections. In the female cattle category, five positive samples were recorded. According to Budiati (2002), this may have happened because to parasite infection occured during the weaning process or when the young animals were transitioning from maternal milk to other feeds, leading to a decline in maternal antibody levels from colostrum.

Negative samples may also occur because the cattle were infected but had low parasite concentrations in blood (low parasitemia levels) at the time of sampling. Thus, the microscopic examination found did not find any *Theileria* sp., leading to what is known as a false negative result. A false negative result occurs when a sampled cattle is actually infected or sick, but the test results indicate otherwise. The limitations of the examination method also play a role. While blood smear examination is a widely used technique for detecting blood parasites, it may be less sensitive when used to test samples with low levels of parasitemia. According to Chauhan (2015), thin blood smear examination is a laboratory test with low sensitivity, making it challenging to identify cattle with low levels of parasitemia. Akbari *et al.* (2018) highlighted various diagnostic methods used to detect the presence of blood parasites, but the most common method is blood smear examination using Giemsa staining on peripheral blood samples. This method is favored for its cost-effectiveness, simplicity, and minimal equipment requirements. However, it may result in misdiagnosis of false negatives in samples with low levels of parasitemia.

Conclusion

Theileria sp. infection was detected in cattle grazing at the Tamangapa landfill, Makassar. From 43 blood samples collected, 12 were positive for *Theileria* sp. The percentage of infected cattle was 27.91%.

Conflict of Interest

We affirm that there are no conflicts of interest, be they financial, personal, or pertaining to any relationships with other individuals or organizations, related to the material discussed in this manuscript.

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