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Potential Spread of *Trichinella* sp. Infection Due to Consumption of Wild Boar Meat among Wild Carnivores in Ragunan Zoo

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ABSTRACT

Trichinellosis is a zoonotic disease spread by consuming raw or undercooked meat containing *Trichinella sp.* larvae. The existence of *Trichinella spiralis* in Indonesia has been documented since the Dutch colonial period; however, information regarding the incidence of trichinellosis in humans and animals in this country remains limited. Recent studies indicate a rise in trichinellosis among animals, especially pigs and wild boars. This can potentially threaten human health and carnivorous wildlife that consume this meat, such us those in *ex-situ* captivity. This study aimed to identify the presence of *Trichinella sp.* larvae in wild boars hunted in Bengkulu Province, which serve as a food source for wild carnivores at the Ragunan Zoo in Jakarta. A total of 44 wild boar meat samples from Bengkulu, along with serum samples from four Bengal Tigers, two Jaguars, and one African Lion from Ragunan Zoo, were analyzed using the Indirect Enzyme-Linked Immunosorbent Assay (ELISA) technique to identify antibodies against *Trichinella sp.* Among the meat samples tested, seven (15.9%) yielded positive results, 35 (79.5%) were negative, and two (4.5%) were classified as dubious. Test on Bengal Tiger sera showed one (25%) was seropositive, while the rest were dubious. Tests on Jaguar and African Lion sera showed seronegative results. The Bengal tigers examined were born at Ragunan Zoo and were exclusively fed wild boar sourced from Bengkulu. Therefore, evidence suggests that *Trichinella sp.* infection in wild boars in Bengkulu may impact the health of wild carnivores conserved at Ragunan Zoo, Jakarta.

Keywords: *Trichinella*, Wild carnivores, Bengkulu, Ragunan Zoo

ABSTRAK

Trichinellosis merupakan penyakit zoonotik yang disebarkan oleh konsumsi daging mentah atau kurang matang yang mengandung larva cacing *Trichinella sp.* Keberadaan *Trichinella spiralis* di Indonesia telah teridentifikasi sejak masa kolonial Belanda, tetapi informasi mengenai kejadian trichinellosis pada manusia dan hewan di negara ini masih sangat terbatas. Sejumlah penelitian terkini mengindikasikan terjadinya peningkatan kasus trichinellosis pada hewan, khususnya babi dan babi hutan. Hal ini tidak hanya berpotensi mengancam kesehatan manusia, tetapi juga satwa liar karnivora yang mengonsumsi daging tersebut, termasuk satwa yang berada dalam penangkaran *ex situ*. Penelitian ini dilakukan untuk mendeteksi keberadaan larva *Trichinella sp.* pada daging babi hutan hasil buruan di Provinsi Bengkulu yang dimanfaatkan sebagai sumber pakan satwa liar karnivora di Taman Margasatwa Ragunan, Jakarta. Sebanyak 44 sampel daging babi hutan asal Bengkulu serta serum dari empat Harimau Bengala, dua Jaguar, dan satu Singa Afrika asal Taman Margasatwa Ragunan diperiksa menggunakan teknik *Indirect Enzyme Linked Immunosorbent Assay* (ELISA) untuk mendeteksi keberadaan antibodi terhadap *Trichinella sp.* Dari seluruh sampel daging yang diuji, tujuh (15,9%) menunjukkan hasil positif, 35 (79,5%) negatif, dan dua (4,5%) dubius. Pengujian serum Harimau Bengala menunjukkan satu (25%) seropositif, sedangkan sisanya dubiuus. Adapun pengujian pada serum Jaguar dan Singa Afrika menunjukkan hasil seronegatif. Harimau Bengala yang diuji merupakan individu yang lahir di Taman Margasatwa Ragunan dan hanya mendapat pasokan pakan berupa babi hutan asal Bengkulu. Oleh sebab itu, terdapat indikasi kuat bahwa infeksi *Trichinella sp.* pada babi hutan di Bengkulu dapat berpotensi memengaruhi kesehatan satwa liar karnivora yang ditangkarkan di Taman Margasatwa Ragunan, Jakarta.

Kata kunci: *Trichinella*, Satwa liar karnivora, Bengkulu, Taman Margasatwa Ragunan

INTRODUCTION

Trichinellosis is a zoonotic disease caused by infection with the *Trichinella sp.* worm. The disease is spread through the consumption of raw or undercooked meat containing *Trichinella sp.* worm larvae. This parasitic worm can infect approximately 150 species of carnivores and omnivores, including humans (Cybulska *et al.* 2016). *Trichinella*'s capacity to infect various hosts contributes to its global distribution, establishing it as a significant foodborne zoonotic agent (foodborne zoonosis) (Pozio 2007; Pozio 2019). Additionally, this ability expands the threat of trichinellosis beyond human health to include wildlife conservation, particularly carnivorous wildlife (Thompson 2018).

The first reported case of trichinellosis in pigs in Indonesia occurred in 1929 within the Tapanuli and Medan regions, currently part of North Sumatra Province (Visser and Manap 1930, Visser and Wolff 1930). Subsequently, infection with *Trichinella sp.* in dogs was reported in a survey in Tapanuli in 1932 (Seijffer 1940, Holtz 1962). Recent research conducted by Angi *et al.* (2014) showed a seroprevalence of trichinellosis in pig farms in Kupang City, East Nusa Tenggara Province, of 0.8%, while a similar study by Setyani *et al.* (2018) in Tangerang Regency, Banten Province, showed a seroprevalence of 1.25%.

Research on wild boars in Central Bengkulu District by Lestari *et al.* (2018) showed a seroprevalence of 68.2%. This finding poses a threat to the *ex-situ* conservation of carnivorous wildlife in Indonesia, considering that wild boar meat is one of the primary food sources in these conservation sites (Zahrah *et al.* 2022; Nurhidayat 2014). This study aimed to investigate the potential transmission of trichinellosis among wild carnivores in Ragunan Zoo, Jakarta, linked to the consumption of feed sourced from wild boars hunted in Bengkulu Province, and its relationship with the incidence of trichinellosis in the area of origin of the wild boars.

MATERIALS AND METHODS

Sample Collection

Wild boar meat samples were collected from frozen wild boar meat received by Ragunan Zoo and supplied by collectors residing in North Bengkulu Regency, Bengkulu Province. The collectors acquired the meat from hunters who captured wild boars in the forests and oil palm plantations of North Bengkulu. The wild boars were skinned, and their carcasses were cut into small pieces weighing 1–2 kg. The meat pieces were packaged in plastic, frozen, and stored in a freezer before being transported to Ragunan Zoo in a refrigerated vehicle and sealed by the Animal Quarantine Office.

Sample collection was conducted four times, with an interval of two to three weeks between collections, following the schedule for meat delivery to Ragunan Zoo from December 2020 to January 2021. Eleven samples weighing 20 grams were randomly collected from the wild boar meat stock at each delivery time. Samples were stored in labeled plastic clips at –30°C before use.

Serum samples were taken from the carnivore serum archive (Bengal tigers, jaguars, and African lions). All animals were bred in captivity at Ragunan Zoo and East Java Park (Table 1). Throughout their lives, the animals were fed on wild boars. Starting in 2015, they were also given additional feed in the form of broiler chicken meat. These wild animal serum samples have permitted access to genetic resources for research purposes from the Jakarta Natural Resources Conservation Agency, No. SK.26/K.13/TU/TSL/02/2021 dated February 19, 2021. Four serum samples were obtained from Bengal tigers (*Panthera tigris tigris*), two from jaguars (*Panthera onca*), and one from an African lion (*Panthera leo*). Like the meat samples, the serum samples were stored in microtubes at –30 °C before analysis.

Table 1 Details of carnivorous wildlife samples from Ragunan Zoo and serological test results for anti-*Trichinella* antibodies

Species	Code	Sex	Age	Origin	Serological test results for Trichinella
Bengal tiger	HB1	Male	19 years old	Born in Ragunan Zoo	Seronegative
Bengal tiger	HB2	Male	12 years old	Born in Ragunan Zoo	Dubious
Bengal tiger	HB3	Male	14 years old	Born in Ragunan Zoo	Seronegative
Bengal tiger	HB4	Male	12 years old	Born in Ragunan Zoo	Seropositive
Jaguar	J1	Female	19 years old	Born in Ragunan Zoo	Seronegative
Jaguar	J2	Male	17 years old	Born in Ragunan Zoo	Seronegative
African lion	S1	Male	14 years old	East Java Park	Seronegative

Sample Preparation

The meat samples were collected through an extraction process before entering the serological testing stage. The extraction process involved grinding the meat with a mortar and pestle, then squeezing it through gauze to obtain the liquid (meat juice). The meat juice was collected in a Petri dish and subsequently transferred to a 10 mL microtube with a micropipette. All collected meat juice samples were preserved at –21°C before analysis.

Indirect ELISA Serology Test

The Indirect ELISA method, utilizing the ID Screen® *Trichinella* Indirect Multi-species kit (ID Vet France), was employed to detect anti-*Trichinella sp.* antibodies in meat juice and serum samples. The test kit identified antibodies against *Trichinella sp.* in samples by utilizing *Trichinella* excretory-secretory (ES) antigens, which demonstrated sensitivity to multiple *Trichinella* species, including *T. spiralis*, *T. pseudospiralis*, *T. britovi*, and *T. nativa*. Antibodies in the sample bound to the kit's ES antigen, which subsequently bound to horseradish peroxidase (HRP)-labeled anti-ES antibodies. The HRP label interacted with a chromogenic substrate, changing color. If antibodies were absent, binding between the ES antigen and HRP-labeled anti-ES antibodies did not occur, leading to no color change. After the designated incubation period was completed, the reaction was terminated by adding a stop solution.

The optical density (OD) of the sample following the aforementioned reaction was measured using an ELISA reader at a wavelength of 450 nm. The average OD values of the positive control (ODPC) and negative control (ODNC) and the OD values of the samples were used to calculate the S/P ratio (S/P%) using Equation 1.

$$S/P\% = \frac{[OD_{sample} - OD_{NC}]}{[OD_{PC} - OD_{NC}]} \times 100 \quad (1)$$

Each sample was classified as positive, negative, or dubious based on the S/P% result. For meat juice, the classification was: positive (S/P%≥30%), negative (S/P%≤25%), dubious (25%<S/P%<30%). For serum, the classification was as follows: positive (S/P%≥60%), negative (S/P%≤50%), and dubious (50%<S/P%<60%).

Data Analysis

The research data were processed and visualized using Microsoft Excel software and then analyzed using descriptive statistics. The total positive meat samples were analyzed concerning the minimum transmission threshold for trichinellosis as established by Fichi *et al.* (2015), utilizing Minitab 18 software. This test was conducted to estimate the potential transmission of trichinellosis from wild boar meat from natural populations based on the proportion of positive samples. The proportion of positive samples obtained was evaluated against the threshold proportion using the following hypothesis, within a 95% confidence interval:

H_0: P=0.00017

H_1: P>0.00017

RESULTS

Detection of Trichinella Infection in Wild Boar Meat Samples from North Bengkulu Regency

The serological test results obtained through the indirect ELISA method on 44 samples from four collections indicated that seven samples were positive (15.9%), 35 were negative (79.5%), and two were classified as dubious (4.5%). The test results for each collection are illustrated in Figure 1. The results demonstrate a consistent presence of positive *Trichinella* samples across all collections, indicating a persistent infection within the wild boar population in the area.

Detection of Anti-Trichinella Antibodies in Serum from Carnivorous Wild Animals at Ragunan Zoo

Serological test results using the indirect ELISA method on archived serum samples from Ragunan Zoo showed one (14.3%) seropositive sample for *Trichinella* originating from a Bengal tiger. The detailed results of the serological tests on Bengal tiger serum showed one sample (25%) seropositive, two samples (50%) seronegative, and one sample (25%) dubious. All test results for Jaguar and African lion serum samples indicated an absence of specific anti-*Trichinella* antibody reactions. The findings and serological test results are detailed in Table 1.

Proportionality Test of Minimum Transmission Rate Through Wild Boar Meat

Fichi *et al.* (2015) indicate that when the prevalence of trichinellosis in wild boars is below 0.017%, the risk of transmission to humans and other animals is negligible. The proportion test conducted in this study showed a p-value close to 0. Consequently, H_0 was rejected, and H_1 was accepted at a 95% confidence interval. The findings suggest a significant probability

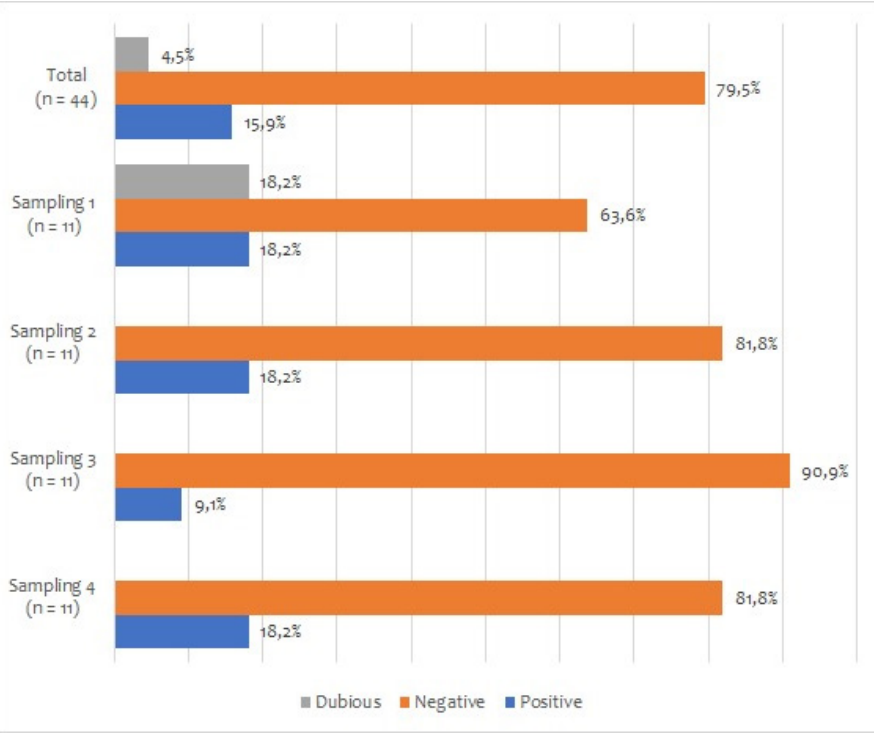

Figure 1 Details of the proportion of serological test results of wild boar meat samples from North Bengkulu Regency for anti-*Trichinella* antibodies

that the wild boar population in the field exceeds the threshold for Trichinellosis, indicating a substantial risk of transmission via wild boar meat sourced from this population.

DISCUSSION

Hunting wild boars has emerged as a source of income for individuals who sell wild boar meat, in addition to its role in controlling pests that harm food crops and plantations (Luskin *et al.* 2013). The meat is sold for consumption by specific communities and to Ragunan Zoo for carnivorous animals (Luskin *et al.* 2013, Nurhidayat 2014).

The detection of anti-*Trichinella* antibodies in wild boar meat sourced from Ragunan Zoo feed suppliers and Bengal tigers that consumed this meat suggests that trichinellosis in the wild may influence the occurrence of similar cases in *ex-situ* conservation settings. This is further supported by the finding that the seropositive tigers for *Trichinella* were captive-bred tigers from Ragunan Zoo that only consumed feed provided by their keepers. Therefore, the sole source of *Trichinella* infection was the feed.

In recent years, broiler chickens have been used as a supplementary nutritional source to address fluctuations in wild boar meat supplies. *Trichinella pseudospiralis* is the sole species capable of infecting birds, and it is endemic to Kazakhstan, Italy, Tasmania, and the United States (Pozio 2005). Therefore, chicken meat does not serve as a source of *Trichinella* infection.

This condition is directly associated with the life cycle of *Trichinella*, where transmission occurs exclusively through cysts found in the meat of infected animals. Upon ingesting meat containing *Trichinella* cysts, the cysts enter the digestive tract and are exposed to acid and pepsin in the stomach, releasing larvae from the cysts. The larvae penetrate the mucosal layer of the small intestine and mature into adult worms during the intestinal phase. After one-week, adult female worms produce larvae. The larvae subsequently migrate to tissues, such as skeletal muscle, forming cysts (Bruschi 2021; Chaudhury 2022).

Tigers with seropositivity for *Trichinella* exhibit no clinical symptoms. However, it cannot be immediately concluded that the detected antibodies originate from a past (inactive) infection. Clinical symptoms resulting from trichinellosis vary widely, ranging from asymptomatic to paralysis, and even death. This is closely related to the location of cyst formation and the stage of *Trichinella* infection in the host. The early phase of infection, when the worms are actively migrating, typically causes symptoms such as fever, headache, diarrhea, and excessive sweating (Dupouy Camet and Bruschi 2007). The nonspecific initial symptoms often result in trichinellosis going unrecognized until later symptoms develop due

to migration or the performance of blood tests. Trichinellosis can cause eosinophilia, leukocytosis, and elevated creatinine phosphokinase levels, which accompany the aforementioned symptoms (Caron *et al.* 2020; Vutova *et al.* 2020; Melese *et al.* 2021).

The primary migration pathway of *Trichinella* within the host is directed towards the muscles, especially the active muscles, including the masseter, diaphragm, extremities, tongue, and ocular muscles. This leads to myalgia, myositis, myopathy, and localized swelling at the cyst formation site. Cyst formation around the eyes may lead to periorbital edema and ocular hemorrhage (Vutova *et al.* 2020; Melese *et al.* 2021). Migration to other organs can cause different symptoms depending on the response of *Trichinella* to the environment within that organ. Migration of *Trichinella* to the liver prevents cyst formation, and the worm larvae die inside the liver. However, the migration activity leaves tissue damage in the form of "curved tunnels" visible in magnetic resonance imaging (MRI). Additionally, antigens left behind by dead larvae can trigger local inflammation (hepatitis) and even systemic inflammation (Xiong *et al.* 2021).

The migration of *Trichinella* to the nervous system, including the brain (neurotrichinellosis), allows the formation of cysts. This may induce symptoms that vary from mild neurological disorders, which are treatable, to severe disorders that can lead to fatality. The symptoms resulting from the migration process or the inflammatory response to the presence of larvae and cysts. Clinical symptoms of neurotrichinellosis generally manifest in the second or third week post-infection, although they may present earlier (Mitrović *et al.* 2019). The prognosis for recovery in hosts with neurotrichinellosis is closely related to the speed of diagnosis and appropriate treatment (anthelmintic and anti-inflammatory agents). Delayed or absent light reflexes, reduced or absent knee reflexes, and generalized tendon reflexes increase the risk of moderate mortality (20-25%). Symptoms such as mydriasis, paraparesis, dysphagia, psychomotor seizures, or delirium increase the likelihood of death by 30–45%. Anisocoria, acalculia, or seizures may also indicate an increased risk of death (Rosca *et al.* 2021).

The annual report of the Bengkulu Agricultural Quarantine Station for 2021 indicated that 68,690 kg of wild boar meat was exported from Bengkulu Province within a single year. This wild boar meat was supplied as feed for carnivorous wildlife at Ragunan Zoo and Semarang Zoo. In this study, the proportion of trichinellosis-positive samples was 15.9%. The proportion test results indicate that the prevalence of wild boars in Bengkulu exposed to trichinellosis exceeds the transmission threshold of 0.017%. It is estimated that at least 10,921 kg of wild boar meat in circulation may harbor *Trichinella* cysts. The parasite poses a significant poses a significant threat to the health of *ex-situ* conservation efforts for carnivorous wildlife; therefore, immediate implementation of control measures is essential.

The ELISA serum test results for the Bengal tiger indicated dubious findings, revealing low concentrations of antibodies. This may have resulted from low-level exposure to *Trichinella* or from exposure that occurred long ago, leading to a reduction in antibody concentrations.

Controlling trichinellosis in wild boars in their natural habitat is not feasible. Consequently, control measures must prioritize the prevention of animal consumption of meat-containing cysts within *ex-situ* conservation facilities. Enhancing the detection of trichinellosis at checkpoints, such as quarantine or procurement departments within *ex-situ* conservation institutions, can achieve this goal. The sensitivity of testing meat samples for *Trichinella* excretory-secretory (ES) antigens using ELISA ranges from 93.1% to 99.2% (Yang *et al.* 2016).

Furthermore, these initiatives must be complemented by surveillance and regular administration of anthelmintics to animals housed in *ex-situ* conservation facilities. Oral treatment with mebendazole at a dose of 83–100 mg/kg BW for 3–5 days in pigs artificially infected with *T. spiralis* significantly reduces the number of larval cysts in tissues compared to untreated controls (Fredericks *et al.* 2024). This treatment method applies to carnivorous animals at elevated risk of *Trichinella sp.* infection due to the consumption of wild boar meat.

The test results and analyses conducted in this study indicate that wild boars from North Bengkulu and Bengal tigers at Ragunan Zoo, which consumed meat from the same supplier, were exposed to trichinellosis. Therefore, *Trichinella* sp. infection in wild boars in Bengkulu could impact the health of carnivorous wildlife at Ragunan Zoo, Jakarta.

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