



Diversity of Flies and Molecular Detection in Blood Sucking Flies in Surra Endemic Area in Indonesia

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Abstract | Blood-sucking flies as mechanical vectors can transmit *Trypanosoma evansi* which causes surra disease. An increasing fly population can accelerate disease transmission and disrupt livestock feeding activities, thereby reducing livestock body weight and milk production. Traditional methods of controlling flies include cleaning the cage, using smoke from a small fire, insecticides, and fly traps. This research aims to determine the diversity of flies that have the potential to act as surra vectors and to detect molecularly the presence/absence of *T. evansi* DNA in the feeding apparatus of flies. The results of research on the diversity of flies that have the potential to be surra vectors based on research location are blood-sucking flies such as *Stomoxys calcitrans*, *Stomoxys sitiens*, *Stomoxys indica*, *Haematobia irritans*, *Tabanus rubidus*, *Tabanus megalops*. Although, molecular detection by PCR with specific primer of 927 flies feeding apparatus in Brebes, Blora, Bantul, Kulon Progo, and Kongaloko Southwest Sumba did not reveal any *T. evansi* DNA. This is because the number of pathogen (*T. evansi*) may be no present or very low parasitemia in flies feeding apparatus and very low population of flies. Flies were discovered to land more frequently on cattle body parts, particularly the head, neck, and abdomen. *Haematobia irritans* flies can serve as mechanical vectors for the transmission of ectoparasit egg, lice, and mite from one animal to another.

Keywords | Cattle, Diversity, Flies, Surra, *Trypanosoma evansi*, Vector

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INTRODUCTION

Trypanosoma evansi has the most diverse host and geographic distribution worldwide. Its epidemiology is more complex due to the variety of its hosts and vectors (Desquesnes *et al.*, 2013). One of the surra vectors that is crucial to the spread of *T. evansi* to its host is the *Tabanus*

and *Stomoxys* flies. Flies facilitate transmission by feeding on multiple animals. An increase in fly population impacts the spread of surra illness. Because they have a habit of biting flies that move while vomiting blood from prior bite wounds, *Tabanus* sp. flies, sometimes known as horse flies, can spread *T. evansi* (Nurcahyo, 2017). According to Afriyanda *et al.* (2019), a rise in the population of stable flies, or

Stomoxys sp., can hasten the spread of disease and interfere with cattle feeding operations, which lowers body weight and milk output.

Fly-related economic losses include weight loss, reduced output of meat and milk, and the potential for blood parasite infections. More than 2,000 cattle perished in Indonesia's worst surra outbreak, which struck Sumba Island between 2010 and 2012. Due to regular contact with surra vectors, farmers residing in locations where surra outbreaks are occurring are very susceptible to acquiring *T. evansi*. It indicated a high level of parasitic infections (high parasitemia) in Sumba Island (Sawitri *et al.*, 2019). Using pesticides on animals or setting traps can help manage fly populations. The Nzi and vavoua traps are the most effective traps for mechanical vectors (Desquesnes *et al.*, 2013). Natural pesticides can be created as an alternative method of controlling *Stomoxys calcitrans* and *Tabanus megalops* using *Plectranthus amboinicus* essential oil (Leesombun *et al.*, 2022). The application of smoke from a small fire as a barrier against fly bites is another conventional method of fly control (Desquesnes *et al.*, 2013). Animal activity to avoid flies includes tail wagging, skin twitching, head movement, and foot stamping. Cows frequently swing their tails to the side or back to effectively remove flies (Choudhary *et al.*, 2019). When flies are present, it's a sign of dirty cages. Consequently, preventive measures can lower the fly population by keeping the cage clean every day.

The diversity of fly species that act as disease vectors is very important to know because they play a role in the transmission of parasitic pathogens that can threaten human and animal health. One of the pathogens is *Trypanosoma evansi* which causes trypanosomiasis. This disease can cause death if not treated properly. Currently, the information available about flies as vectors in animal cages is very limited. This study addresses the gap in molecular data on *T. evansi* in Indonesian flies, a significant limitation in vector management strategies. Identifying parasitemia levels in fly vectors is critical for creating more effective vector management strategies, as high parasitic loads in flies are directly associated to disease transmission. Furthermore, studying the diversity of fly species involved in transmission would help to optimize control tactics, reducing surra's impact on animal health and the economy. This research will close a significant gap in vector management by identifying the presence of *T. evansi* DNA in fly feeding apparatus, hence decreasing the spread of this devastating sickness. Molecular identification and detection by polymerase chain reaction (PCR) of *T. evansi* in fly vectors is important for the spread of surra disease. Based on this context, a research was performed to determine the diversity of flies as vectors of *T. evansi* in order to limit the impact and choose effective control measures. In addition, the presence or absence of *T. evansi* DNA in fly feeding apparatus was determined molecularly by PCR.

ETHICAL COMMITTEE

This research has ethical clearance No. 00014/V/UN1/LPPT/EC/2023.

MATERIALS

The flies were captured in Blora (8,934 flies), Brebes (804 flies), Bantul (165 flies), Kulon Progo (115 flies), and Kongaloko Southwest Sumba (381 flies). Data of flies feeding apparatus were detected by PCR in Blora (289 flies), Brebes (235 flies), Bantul (131 flies), Kulon Progo (101 flies), and Kongaloko Southwest Sumba (171 flies). The total number of flies collected and detected by PCR from different sites (Blora, Brebes, Bantul, Kulon Progo, and Kongaloko Southwest Sumba) during the research period are shown in Table 1. External parasites transmitted by *Haematobia irritans* can bring ectoparasite egg, lice, and mite shown in Figure 1.

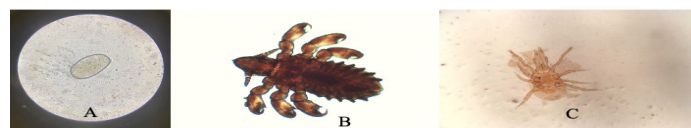


Figure 1: External parasites isolated in *Haematobia irritans*; A: Ectoparasite egg; B: Lice; C: Mite.

RESEARCH LOCATION AND SAMPLING TECHNIQUE

Locations for fly-catching were Tonjong, Brebes, and Blora Central Java; Bantul and Kulon Progo, Yogyakarta; and Kongaloko Karuni Village, Tambolaka, Southwest Sumba. The research was performed from April 2022 to October 2024.

Flies were caught using Nzi traps with urine and animal feces attractants installed $\pm$ 5 meters from the cage. Fly collection also used the insect net (sweepnet) or hand collecting methods. Flies were sorted by size and shape then identified in the field using an identification book. Flies were distinguished between blood-sucking and non-blood-sucking flies, and flies were counted based on fly species and then stored in 70% ethanol.

IDENTIFICATION OF FLIES

Identification of *Stomoxys*, *Tabanus*, *Musca domestica*, and *Haematobia irritans* can be made using various characteristics such as wing venation, abdominal pattern, and eye spacing. The several journals to identified of *Stomoxys*, *Tabanus*, *Musca domestica*, and *Haematobia irritans* based on the methods of research Qudsiyati *et al* (2023).

ISOLATION OF PARASITE FROM EXTERNAL SURFACE OF FLIES

The flies were collected in sterile specimen vials labeled

with the date and location of the isolation. Flies were retrieved and shook in normal saline for 3 minutes to eliminate parasites from their outer surface. The solution was transferred to a 1.5-ml eppendorf tube and centrifuged at 3,000 rpm for 5 minutes. The supernatant was discarded, and the sediment was transferred to a glass plate dyed with Lugol's iodine. It was examined using a light microscope at 10X for parasite detection and 40X for identification. To maximize the chances of parasite discovery, triplicate slides were made for each sample.

DNA EXTRACTION

Extraction of the mouthpart of flies with Favorgen kit. The procedure of Favorgen kit such as cut the tissue sample (head and proboscis of flies) to a sterile microcentrifuge tube in pools of 5 same species flies. DNA was extracted from pooled fly samples following the Favorgen kit protocol.

MOLECULAR DETECTION

Polymerase chain reaction (PCR) is used to identify *T. evansi* from the mouthpart DNA of flies. Forward primer 5'-CTG TTG ACA TGG GAG ATG AG-3' and reverse primer 5'-GCC TTT CCC ATT TCT CTT CC-3' were utilized for DNA amplification by PCR. A five-minute 94°C heating phase preceded 35 cycles of 30 seconds at 94°C, 30 seconds at 56°C, and 30 seconds at 72°C in the PCR. A final five-minute extension step was conducted at 72°C (Qudsiyati *et al.*, 2024). Genetica Science sequenced the results of the PCR. The nucleotide Basic Local Alignment Search Tool (BLAST) was used to validate the sequences that were found by the PCR investigation.

RESULTS AND DISCUSSION

Flies serve as mechanical vectors, transferring infections from sick cattle to healthy animals by sucking blood from the livestock's body. Flies are ectoparasites that belong to the Diptera order and undergo complete metamorphosis. The fly population can grow if it breeds in a tropical climate like Indonesia. Furthermore, the unclean condition of the cage, caused by piles of cattle waste and unused feed, serves as a breeding ground for flies. Stable flies frequently disrupt and suck the blood of livestock, resulting in painful bites and blood loss. These flies actively suck blood during the day. Blood-sucking flies are the primary vector for mechanical transmission of *T. evansi*, as the protozoa does not grow inside the body of the fly. Flies can transport *T. evansi* by sucking blood from animals and quickly moving on to new host. Flies cause blood loss, illness transmission, and pain in animals, resulting in reduced body weight and meat production.

Stomoxys is similar in size to house fly but can be easily

distinguished from its mouthparts. *Stomoxys calcitrans* was found to be the most abundant species followed by *S. sitiens*, *S. indica*, *S. bengalensis* (Masmeatathip *et al.*, 2006). *Stomoxys calcitrans* was captured throughout the year and was the most abundant species (Phasuk *et al.*, 2013). The high presence of *Stomoxys* flies may be due to suitable environmental conditions such as humidity, rainfall, and ambient temperature to maintain a good breeding habitat (Shety *et al.*, 2022).

The majority of the daily activity of the captured flies was seen in the morning, from 09.00 to 13.00. The rainy season in January in Indonesia has the greatest fly population of the year. When the humidity and rainfall levels are right, fly larvae can develop into adults in the right environmental conditions. Proper handling of fly populations should be done when the population is high (Nurcahyo, 2017). The activity of *Stomoxys calcitrans* is higher during the day. Early in the morning and right before dusk is when *Stomoxys sitiens* and *S. indicus* are most active (Lorn *et al.*, 2020).

Tabanus sp. is also known as horse fly (Diptera: Tabanidae). Urine from animals (cows, horses, sheep), octenol and phenol are effective attractants for Tabanidae (Baldacchino *et al.*, 2012). *Tabanus* sp. is rarely found in stabled horses, while many flies are found in horses tied in gardens or let loose in fields. The presence of *Tabanus* species in three different habitat types, namely primary forests, secondary forests, and villages, shows that the population of horse flies in rural areas is higher than in other habitats (Changbunjong *et al.*, 2018).

This study was conducted in five locations with dominant species caught from Bantul, *Stomoxys calcitrans* (70.91%); Kulon Progo, *Tabanus megalops* (31.30%); Kongaloko Southwest Sumba, *Musca* sp. (98.16%); Brebes, *Haematobia irritans* (72.14%) and Blora, *Haematobia irritans* (93.36%). These fly species are easily adaptable in these areas. Differences in environments between research locations indicate the diversity of fly species captured. *Musca domestica* is more commonly seen in research areas close to residential areas. The population of stable flies rose markedly in pastures or agricultural lands when cattle manure slurry was used. This is because the fly's stable antennae have olfactory sensilla that react to odors associated with hosts and their environments (Tangtrakulwanich *et al.*, 2015).

Qudsiyati *et al.* (2023) study findings (data not yet published). It is known that there is no impact of the fly population at different altitudes if the Mann Whitney test is performed. Altitude has no effect on the fly population since other factors such as vegetation type and flies' capacity to adapt to their surroundings are more important. Dominant vegetation with wild plants is positively con-

nected with increased fly numbers and species richness. Flies such as house flies (*Musca domestica*) and stable flies (*Stomoxys calcitrans*) can adapt to a variety of environmental conditions (lowlands or highlands) as long as temperature, humidity, and food availability are favorable. The amount of flies captured at both sites increases every day between 14.00 and 15.00 WIB. According to the T-test, the average species data for Andini Mulyo, Bantul and Tani Maju Jaya, Kulon Progo's *Stomoxys sitiens* and *Stomoxys indica* fly populations do not significantly differ between the two locations. Within the fly data group, the number of flies in the two locations differs significantly (*Stomoxys calcitrans*, *Tabanus rubidus*, *Musca domestica*, and *Haematobia irritans*). According to the overall number of flies, there was no discernible difference between the two locations. The Pearson correlation test results showed that there was no relationship between the number of flies and either temperature or humidity, according to the specifications of the total number of flies versus temperature and humidity. The T-test showed no significant difference in temperature, while there was a significant difference in humidity.

In this study, flies were found to land more on livestock (cow) body parts, specifically the head, neck, and abdomen. This is because the body part has a lot of sweating (body odor), which attracts flies. Livestock's head and neck are easily accessible to flies and have a lot of blood vessels near the skin's surface. The lower stomach area is frequently infested with flies because the livestock frequently lies on feces or leftover food.

Flies caught directly using sweep nets in Blora were primarily *Haematobia irritans*, and their exterior surfaces were examined for parasites. The findings of the test revealed ectoparasite egg, lice, and mite (Figure 1). This discovery shows that *Haematobia irritans* flies can act as a mechanical vector for the dissemination of ectoparasites like mites from one animal to another.

This study captured 10.399 flies from five location and detected *T. evansi* with specific primer of 927 flies feeding apparatus with PCR. Despite detecting 927 flies feeding apparatus across five sites, no *T. evansi* DNA was detected. The results of this study were obtained when the parasitemia condition and the fly population was low.

The study conducted by Phetkarl *et al.* (2023) gathered 189 biting flies, comprising five tabanid species (*Tabanus rubidus*, *Atylotus cryptotaxis*, *Tabanus mesogaeus*, *Chrysops dispar*, and *Tabanus megalops*) and four *Stomoxys* species (*Stomoxys sitiens*, *Stomoxys bengalensis*, *Stomoxys indicus*, and *Stomoxys calcitrans*). The most prevalent species among the flies gathered was *Stomoxys calcitrans*, which accounted for 58.7% (n=111). The 18S rRNA gene of *Babesia* /*Theileria* species was detected in 10 (12.4%) of the 81 samples (individu-

als and groups) that were subjected to Polymerase Chain Reaction (PCR). In none of the samples was *Trypanosoma* DNA found.

Sontigun *et al.* (2022) used PCR to detect DNA *Trypanosoma* spp., *Anaplasma* spp., *Theileria* spp., *Ehrlichia* spp., and *Babesia* spp. in the blood of cattle and Tabanid flies. A total of 279 female tabanid flies from five species were collected: *Chrysops fuscomarginalis*, *Chrysops dispar*, *Tabanus mesogaeus*, *Tabanus rubidus*, and *Tabanus megalops*. *Tabanus megalops* was the most prevalent species, accounting for 89.2% of the collected flies (n = 249). Microscopy was less sensitive than PCR for detecting blood parasites. The PCR approach demonstrated that 76.6% of *T. megalops* included at least one pathogen (*Babesia*, *Anaplasma*, *Theileria* or *Ehrlichia*). Furthermore, all beef cattle tested positive for various diseases (*Babesia bovis*, *Babesia bigemina*, *Theileria* spp., *Ehrlichia* spp., and *Anaplasma marginale*).

The absence of *Trypanosoma* DNA in the blood of cattle and *T. megalops* flies was one of the findings of the research conducted by Sontigun *et al.* (2022). Although the amount of each pathogen per insect was not determined in this investigation, the PCR results for every blood parasite species examined were negative. This implies that there might not be many pathogens. Furthermore, there were fewer tabanids caught than there were host animals in the area (more than 200 animals). As a result, *T. megalops* can have a low mechanical vector. Because of their big mouths, long-distance movement, and irregular feeding schedules, tabanid flies act as mechanical vectors. The pathogen titer, vector density, host density, and pathogen survival in the vector's mouth and intestines all affect the likelihood of successful mechanical hemopathogen transmission by tabanids (Sontigun *et al.*, 2022).

Trypanosoma DNA was detected in 9.82% of Zambian tabanid fly vectors (14/157). Only *Tabanus taeniola* (17.07%) and *Tabanus par* (87.50%) were found to be infected with *Trypanosoma*. Numerous factors influence the parasite's mechanical transmission by tabanid flies. First, for the tabanid fly to get contaminated during a blood meal, the host's blood must have a high degree of parasitemia. Second, the vector fly and the host animal must be in close proximity to one another and there must be a large density of possible mechanical vectors. Third, a fly's biology (the size of its mouthparts) indicates that more parasites are mechanically transmitted by larger mouthparts. Furthermore, the fly can regurgitate contaminated blood into its host, allowing for delayed transmission. The parasite survival duration in the mouthparts is 24 hours, whereas it is 5–7 days in the crop and stomach (Taioe *et al.*, 2017). *Trypanosoma evansi* DNA was initially discovered by molecular detection (PCR) in the feeding apparatus of South American *Dichelacera alcornis* and *Dichelacera januarii* in the work by Ramos *et al.* (2023).

Table 1: The diversity and molecular detection of flies during the research period.

Location of Sampling	Environmental conditions	Fly species caught	Molecular detection (PCR) on flies	Result
Andini Mu-lyo, Bantul, Yogyakarta	Near residential areas and rice field	<i>Stomoxys calcitrans</i> (117 flies/ 70,91%); <i>Stomoxys sitiens</i> (1 fly/0,61%); <i>Stomoxys indica</i> (13 flies/7,88%); <i>Tabanus rubidus</i> (3 flies/1,82%); <i>Haematobia irritans</i> (2 flies/1,21%); <i>Musca domestica</i> (29 flies/17,58%).	<i>Stomoxys calcitrans</i> (115 flies), <i>Stomoxys sitiens</i> (1 fly), <i>Stomoxys indica</i> (10 flies), <i>Tabanus rubidus</i> (3 flies), <i>Haematobia irritans</i> (2 flies).	Negative or It did not find <i>T. evansi</i> in apparatus feeding of flies species.
Tani Maju Jaya, Kulon Progo, Yogyakarta	Near residential areas and wild plants	<i>Stomoxys calcitrans</i> (17 flies/ 14,78%); <i>Stomoxys sitiens</i> (1 fly; 0,87%); <i>Stomoxys indica</i> (28 flies/ 24,35%); <i>Tabanus megalops</i> (36 flies/ 31,30%); <i>Tabanus rubidus</i> (17 flies/ 14,78%); <i>Musca domestica</i> (5 flies/ 4,35%); <i>Haematobia irritans</i> (10 flies/ 8,7%); <i>Tabanus</i> sp. (1fly/ 0,87%).	<i>Stomoxys calcitrans</i> (15 flies); <i>Stomoxys sitiens</i> (1 fly); <i>Stomoxys indica</i> (25 flies); <i>Tabanus megalops</i> (35 flies); <i>Tabanus rubidus</i> (15 flies); <i>Haematobia irritans</i> (10 flies).	Negative or It did not find <i>T. evansi</i> in apparatus feeding of flies species.
Kongaloko, Southwest Sumba	Near residential and campus areas	<i>Musca</i> sp. (374 flies/98,16%), <i>Tabanus megalops</i> (1 fly/0,26%), <i>Stomoxys calcitrans</i> (6 flies/1,57%).	<i>Musca</i> sp. (165 flies), <i>Tabanus megalops</i> (1 fly), <i>Stomoxys calcitrans</i> (5 flies).	Negative or It did not find <i>T. evansi</i> in apparatus feeding of flies species.
Brebes, Central Java	Rice field	<i>Stomoxys calcitrans</i> (140 flies/17,41%); <i>Haematobia irritans</i> (580 flies/72.14%); <i>Tabanus megalops</i> (12 flies/1.49%); <i>Tabanus rubidus</i> (27 flies/3.36%), <i>Musca domestica</i> (45 flies/ 5,60%).	<i>Stomoxys calcitrans</i> (55 flies); <i>Haematobia irritans</i> (155 flies); <i>Tabanus megalops</i> (10 flies) and <i>Tabanus rubidus</i> (15 flies).	Negative or It did not find <i>T. evansi</i> in apparatus feeding of flies species.
Blora, Central Java	Tobacco and corn field	<i>Haematobia irritans</i> (8.609 flies; 96,36%), <i>Tabanus megalops</i> (19 flies; 0,21%), <i>Tabanus rubidus</i> (4 flies; 0,04%), <i>Stomoxys calcitrans</i> (30 flies; 0,34%), <i>Musca domestica</i> (272 flies; 3,04%).	<i>Haematobia irritans</i> (240 flies), <i>Stomoxys calcitrans</i> (30 flies), <i>Tabanus megalops</i> (15 flies), <i>Tabanus rubidus</i> (4 flies).	Negative or It did not find <i>T. evansi</i> in apparatus feeding of flies species.

The findings of this study (Table 1) are similar to the findings of Phetkarl *et al.* (2023) and Sontigun *et al.* (2022) that used PCR to find no *Trypanosoma* in flies in Thailand because the number of pathogens (*Trypanosoma*) in flies may be very low, the vector density (number of flies caught) is low, the survival of pathogens (*Trypanosoma* spp.) in the vector's mouth and intestines, host density, and pathogen titer. In contrast, Taioe *et al.* (2017) reported that *Trypanosoma* was detected in *Tabanus taeniola* and *Tabanus par*, but exclusively in these two species. In this investigation, no *Tabanus* species (*Tabanus taeniola* and *Tabanus par*) were discovered in Indonesia. This is similar to what occurred in the Ramos *et al.* (2023) investigation, which detected no vectors of *Dichelacera januarii* or *Dichelacera alcicornis* in Indonesia. The type of vector present in the environment also affects the likelihood of *Trypanosoma* in flies. This happens when the flies, *Stomoxys* and *Tabanus megalops*, which are common in Thailand, do not have *Trypanosoma*. These vectors are likewise prevalent in this study location, and the findings also indicate that flies do not harbor *Trypanosoma*.

Crooft *et al.*, (2017) study had two samples SU-318 and SU-319 isolated from tabanid flies collected in the field, positive in TBR-PCR but negative in ITS1-PCR. This is because the amount of DNA is very low due to very low parasitemia levels thus not enough to perform PCR, failure of ITS-1 amplification may be a specific mutation in the ITS rDNA region for these samples that prevents primer

annealing and amplification procedures, and invertebrate vectors may have different substances contained in the DNA preparation may have some inhibitory effects on the PCR reaction, different from DNA isolated from mammalian hosts.

The vector biting rate, which is calculated as the inverse of the interval between consecutive blood feedings, is one of the most crucial variables in the management of vector-borne infections. The vector species determines the trypanosomes' ability to survive outside of their host. *Trypanosoma evansi* is kept in camel herds without infecting other livestock nearby because motile and viable parasites of the species live on the proboscis of *Stomoxys* spp. for approximately five to seven minutes after eating (Rodriguez *et al.*, 2014).

CONCLUSION AND RECOMMENDATIONS

Bloodsucking flies, including *Stomoxys calcitrans*, *Stomoxys sitiens*, *Stomoxys indica*, *Haematobia irritans*, *Tabanus rubidus*, and *Tabanus megalops*, have been identified as probable surra vectors based on research site. However, no *T. evansi* DNA was found in the molecular detection of blood-sucking flies' feeding apparatus in Brebes, Blora, Bantul, Kulon Progo, and Kongaloko Southwest Sumba. Governments should emphasize improving the management of livestock

traffic, upgrading vector surra research such as research on the diversity of flies as surra vectors, research related to the potential of plants to become insecticides, developing vector management strategies tailored to Indonesian farms, and providing training programs to prevent blood-sucking flies from feeding on livestock. Future research should confirm these findings such as identify the variety of flies with larger sample sizes that could serve as surra vectors at different study sites by PCR or other advanced molecular techniques.

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NOVELTY STATEMENT

The novelty of this study found that there was no *T. evansi* DNA found in the bloodsucking flies' feeding apparatus in Brebes, Blora, Bantul, Kulon Progo, and Kongaloko Southwest Sumba, according to molecular detection. New information on *Haematobia irritans* as mechanical vector to transfer ectoparasite egg, lice, and mite from one animal to another.

AUTHOR'S CONTRIBUTIONS

Nila Qudsiyati : Writing original draft, conceptualization, data curation, formal analysis, visualization.

Raden Wisnu Nurcahyo : Conceptualization, funding acquisition, supervision, project administration.

Dwi Priowidodo : Investigation, Methodology, supervision, validation.

Soedarmanto Indarjulianto : Data curation, investigation, supervision, validation.

CONFLICT OF INTEREST

All authors declare that they have no conflicts of interest.

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