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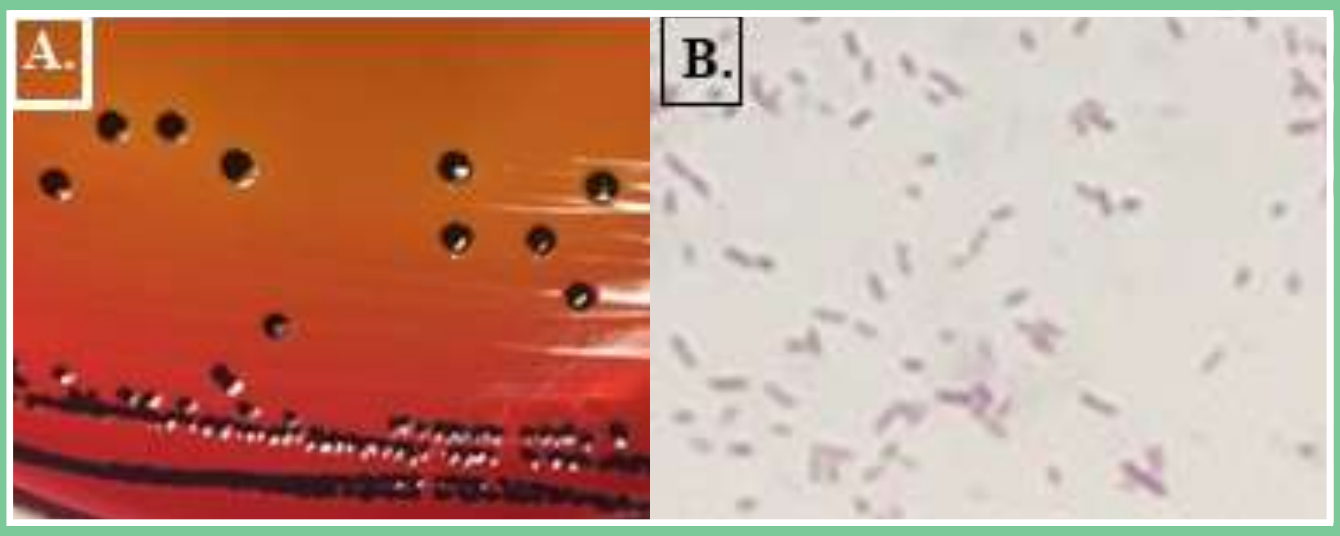

Faculty of Veterinary Medicine, Universitas Gadjah Mada,
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Editorial Team

TABLE OF CONTENS

Figure: Morphology of S.typhimurium colonies colored red with a black dot in the middle on XLD media (A) and Morphology of short rods and red colored with Gram staining (B)

Greetings from the Editor

Dear our respected IJVS readers,

All Editors and the Editorial Team of the Indonesian Journal of Veterinary Sciences would like to express the gratitude and high appreciation to the authors for the articles that have been sent to us. This September issue (Volume 2, number 2, 2021) contains 6 articles on the topic of herbal potency against fungi and bacteria, herbal toxicity testing, case studies of dermatophytosis in dogs, molecular and serological diagnosis of Bovine Herpes Virus, and the effect of the dry period on productivity and health of dairy cows.

We hope that IJVS will continue to publish by presenting quality articles in the field of Animal Health and science related to Animal Health, and can provide this scientific insight to all readers at the national and international levels.

Best Regards

Editor In Chief

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Antifungal Activity Test of the Lavender Essential Oil (*Lavandula Angustifolia)* against *Trichophyton Mentagrophytes*

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Abstract

Dermatophytosis is a zoonotic skin disease generally found in pets and humans caused by fungal colonization of dermatophytes, such as *Trichophyton mentagrophytes*. Treatment for dermatophytosis is still a challenge due to the antifungal resistance and toxicities of antifungal drugs. Lavender essential oil (*Lavandula angustifolia*) contains active compounds such as *linalyl acetate*, *linalool*, *cineole*, *camphor*, and *lavandulyl acetate* which are the active chemicals that inhibit the growth of fungi. This research aims to determine antifungal activity test of lavender essential oil in inhibiting the growth of the fungus *T. mentagrophytes*. The antifungal activity of lavender essential oil was investigated using agar well diffusion method which utilizes *Sabouraud Dextrose Agar* (SDA) as a medium with a variety of lavender essential oil concentrations (5, 10, 20, 40, 60, 80, and 100%). The agar is incubated and the inhibition zone is measured every 24, 48, and 72 hours. The results were descriptively analyzed based on the inhibition zone formed around the agar well. The results show that lavender essential oil at the concentrations of 5, 10, 20, 40, 60, 80, and 100% were able to inhibit the growth of *T. mentagrophytes*. This result concludes that lavender essential oil at the concentration of 5% is considered as the effective concentration for dermatophytosis treatment because it has the least level of toxicity.

Keywords: antifungal activity test, dermatophytosis, lavender essential oil, *Trichophyton mentagrophytes*

Introduction

Dermatophytosis is a skin ailment that commonly afflicts animals and poses a zoonotic threat to humans (Bond, 2010). This disease is caused by colonization of dermatophyte fungi that attack tissues containing keratin such as the stratum corneum of the skin, nails, and hair (Rosita & Kurniati, 2008). According to Bond (2010), afflicted animals will exhibit lesions that manifest as a combination of alopecia, papules, erythema, crusts, and scaling. Dermatophytosis symptoms in dogs and cats are characterized by baldness and hair loss that forms rings, hence the popular term "ringworm." Three genera, namely Epidermophyton, Trichophyton, and Microsporum, are responsible for causing dermatophytosis (Outerbridge, 2006).

Trichophyton mentagrophytes is a species of dermatophyte fungi that is widespread worldwide. This zoophilic species can infect humans and domestic animals such as cats, dogs, sheep, cows, horses, pigs, rodents, and monkeys. *T. mentagrophytes* can infect tissues such as hair, nails, or skin and subsequently develop into cylindrical lesions with smooth-walled macroconidia and characteristic microconidia.

Modern dermatophyte treatment has significantly developed, with cure rates reaching 80-90%. Effective treatment requires the regular use of drugs for the recommended period of time. The use of lavender essential oil is intended to provide a safer treatment alternative to the azole group. Essential oil is a natural extract derived from plants, particularly flowers, leaves, wood, seeds, or flower buds (Harahap et al., 2019).

Lavender essential oil has been reported to be active in fighting many species of fungi such as *Candida albicans*, *Aspergillus strains*, and *Cryptococcus neoformans*. The high content of 1,8-cineole, fenchone, and trans-α-necrodyl

acetate indicates antifungal activity against dermatophytes, yeast strains, and *Aspergillus*, with MIC ranging from 0.16 to 0.31 µL/mL. The content of carvacrol and (Z)-ocimene is effective in combating dermatophytes and *Cryptococcus neoformans*, with MIC and MLC values of 0.16 µL/mL and 0.32 µL/mL. The antifungal mechanism of lavender essential oil acts through the cytoplasmic cell membrane and causes disruption and ultimately cell death in fungal cells (Salehi et al., 2018). The purpose of this study was to determine the effectiveness of lavender essential oil against *Trichophyton mentagrophytes.*

Material and Method

The research was conducted at the Prof. Soeparwi Animal Hospital and Pharmacology Laboratory, Department of Pharmacology, Faculty of Veterinary Medicine, Gadjah Mada University, this research used to investigate the antifungal inhibition properties of Young Living Essential Oil lavender against *T. mentagrophytes*. The study was carried out from August 2021 to February 2022. The lavender essential oil was diluted with V-6 vegetable oil™ to various concentrations of 5%, 10%, 20%, 40%, 60%, 80%, and 100%. Different dilution formulations were used to test lavender essential oil with varying active compound content. The efficacy of lavender essential oil was evaluated using well diffusion testing, and its activity was compared to oils with different profiles. Lavender essential oil was applied topically using Young Living Essential Oil® as a carrier oil (Buckle, 2003).

Table 1. Multilevel dilution formulation of Lavender essential oil

Concentration	Lavender Essential Oil (µl)	Vegetable oil (µl)
100%	100	-
80%	80	20
60%	60	40
40%	40	60
20%	20	80
10%	10	90
5%	5	95

Note: Lavender essential oil is mixed with vegetable oil according to the ratio in Table1.

Sebazole® shampoo is a topical treatment for dermatophytosis in animals caused by dermatophytes. It contains 35 mg/mL econazole nitrate, 19.4 mg/mL sulphur as sodium thiosulfate, 10 mg/mL sodium salicylate, and 5 mg/mL chloroxylenol. Sebazole® Virbac with concentration 100 µl was given as the positive control.

The testing was carried out using the agar well diffusion method in SDA medium. A cotton swab was dipped in *T. mentagrophytes* suspension and spread evenly onto Sabouraud Dextrose Agar (SDA) medium. This process was repeated twice, with the swab being rotated by 60º each time to ensure even distribution (Scorzoni et al., 2007). Two wells with a diameter of 0.9 mm were made in each plate using a 68-75 µl micropipette tip. Lavender essential oil was prepared at concentrations of 100, 80, 60, 40, 20, 10, and 5% and added to the wells using a 100 µl micropipette, alongside a positive control of Sebazole® shampoo and a negative control of vegetable oil. The positive and negative controls were also added to the wells in 100 µl quantities. The plates were then incubated at 27°C for 24, 48, and 72 hours in an incubation chamber (Tahir et al., 2016). The effectiveness of the antifungal activity of lavender essential oil was determined based on the clear zone (inhibition zone) that appeared around the wells, which was measured in millimeters using a vernier caliper every day for 3 days.

The diameter measurement results are entered into the inhibition zone measurement formula:

$$ROW = \frac{absolute\ organ\ wt.}{b.\ wt.\ of\ the\ animal\ on\ sacrifice\ day}\ x\ 100$$

Note:
VD : vertical diameter (mm)
DW : diameter of the well (mm)
HD : horizontal diameter (mm)

The antifungal response inhibition zone diameter is classified into four categories based on its inhibitory activity. If the diameter of the inhibition zone is more than 20 mm, it is classified as very strong. If it is between 11-20 mm, it is classified as strong. If the diameter is between 5-10 mm, it is classified as medium, and if it is less than 5 mm, it is classified as weak (Davis and Stour, 1971).

The data was analyzed using statistical methods. One-Way Analysis of Variance (ANOVA) was used for normally distributed data, while the non-parametric Kruskal Wallis test was used for non-normally distributed data. The aim was to determine if there was a significant impact of administering different concentrations of lavender essential oil on the diameter of the inhibition zone against *T. mentagrophytes*.

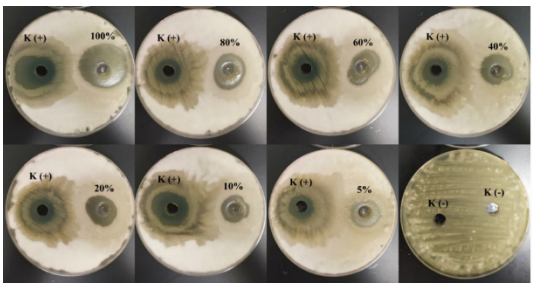
Result and Discussion

Based on the results of the study, lavender essential oil tested in vitro has been shown to have the ability to inhibit the growth of *T. mentagrophytes* fungus. This is evidenced by the formation of inhibition zones around wells that have been treated with lavender essential oil at various concentrations, as shown in Table 2.

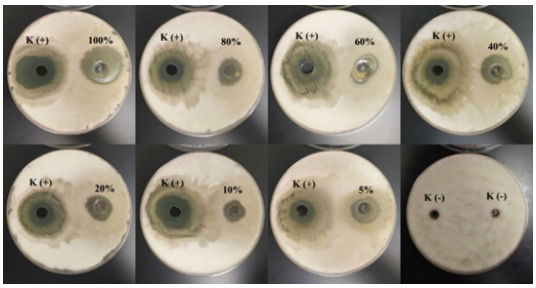
Table 2. Mean diameter of the inhibition zone (mm) and standard deviation (mm) of lavender essential oil on the growth of *T. mentagrophytes*

[1], A

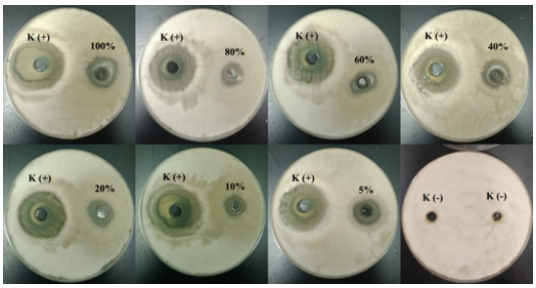
Mean and Standard Deviation of Inhibition Zone of Lavender Essential Oil (mm)				
Treatment	Day 1	Day 2	Day 3	Mean
Control (+)	29,25 ± 2,78	27,40 ± 1,99	23,20 ± 0,65	26,62 ± 3,21
100%	27,90 ± 2,48	25,40 ± 3,38	22,65 ± 3,67	25,32 ± 3,71
80%	24,50 ± 2,77	22,25 ± 1,59	20,40 ± 2,41	22,38 ± 2,75
60%	22,95 ± 1,55	19,75 ± 1,92	18,90 ± 1,77	20,53 ± 2,43
40%	22,35 ± 1,93	19,30 ± 4,43	17,75 ± 3,18	19,8 ± 3,67
20%	19,15 ± 3,10	14,60 ± 2,30	12,48 ± 5,29	15,41 ± 4,53
10%	18,3 ± 1,85	14,20 ± 2,15	12,40 ± 2,04	14,97 ± 3,16
5%	15,70 ± 2,18	13,75 ± 1,72	12,10 ± 1,59	13,85 ± 2,29
Control (-)	0 ± 0	0 ± 0	0 ± 0	0 ± 0



(a)



(b)



(c)

Figure 1. (a) Inhibition zone formed by the antifungal activity of lavender essential oil against *T. mentagrophytes* on the first day on Sabouraud Dextrose Agar (SDA) media. (b) Inhibition zone formed by the antifungal activity of lavender essential oil against *T. mentagrophytes* on the second day on Sabouraud Dextrose Agar (SDA) media. (c) Inhibition zone formed by the antifungal activity of lavender essential oil against *T. mentagrophytes* on the third day on Sabouraud Dextrose Agar (SDA) media. K (+) = positive control; 100% = 100% concentration; 80% = 80% concentration; 60% = 60% concentration; 40% = 40% concentration; 20% = 20% concentration; 10% = 10% concentration; 5% = 5% concentration; K (-) = negative control.

The inhibition zones that appeared on Sabouraud Dextrose Agar (SDA) media due to the antifungal activity of lavender essential oil against *T. mentagrophytes* on the first, second, and third day can be seen in Figure 1.

Table 2 displays the mean results obtained from the measurement of the inhibitory zone of lavender essential oil on the growth of *T. mentagrophytes*. The table demonstrates that no inhibitory zone was formed in the negative control, whereas the positive control produced the largest mean diameter of the inhibitory zone. The lavender essential oil at different concentrations exhibited varying mean diameter of the inhibitory zone. The mean inhibitory zone diameters of lavender essential oil at concentrations of 100, 80, 60, 40, 20, 10, and 5% were 25.32 ± 3.71; 22.38 ± 2.75; 20.53 ± 2.43; 19.8 ± 3.67; 15.41 ± 4.53; 14.97 ± 3.16; and 13.85 ± 2.29 mm, respectively, while that of the positive control was 26.62 ± 3.10 mm. The results of the mean data indicate that lavender essential oil possesses antifungal activity against *T. mentagrophytes* and continues to retain its capability to inhibit the growth of *T. mentagrophytes* at a concentration of 5%.

The correlation results between the inhibitory zone against *T. mentagrophytes* fungi and the concentration of lavender essential oil at 5, 10, 20, 40, 60, 80, and 100% on the first day, second day, and third day can be seen in Figure 2.

Figure 2 depicts the antifungal activity of lavender essential oil against *T. mentagrophytes* at various concentrations. The figure illustrates differences in antifungal activity across several variations of lavender essential oil concentrations, namely 5%, 10%, 20%, 40%, 60%, 80%, and 100%, as well as positive and negative controls. The positive control exhibited an average inhibition zone of 29.25 mm on the first day, 27.40 mm on the second day, and 23.30 mm on the third day, while the negative control showed an average inhibition zone of 0 mm. As shown in Figure 2, the average inhibition zone increased as the concentration of lavender essential oil increased, with respective values on the first day of 15.70 mm, 18.30 mm, 19.15 mm, 22.35 mm, 22.95 mm, 24.50 mm, and 27.90 mm for 5%, 10%, 20%, 40%, 60%, 80%, and 100% concentrations, respectively. On the second day, the respective values were 13.75 mm, 14.20 mm, 14.60 mm, 19.30 mm, 19.75 mm, 22.25 mm, and 25.40 mm, while on the third day, they were 12.1 mm, 12.4 mm, 12.48 mm, 17.75 mm, 18.9 mm, 20.40 mm, and 22.65 mm.

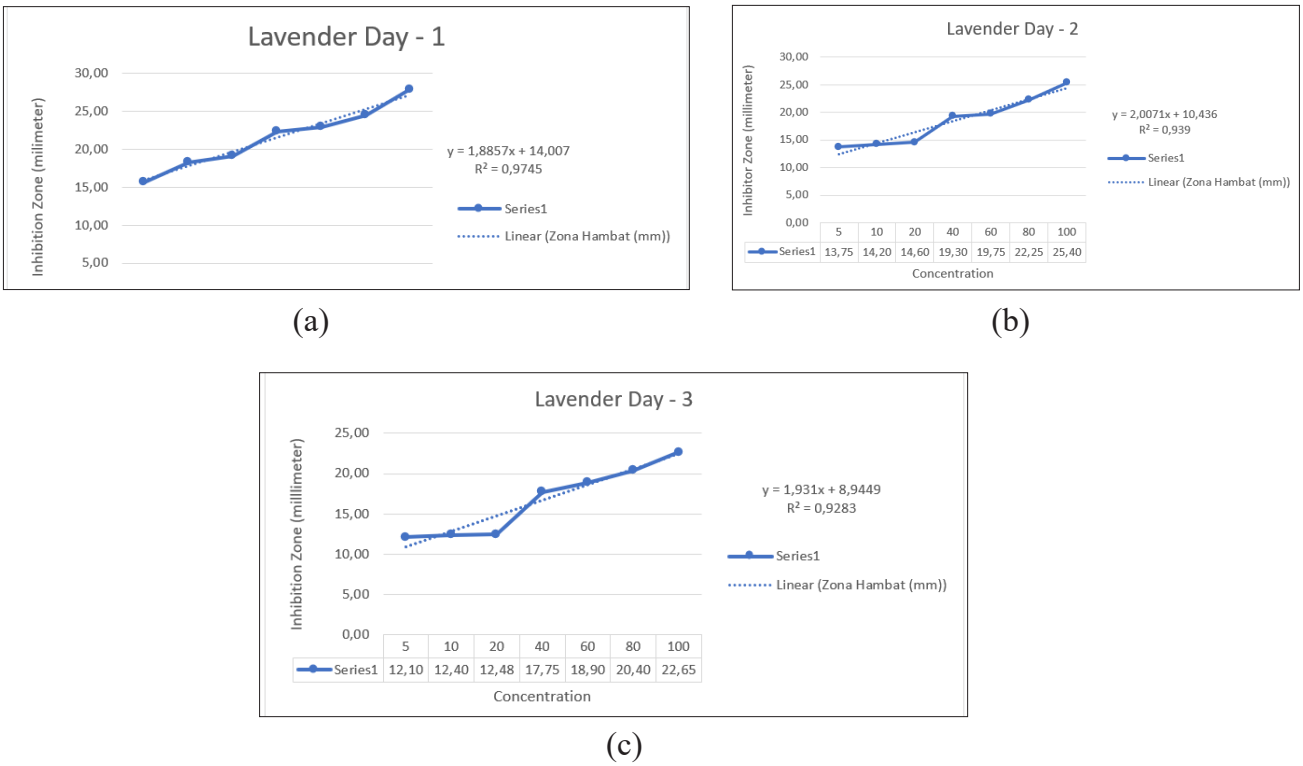


Figure 2. Correlation between the inhibition zone on *T. mentagrophytes* and the concentration of lavender essential oil at 24 hours (a); 48 hours (b); and 72 hours (c)

Table 3. Behavioral and physical observations in mice given daily oral doses (28 days) of Euphorbia hirta ethanolic leaf extract.

Observations	Control, n=5	*Euphorbia hirta* leaf extract	
		500 mg/kg, n=5	1000 mg/kg, n=5
Skin color	n	n	n
Piloerection	-	-	-
Alopecia	-	-	-
Tachypnea	-	-	-
Stool	n	w, 3/5	w, 3/5
Lacrimation	-	-	+, 3/5
Retching	-	-	-
Corneal Opacity	-	-	+, 3/5
Vocalization	-	-	-
M. membrane	n	n	n
Palpebral opening	n	n	n
Exophthalmos	-	-	-
Tremor	-	-	-
Staggering gait	-	-	-
Grip	n	N	n
Opisthotonus	-	-	-
Alertness	n	N	n
Touch response	n	n	n
Morbidity	-	-	-
Mortality	-	-	-

Legend: (n) normal; (-) absent; (+) present, (w) watery

[1], A

Figure 2 shows the relationship between x variable, which is the logarithm of lavender essential oil concentration (%), and y variable, which is the inhibition zone (mm). The calculation results on the first day yielded values of a = 14.007 and b = 1.8857 with an R^2 value of 0.9745, indicating a strong positive correlation between the two variables. On the second day, the calculation resulted in values of a = 10.436 and b = 2.0071 with an R^2 value of 0.939, indicating a strong positive correlation between the two variables. The calculation on the third day yielded values of a = 8.9449 and b = 1.931 with an R^2 value of 0.9283, indicating a strong positive correlation between the two variables. The inhibitory effect of lavender essential oil on the growth of *T. mentagrophytes* increases with increasing concentration of lavender essential oil, in accordance with Sugiyono (2012) statement that an R2 value of > 0.80 - 1.000 indicates a very strong relationship between the two variables.

The analysis of the antifungal activity test at various concentrations of essential oils on the first day was conducted using Kruskal-Wallis

Table 4. Body weights of mice given daily oral doses of Euphorbia hirta ethanolic leaf extract for 28 days.

Treatment	Mean Body Weight (grams ± SD)					% Difference in weight
	D0	D7	D14	D21	D28	
Control	21.82 ±4.03	23.91 ± 2.72	24.88 ± 2.32	25.94 ± 2.42	26.16 ± 1.73	+19.89
500 mg/kg	20.91 ±1.63	20.85 ± 1.96	20.22 ± 1.62	19.96 ± 1.65	20.44 ± 2.39	-2.25
1000 mg/kg	22.28 ± 2.59	21.47 ± 2.03	19.38 ± 4.73	20.47 ± 3.32	20.17 ± 3.55	-9.47
p-values, ns	0.94	0.42	0.69	0.52	0.26	

Legend: Values are expressed as mean ± SD; mean values within a column are not significantly different; ns – not significant, p>0.0.5

statistical test and One Way ANOVA test. The result of Kruskal-Wallis statistical test on the first day showed a significance value of 0.000, where the significance value is smaller compared to p (0.05), and the result of One Way ANOVA statistical test on the second and third days obtained a significance value of 0.000, where the significance value is smaller compared to p (0.05). Thus, the interpretation is that H0 is rejected and H1 is accepted, so it can be concluded that there is a significant difference in the antifungal potential of lavender essential oil at each concentration given.

The linear data shows a difference in the ability to inhibit the growth of fungi at each concentration. There is a decrease in the diameter of the inhibition zone from the lowest concentration to the highest concentration. The difference in the diameter of the inhibition zone can be caused by different active compound contents in each concentration.

According to Pedraza Chaverri et al. (2008), higher concentrations of lavender essential oil will result in larger zones of inhibition. When a solution has a high concentration, it will also contain higher levels of active ingredients. The numerous active compounds found in lavender essential oil increase its antifungal potential, as evidenced by the larger diameter of the zones of inhibition. The active compounds in lavender essential oil with antifungal potential are linalyl acetate, linalool, cineole, camphor, and lavandulyl acetate (Erland & Mahmoud, 2016). Linalyl acetate actively inhibits microorganism growth and inhibits the extension of fungal hyphae, while linalool has potent fungicidal activity. Linalool inhibits fungal growth by modulating the mevalonate pathway, altering intermediary molecules at the cellular level in eukaryotic cells, disrupting the cell membrane, and modulating functions related to cell permeability and signaling by creating deformities and holes in the cell wall (D'Auria et al., 2005; Rehab & Zeinab, 2016; Salehi et al., 2018).

Cineole in lavender essential oil has strong fungicidal properties. According to D'Auria et al. (2005), cineole has higher antifungal activity than camphor. Cineole works by altering the properties of fungal cell walls and triggering compensatory transcriptional responses to cell wall damage, thereby inhibiting fungal cell survival. Although cineole and linalool are minor components of lavender essential oil, they can synergize to inhibit microbe growth, according to Burt (2004).

The topical use of lavender essential oil requires knowledge of its properties and how to use it, especially its dosage and toxicity level. Lavender essential oil used topically affects the central nervous system and lymphatic circulation system as soon as it enters the dermis layer and circulates to every cell in the body (Koensoemardiyah, 2010). The effects of topical lavender essential oil exposure on cats may include hypersalivation, depression, lethargy, ataxia, weakness, tremors, muscle fasciculations, paresis, and abdominal discomfort (Bates, 2018).

Based on the data analysis, lavender essential oil with a concentration of 5% has effective antifungal properties against *T. mentagrophytes* and the lowest toxicity level, making it a safe and economical herbal remedy for treating dermatophytosis fungal infections such as *T. mentagrophytes*.

Conclusion

The research has demonstrated that lavender essential oil exhibits antifungal properties against *T. mentagrophytes* in vitro. We conducted statistical analysis which revealed a noteworthy distinction in the size of the inhibition zone between various concentrations of lavender essential oil, with a significance level of less than 0.05. Additionally, we observed a proportional increase in the diameter of the inhibition zone as the concentration of lavender essential oil increased. However, it should be noted that the diameter of the inhibition zone decreased after a 72-hour incubation period compared to the results obtained after 48 hours and 24 hours of incubation.

Acknowledgment

We express our gratitude to the Department of Pharmacology at Universitas Gadjah Mada for providing us with a venue to carry out our research. We also extend our appreciation to Dr. drh. Agustina Dwi Wijayanti, M.P., and Dr. Aria Ika Septana, M.V.P.H., for their guidance and support during the research process.

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Antibacterial Activities of Honey, Papaya Leaf, Basil Leaf, and Temu Ireng Extract on the Growth of *Salmonella typhimurium*

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Abstract

Indonesia is the second major nation in a term of its biodiversity after Brazil. Indonesia's rich biodiversity makes Indonesian herbs a prospect of developing herbal medicines for the benefit of health and industrial products. Honey, papaya leaves, basil leaves, and temu ireng are natural and herbal that have potential as an antibacterial. *Salmonella typhimurium* is a paratyphoid-causing bacteria that can harm the poultry industry. Paratyphoid is a disease that can cause weight loss and production in chickens. This study aimed to determine the antibacterial activity of honey (Kupang honey and Sumba honey), papaya leaves, basil leaves, and temu ireng to the growth of *S. typhimurium.* Reidentification of *S. typhimurium* was carried out to confirm the colony morphology, cell morphology, and biochemical characteristics. Bacteria were then cultured in Brain Heart Infusion and incubated at 37°C for 24 hours. The bacterial culture was centrifuged at a speed of 5000 rpm for 10 minutes. The precipitate formed is resuspended with sterile Phosphate Buffer Saline. The bacterial suspension is made with a concentration of 1.5 x 10^8 CFU/mL. Antibacterial activity of honey (100%), ethanol extract (100%) and aquadest extract (33,3%) from papaya leaves, basil leaves, and temu ireng against *S. typhimurium* were carried out by modified Kirby-Bauer disk diffusion method. The results of this study show that Kupang honey, Sumba honey, ethanol extract (100%) and aquadest extract (33,3%) from papaya leaves, basil leaves, and temu ireng have low antibacterial activity (<6mm). Whereas Gunung Kidul Lanceng honey (7.37mm), Lombok black honey (6.74 mm), Lombok white honey (7.74mm) and commercial honey (7.29) have moderate antibacterial activity.

Keywords: basil leaves, honey, papaya leaves, *Salmonella typhimurium,* temu ireng

Introduction

Indonesia is a country rich in biodiversity. This country is known as the second country after Brazil which has the largest biodiversity in the world. There are 30,000 types of herbs in Indonesia out of a total of around 40,000 known types of herbs in the world (BPPP, 2017; Directorate General, 2014). Abundant biodiversity makes herbs one of Indonesia's mainstay commodities (Putri *et al.,* 2014). According to the Directorate General (2014), Indonesia has prospects for developing herbal medicines for health purposes and industrial products. Herbs are not only used in human health, but herbs have started to be used in the veterinary world. Apart from having many herbal benefits it is easy to get.

Papaya leaves, basil leaves, and Intersection ireng are medicinal plants that are easy to find in Indonesia. These three herbs contain alkaloids, saponins, and flavonoids which have potential as antibacterials (Sudarwati, 2018; Angelina *et al.,* 2015; Baharun *et al.,* 2013). Apart from herbs, honey is a traditional medicine that can be used to treat digestive tract infections. Honey has flavonoid compounds and mechanisms of acidity, osmotic pressure, and hydrogen peroxidase enzymes which are antibacterial (Mulu *et al.,* 2005; Mundo *et al.,* 2004).

Salmonella typhimurium is a bacterium that causes paratyphoid which can harm the poultry industry. Paratyphoid is a disease that can cause weight loss and production in chickens. This disease can cause mortality in young chickens.

These bacteria are bacteria that contaminate feed and can live in the environment for quite a long time (Saif *et al.,* 2008; Tabbu, 2000).

Antibiotic Growth Promoter (AGP) is commonly used in animal feed to prevent infection from pathogenic bacteria (Etikaningrum and Iwantoro, 2017). Control for Disease Control (CDC) estimates that around 40% of antibiotics in the world are used as AGP. Antibiotics added to animal feed range from 2.5 to 12.5 mg/kg which is a low dose (Noor and Masniari, 2005). According to a study conducted by Etikaningrum and Iwantoro (2017) many animal products such as meat and eggs were found to have antibiotic residues. The residue is caused by excessive use of antibiotics and inappropriate use. Humans indirectly consume antibiotic residues contained in animal products, so that resistance does not only occur in animals but also in humans (Barton, 2000). Therefore, the Indonesian government has made regulations regarding the prohibition of the use of AGP in livestock. This is regulated in the Regulation of the Minister of Agriculture of the Republic of Indonesia Number 14/PERMENTAN/PK.350/5/2017 concerning the classification of veterinary drugs.

The existence of regulations regarding the prohibition of the use of AGP, the availability of Indonesia's natural biodiversity that is easy to obtain, and is known to have many uses, makes herbal a subject that can be explored more deeply for its benefits. The content of antibacterial compounds contained in the six honey, papaya leaves, basil leaves and temu ireng is expected to inhibit S. typhimurium. The three herbs are expected to be used as an alternative to AGP so as to increase selling value.

Materials and methods

This study used the pathogenic bacterium *Salmonella typhimurium* from the Center for Veterinary Research (IRCVS, Bogor, Indonesia). The bacterial media used were: Xylose Lysine Deoxychocolate Agar (XLD, OxoidTM), Mueller Hinton Agar (MHA, MecrkTM), Broth Heart Infision (BHI, MerckTM), Indole, Methyl Red, Voges Proskauer, citrate, sucrose, glucose, sorbitol, lactose, mannitol, maltose, and Tripple Sugar Iron Agar (TSIA). Kovach's reagent, methyl red, alpha naptol, 40% KOH, H2O2 were used as reagents for biochemical tests. Bacterial staining using crystal violet, lugol, alcohol, and safranin. Other materials used were blank discs (OxoidTM), antibiotic discs Trimethopim (W 5µg, OxoidTM), Phosphate Buffer Saline (PBS, Sigma-aldrich®) and sterile distilled water. Papaya leaves (Carica papaya L.), basil leaves (Ocimum tenuiflorum L.), and rhizome of temu ireng (Curcuma aeruginosa Roxb.) used came from different areas. Papaya leaves were obtained from Magelang City, Central Java; basil leaves were obtained from Pakem District, Sleman, Yogyakarta; and temu ireng rhizome obtained from Kaliurang, Sleman, Yogyakarta. The three plants were identified at the Plant Systematics Laboratory, Faculty of Biology, Gadjah Mada University, with certificate number 01417/S. Tb./X/2018; 01428/ S.Tb/ XI/2018; and 01437/ S.Tb/ XII/ 2018. The honey used is honey from Kupang, Lanceng Gunung Kidul honey, Lombok black honey, Lombok white honey, Sumba honey, and Commercial honey. The tools used were petri dishes, test tubes, glass slides, microscopes, loops, Bunsen lamps, syringes, conical tubes, ependorf tubes, calipers, and tweezers.

Re-identification of *S. typhimurium* was carried out by culturing the bacteria on XLD media which is a selective and differential medium for Salmonella sp. and incubated at 37oC for 18-24 hours. Bacteria growing on XLD media were observed for colony morphology and Gram stained to confirm cell shape. Appropriate colony forms were cultured on BHI medium and incubated at 37^{o}C for 18 – 24 hours. After the bacteria grew, the bacteria were identified by biochemical tests. Salmonella thyhimurium biochemistry tests include catalase, oxidase, motility, indole, MR-VP, and citrate (IMViC) tests, urea, Triple Sugar Iron Agar, and carbohydrate fermentation tests (glucose, sucrose, lactose, sorbitol, maltose, and mannitol).

The ethanol extracts of papaya leaves, basil leaves, and temu ireng rhizomes were carried out in the extraction process at the Integrated Research and Testing Laboratory (LPPT) of Gadjah Mada University. The method used is the maceration extraction method. Extraction was carried out using 96% ethanol solvent. Aquades extract was carried out by mixing simplicia with sterile distilled water to a concentration of 33.3% (w/v).

The solution was allowed to stand for 24 hours at room temperature while stirring occasionally, then filtered aseptically. The process of making papaya leaf simplicia, basil leaves, and temu ireng rhizomes was carried out at LPPT Gadjah Mada University. The honey used is a type of honey from different origins, including Kupang honey, Gunung Kidul Lanceng honey, Lombok black honey, Lombok white honey, Sumba honey, and Commercial honey. Honey is put into the Ependorf tube.

S. typhimurium isolates were cultured in BHI and incubated at 37oC for 24 hours. The bacterial culture was centrifuged at 5000 rpm for 10 minutes. The precipitate formed was resuspended with sterile PBS. The suspension was made at a concentration of 1.5 x 108 CFU/mL. The sensitivity test for S. typhimurium was carried out using the Kierby-bauer disk diffusion method with some modifications. Blank disks were immersed in ethanol extract with a concentration of 100%, distilled water extract with a concentration of 33.3%, and honey with a concentration of 100%, respectively. The soaked disks are placed on the surface of the MHA media which has been cultured with S. typhimurium bacteria with sufficient distance between the disks one to another (Prakasita *et al.,* 2019). Trimethoprim antibiotic disks (W 5µg, OXOIDTM) were used as positive controls and blank discs soaked in sterile distilled water were used as negative controls. The media was incubated at 37oC for 18-24 hours. The clear zone formed around the disk is measured using a caliper.

Data analysis from the test results was carried out using descriptive analysis. The results of the re-identification of bacteria were compared with the results of identification that had been carried out previously in the existing literature. Data on the test results for the antibacterial activity of honey, 100% ethanol extract and 33.3% distilled water extract from papaya leaves, basil leaves, and Intersection ireng are presented in tabular and graphical form by comparing the diameters of the inhibition zones of honey and herbs.

Results and Discussion

*Salmonella typhimurium i*s one of the pathogenic bacteria that is in the digestive tract of chickens. This bacterium is one of the causes of paratyphoid fowl (Markey *et al.,* 2013). This disease can be detrimental to chicken farmers because it causes a decrease in body weight and production of chickens (Tabbu, 2000). The use of AGP in feed additives is used to minimize infection in poultry but the use of antibiotics in the long term can cause antibiotic resistance in animals and indirectly in humans. The government has issued Regulation of the Minister of Agriculture of the Republic of Indonesia Number 14/PERMENTAN/PK.350/5/2017 concerning the classification of veterinary drugs.

The use of natural materials such as honey from different places, ethanol extract and distilled water from papaya leaves, basil leaves, and temu ireng rhizomes, in this study aims to determine antibacterial activity against the growth of S. typhimurium bacteria. The antibacterial activity was seen by the Kierby-bauer disk diffusion test of ethanol extracts of papaya leaves, basil leaves, and Intersection ireng with a concentration of 100%; distilled water extract of papaya leaves, basil leaves, and Intersection ireng with a concentration of 33.3%; and honey with a concentration of 100%.

Re-identification of *S.typhimurium* was carried out to ensure that the bacteria used were

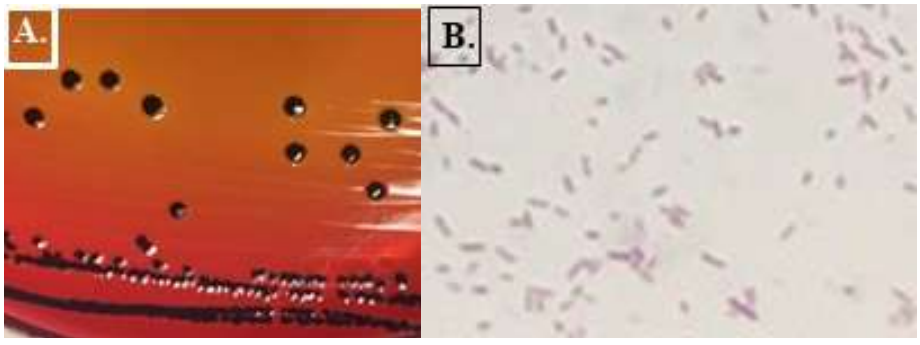

Figure 1. Morphology of S.typhimurium colonies colored red with a black dot in the middle on XLD media (A) and Morphology of short rods and red colored with Gram staining (B)

true and pure. *S.typhimurium* isolates cultured on XLD media, after incubation at 37oC for 18-24 hours, showed a round colony morphology with black dots (Figure 1). These results are in accordance with the statement of Leboffe et al. (2011) and Markey et al. (2013).

Appropriate colonies were followed by biochemical tests with catalase, oxidase, IMViC, motility, urease, TSIA, and carbohydrate fermentation test. The biochemical test results of *S. typhimurium* are in accordance with the research conducted by Markey et al. (2013), these results are presented in Table 1.

Table 1. Biochemical test results for S.typhimurium

Test	Result	Reference
Katalase	+	+
Oksidase	-	-
IMViC		
Indol	-	-
Methyl Red	+	+
Voges-proskauer	-	-
Citrate	+	+
Motilitas	+	+
Urease	-	-
TSIA		
Slant	Red	Red
Butt	Yellow	Yellow
H_2S	+	+
Fermentasi Karbohidrat		
Glukosa	+	+
Sukrosa	-	-
Laktosa	-	D
Sorbitol	+	+
Manitol	+	+
Maltosa	+	+

d = 26 -75% Positive (Markey *et al.,* 2013)

The herbal activity of papaya leaves, basil leaves, and rhizome of temu ireng and honey was examined using the Kierby-bauer disk diffusion method with modifications and carried out three times. The results showed that the antibacterial activity of ethanol extract with a concentration of 100% from papaya leaves, basil leaves, and the rhizome of Intersection Ireng on the growth of *S. typhimurium* showed an inhibition zone diameter of <6mm (Figure 3a). Aquades extract with a concentration of 33.3% also showed the same antibacterial activity (Figure 3b). Three times the test was carried out on each extract showed the diameter of the inhibition zone was less than 6 mm. The average results of three repetitions are presented in Table 2.

Table 2. Diameter of inhibition zone of papaya leaf herbal extracts, basil leaves and Intersection ireng on the growth of *S.typhimurium*

Ekstract	Inhibition zone diameter (mm)		
	Papaya leaf	Basil leave	Temu ireng
Ethanol (100%)	< 6	< 6	< 6
Aquades (33,3%)	< 6	< 6	< 6

According to Prakasita et al. (2019) antimicrobial activity was divided into high (> 11 mm), moderate (6 – 11 mm), and low (< 6 mm). These results indicate that the zone of inhibition of the three herbal extracts is categorized as low, making it less effective (Figure 3). Several previous studies have been carried out using different bacteria. Research conducted by Putri et al. (2016) stated that aquades extract of papaya leaves with concentrations of 5%, 20%, 35%, 50%, 65%, and 80% could not inhibit *P. aeruginosa* bacteria. Different results were presented by the research by Tuntun (2016) and Sudarwati (2018) which stated that the ethanol extract of papaya leaves could inhibit the growth of Salmonella typhi, Escherichia coli, and Staphylococcus aureus. Antibacterial activity test of ethanol extract of basil leaves was also carried out by Solikhah et al. (2016), the results of his research stated that ethanol extract with a concentration of 100% could inhibit the growth of *Staphylococcus aureus* but could not inhibit the growth of *E.coli*. The results of this study indicate that the antibacterial activity of an herb can be determined from the type of bacteria that is inhibited. The 100% concentration of ethanol extract of basil leaves could not inhibit Gram-negative *E. coli* but could inhibit Gram-positive *S. aureus* bacteria. The theory is explained by Brooks et al. (2007) that the type of bacteria can affect the antibacterial activity of herbs. In this study, *S. typhimurium* is a Gram-negative bacterium and its growth is not inhibited by 100% ethanol extract of basil leaves. According to Rathnayaka (2013) distilled water extract from basil can inhibit *E. coli, P. aeruginosa*, and *S. typhimurium*. Research on the antibacterial activity of temu ireng extract has not been carried out much. According to Suryani (2005) Intersection blackbird can inhibit *E. coli, Shigella dysenteriae*, and *Vibrio cholerae*. Research conducted by

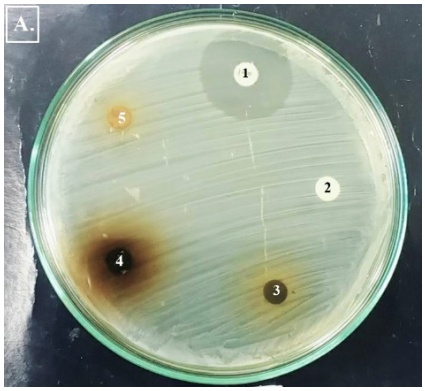

Figure 1. Growth of S. typhimurium on MHA media with trimethoprim discs (1); sterile distilled water (2); 100% concentration of papaya leaf ethanol extract (3); basil leaves (4); and meeting ireng (5) [A]

Baharun et al. (2013) stated that the essential oil of Intersection Ireng could inhibit *S. aureus* bacteria but in *B. subtilis* it had weak activity.

Many factors can affect the antibacterial activity of herbs. The manufacture of extracts greatly influences the secondary metabolites contained in herbs which are influenced by the environment in which the plants grow (Verpoorte and Alfermann, 2000). The content of secondary metabolites found in herbs is influenced by environmental factors such as climate, weather, altitude, and soil fertility (Widaryanto and Nur, 2018). The age of the plant and the part of the plant extracted also affects its antibacterial activity. The papaya leaves used are fresh old papaya leaves (Tuntun, 2016) while the basil leaves used are basil leaves and stems (Solikhah *et al.,* 2016).

The honey used comes from different places and/or different types. The honey used includes Kupang honey, Lanceng Gunung Kidul honey, Lombok black honey, Lombok white honey, Sumba honey, and commercial honey. The same test was carried out to see the activity of the six honeys with a concentration of 100% on the growth of *S. typhimurium*. The inhibition zone (clear zone) formed around the disk shows the activity of honey on bacterial growth (Figure 4). The average results of three repetitions of the honey diffusion test against *S. typhimurium* are presented in Table 3.

Lombok white honey has the largest diameter of the inhibition zone (7.74 mm) compared to other honeys. Kupang honey and Sumba honey do not have low inhibition (<6mm). Gunung Kidul

Table 3. Diameter of honey inhibition zone on the growth of S.typhimurium

Honey	Inhibition Zone Diameter (mm)					
	1	2	3	4	5	6
100%	< 6 ±.00	7.37 ±0.52	6.74 ±0.42	< 6 ±.00	7.29 ±.60	7.74 ±.16

Description: Kupang honey (1); honey Lanceng Gunung Kidul (2); Lombok black honey (3); Sumba honey (4); Super Archipelago honey (5); Lombok white honey (6)

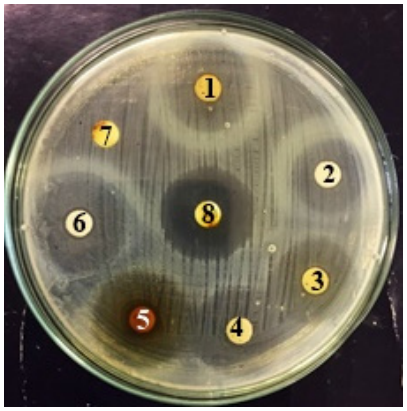

Figure 3. Growth of S. typhimurium on MHA medium with Kupang honey disc (1); honey Lanceng Gunung Kidul (2); Lombok black honey (3); Sumba honey (4); honey (5); Lombok white honey (6); sterile distilled water (7); trimethoprim (8)

Lanceng honey, Lombok black honey, commercial honey, and Lombok white honey have moderate antibacterial activity (6 – 11 mm) while Kupang honey and Sumba honey have low antibacterial activity. Other research says that honey has antibacterial activity against *P. fluorescens and P. putida* (Andriani *et al.,* 2012). According to Yuliati (2017) honey can inhibit the growth of *S. aureus* and *P. aeruginosa*. Honey is antibacterial because it has acidity, osmolarity, hydrogen peroxidase enzymes, aromatic acids, and phenols (Mundo *et al.,* 2004). The antibacterial activity of honey is influenced by the type of honey produced by bees. The source of the nectar will affect the color, taste, and components of honey (Andriani *et al.,* 2012). The results of this study prove that honey from different places has different antibacterial activities.

Conclusion

Gunung Kidul Lanceng honey, Lombok black honey, Lombok white honey, and commercial honey with a concentration of 100% had moderate antibacterial activity while Kupang honey and

Sumba honey had low antibacterial activity against the growth of *S. typhimurium*. 100% ethanol extract and 33.3% distilled water from papaya leaves, basil leaves, and temu ireng rhizomes have antibacterial activity against the low growth of *S. typhimurium*.

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Acute Toxicity Test of Tabar Kedayan Root Extract (*Aristolochia Foveolata Merr.*) In Swiss Strain Female Mice (*Mus Musculus*)

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[1], A

Abstract

The Tabar Kedayan (TK) is a plant known as an antidote for poison in the Dayak community. The part of the TK plant used widely for medication is the root. Empirically, it is known to have benefits as an antidote, anti-diarrheal, anti-pain, and anti-cancer. However, the toxicity of TK is unknown. This study was conducted to determine the toxic levels of TK root extract using the OECD 423 method. The toxicity test is a procedure used to determine the toxic levels of a chemical compound in the body through physical changes, behavior, and death of experimental animals. The experimental female mice used in the study were divided into two groups: the control group and the treatment group. Both groups contained three mice. The treatment group was given 300 mg/kg BW of TK extract orally, and the control group was given 10 ml/Kg body weight water. The treatment dose was then increased to 2000 mg/kg BW with new experimental animals because the treatment group did not show symptoms of acute intoxication at the first dose. The experimental animals were observed for 14 days, and the results showed no sign of symptoms of acute intoxication, so the TK root extract was categorized as category 5, which was not classified, with an LD50 > 2000-5000 mg/kg BW.

Keywords: acute toxicity text, LD50, Tabar Kedayan root extract, mice

Introduction

The Dayak people in Malinau District have a plant empirically believed to be a medicinal plant, namely the root of Tabar Kedayan (*Aristolochia foveolata* Merr.). Tabar Kedayan root is a plant-believed to be an anti-poison to neutralize insect poisons, snake venom, and venomous animal bites (Liwun, 2009). Tabar Kedayan root also has benefits for treating intestinal pain, gallbladder pain, arthritis, gout, and rheumatism, boosting the immune system, and reducing pain at the start of menstruation. The study by Sapri et al. (2016) showed that the root of TK has analgesic properties. Tabar Kedayan root extract doses of 25 mg/20 g BW and 50 mg/20 g BW have analgesic properties equivalent to 0.05% tramadol, while the 100 mg dose has more potent analgesic properties than 0.05% tramadol.

The TK root extract has many properties and benefits that the people of North Kalimantan empirically trust, so further testing is performed regarding the toxicity of the TK root extract before it is made into an herbal medicinal product. The acute toxicity test is the first before other toxicity tests are performed. The acute toxicity test is carried out by administering the dose according to standard instructions, then observing the first 4 hours after administration and continuing to observe toxic symptoms every 24 hours for 14 days using the Organization for Economic Cooperation and Development (OECD) 423 method. The OECD method is the test used to determine the potential for toxicity. Toxic symptoms were observed using female mice as experimental animals. Anonymous (2001) states female mice are more sensitive to toxicity exposure for toxicity tests. The toxicity test results in the LD50 form the basis for doses that cause toxic effects so people can determine the safe doses. There is no supporting data regarding acute toxicity tests on TK root extract, so research

is needed to obtain complete information regarding the safety category of drug doses and the potential for further toxicity tests.

Materials and Methods

This research was conducted at the Laboratory of the Department of Pharmacology FKH UGM, using experimental animals, namely female Swiss mice aged 2-3 months with an average weight of 28.5 grams. The materials in this study were tabar kedayan root Simplicia extracted with 70% alcohol solvent, aqua distillate, 1 ml syringe (OneMed®), latex gloves (Sensi®), masks (Sensi®), labels, plastic, aluminum foil, rubber, picric acid, filter paper.

The tools used during the study were metabolic cages, drinking bowls, water baths (Julabo PC®), mortars, tampers, sonde (oral cannula), HI 301N magnetic stirrers (Hanna Instrument®), 1000 ml beakers (Pyrex®), 100 ml beakers (Pyrex iwaki® te-32), 50 ml Duran beaker (Schott®), 25 ml measuring cup (Pyrex®) 100 ml measuring cup (Pyrex®), 50 mm funnel (Herma®), glass stirrer, digital balance (Ohaus®), ram, knife, cutting board, tray, tin, oven (Jouan®), blender (Panasonic®), 1000 ml Erlenmeyer flask (Pyrex®), plastic pot, 10 ml volumetric flask (Pyrex iwaki®), glass jar, and dropper.

The TK root was simply washed and then drained. The TK root was cut into small pieces using a knife and then placed in a baking dish. The TK roots were air-dried for a day and then put in the oven at 55°C for a day. The root has been baked, blended, and then weighed to determine the weight of the simplicial. The simplicial powder of the TK root was put in two pieces of 1000 ml beaker glasses then 70% alcohol was added. The mixture of TK root Simplicia powder and 70% ethanol was stirred using a magnetic stirrer for 30 minutes and then allowed to stand for 24 hours. The mix of TK root Simplicia powder and 70% ethanol was filtered using filter paper and put in an Erlenmeyer. The filtered liquid was evaporated in a water bath at 55°C until a thick extract was obtained. The thick extract was put in a plastic pot. Evaporation of the extract was repeated for four days.

The experimental animals were divided into two groups: a treatment group of three mice and a control group of three mice. The control group was given distilled water at 10 ml/kg BW treatment group was given TK extract with an initial dose of 300 mg/kg BW orally. Experimental animals were observed intensively for 4 hours and continued every 24 hours for 14 days. Animals were kept for physical changes, behavior, and death. If the animals show no toxicity, the dose is increased to 2000 mg/kg BW, and then observed for 14 days. The experimental animals were analyzed quantitatively and qualitatively by observing behavior and mortality and then determining the toxicity category of LD_{50} regarding OECD guideline.

Result and discussion

An acute toxicity test is a test that is used to ensure the safety of a compound. Based on OECD 423, toxicity tests can provide information about the potential toxicity of a compound and its effect on several parameters such as age, sex, route of administration, and environmental factors. Toxicity tests can determine variables between species and between strains of microbial animals and the reactivity of animal populations. Physical observations include changes in skin and hair, eyes, mucous membranes, respiration, blood circulation, nervous system, and behavior patterns.

The quantitative data obtained from the acute toxicity test is the $_{LD50,}$ while the qualitative data is in the form of changes in behavior and symptoms of the toxic effects of the test compounds that appear. LD_{50} is defined as a single dose of a substance that is statistically expected to kill 50% of experimental animals. The LD_{50} data obtained is used to determine the toxicity potential of the acute compound relative to other compounds, or it can also be used to estimate doses for other toxicological tests (Donatus, 2005).

Classification of toxicity level categories from OECD 423 (2001) in Table 1.

a. Category 1 (0-5 mg/kg)
b. Category 2 (5-50 mg/kg)
c. Category 3 (50-300 mg/kg)
d. Category 4 (300-2000 mg/kg)
e. Category 5 (2000-5000 mg/kg Unclassified)

The LD_{50} value can be related to the ED_{50} value (a dose that can provide a therapeutic effect

Table 1. Globally harmonized classification system (GHS) category with $_{LD50}$ value

The number of test animals per step	Number of dosages (mg/kg BW)	Number of test animals	GHS category (mg/kg BW)	LD_{50} *cut-off* (mg/kg BW)
3	5	2-3*	Category I (>0-5)	5
3	50	2-3*	Category II (>25-50)	25-50
3	300	2-3*	Category III (>50-300)	200-300
3	2000	2-3*	Category IV (>300-2000)	500 (if 3*) 1000 (if 2*) 2000
3	2000	0-1*	Category V (>2000-5000)	2500 (if 1*) 5000 (if 0*) (unclassified)
3	5000	0*	Category VI (unclassified)	5000 (if 0*) (unclassified) [1], A

(*)test animals show toxic/death symptoms

in 50% of the population) to determine the IT (Therapeutic Index). The therapeutic index is the ratio between the dose that can cause death and the dose that can cause a therapeutic effect. The drug is considered relatively safe if the ratio between LD50 and ED50 is large. In addition, the LD_{50} is useful for determining the relationship between dose and the emergence of toxic effects such as behavior and death, knowing symptoms of acute toxicity which are useful in diagnosing poisoning, fulfilling regulatory requirements if the test substance is to be developed into a commercial drug (Priyanto, 2009).

Balazs (1970) suggested that there were toxic symptoms that could be observed in experimental animals in Table 2.

dose of 300 mg/kg BW. This dose was chosen because there is no information regarding the toxicity of the tabar kedayan root extract. The test compound was administered orally to three mice in the treatment group. Three female mice in the control group were treated with the same volume

Table 2. Toxic symptoms that can be observed in experimental animals

Organ system	Observation and inspection	General intoxication symptom
Central nervous system and somatomotor	Behavior	Changes in behavior toward observers, unusual vocalizations, restlessness
	Movement	Twitches, tremors, ataxia, catatonia, paralysis, convulsions, forced movements
	Reactivity to various stimuli	Excitement, passivity, anesthesia, hyperaesthesia
	Cerebral and spinal reflects.	Weak, none
	Muscle Toni	Stiffness, softness
Autonomy nervous system	Pupil size	Miosis, mydriatic
	secretion	Salivation, lacrimation
Respiration system	rate of breath	Bradyspnea, dyspnea
Gastrointestinal system	Intestine problems	Diarrhea, constipation, flatulence, contractions
	Feces consistency and color	Shapeless, black color
Skin and coat	Color, wholeness	Softness, redness, blistering, pylorrection
Mucosal membrane	Conjunctiva, cavum oris	Congestion, bleeding, cyanosis, yellowness
Eye	Palpebral	
	Eyeball	Exophthalmos
	Clarity	Opacity
etc	General condition	Abnormal body condition

Table 3. Results of physical, behavioral, autonomic, and somatomotor observations of TK root extract at 300 mg/kg BW and 2000 mg/kg BW.

Parameter	Observation	Aquades 10 ml/kg BB		300 mg/kg BB		2000 mg/kg BB	
		4 hours	14 days	4 hours	14 days	4 hours	14 Days
Physical appearance	Hair Color	-	-	-	-	-	-
	Eyes color	-	-	-	-	-	-
	Urine color	-	-	-	-	-	-
Behavior	Grooming	-	-	-	-	-	-
	Restless	-	-	-	-	-	-
	Passive	-	-	-	-	-	-
	Tremor	-	-	-	-	-	-
	Convulsions	-	-	-	-	-	-
Autonomy nervous system	Defecation	-	-	-	-	-	-
	Urination	-	-	-	-	-	-
	Piloerection	-	-	-	-	-	-
	Diarrhea	-	-	-	-	-	-
	Lacrimal activity	-	-	-	-	-	-
Somatomotor nervous system	Walking	-	-	-	-	-	-
	Body position	-	-	-	-	-	-
	Extremity position	-	-	-	-	-	-

Explanation: (+) show intoxication symptoms, (-) no symptoms

of distilled water. The results did not find any toxic symptoms or death in mice during the observation period. The dose was then increased to 2000 mg/kg BW and observed for 14 days. Tabar kedayan root extract was administered orally to three different mice. The results also found no toxic symptoms or death, as seen in Table 3.

Observation of physical parameters for acute toxicity testing of TK root extract doses of 300 mg/kg BW and 2000 mg/kg BW did not cause toxic symptoms. The physical parameters observed were hair, eye, and urine. The results of group observations before and after the experimental animal treatment showed that all mice have clean and white hair color. No visible dirty and dull hair was shown during the experiment. According to the results of Fitria's research (2019), unhealthy mice show physical and behavioral changes, namely coarse hair, dirty tails, and muzzles, due to reduced activity and grooming behavior. Observation of eye color showed that the mice had clear, red healthy eyes before and after treatment. According to Sirois (2016), mice have porphyrin in the Harderian gland, which causes a red color in the mice's eyes. The clinical signs of restless behavior, passivity, tremors, and convulsions in this study were observed visually.

The behavior of the mice was expected, and they did not show agitated behavior. The group of mice treated at 300 mg/kg BW showed inactive behavior for a few minutes and then became active. The group of mice treated with 2000 mg/kg BW showed inactive behavior after 5 minutes of administration of TK root extract and then became active. The mice were active, and no nerve disturbance was observed, resulting in uncontrolled movements such as tremors and convulsions, as seen in Figure 1. According to the results of an acute toxicity study by Kumar et al. (2017), mice experiencing symptoms of toxicity will exhibit uncontrollable shaking leg behavior (tremor) and muscle nerves contracting and rapid relaxation (convulsions) due to the stimulus effect on the Central Nervous System (CNS). Tabar Kedayan root extract in this study does not interfere with the Central Nervous System (CNS), so it does not cause symptoms of behavior changes.

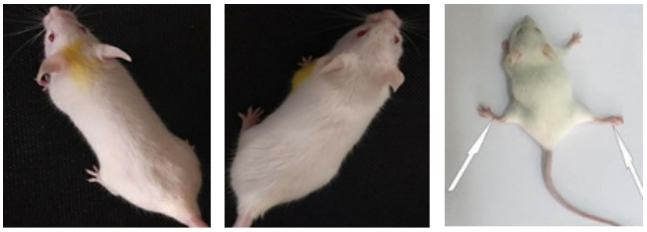

Figure 1. Observations of mice treated with 2000 mg/kg BW (left) and control mice with distilled water 10 ml/kg BW showed a normal gait and proportional limb position (middle) compared to mice given acrylamide 10 mg/kg BW experiencing muscle weakness and gait abnormal day 45 (right) (Lashein et al., 2018).

The OECD (2001) stated that female mice have a higher sensitivity to toxicity than male mice. Based on these results, TK root extract has a potential LD_{50} >2000-5000 mg/kg BW in Swiss female mice, and according to the Globally Harmonized Classification System (GHS) category in the OECD Guideline 423, it is included in category five or not classified.

Conclusion

Tabar kedayan root extract given to Swiss-strain female mice at doses of 300 mg/kg BW and 2000 mg/kg BW did not cause toxic effects and did not cause death. Tabar Kedayan root extract has an LD_{50} >2000-5000 mg/kg BW and belongs to category 5.

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Review

Canine Dermatophytosis in Indonesia, a Tropical Country, Compared to the Non-tropical Countries

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Abstract

One of the diseases that commonly affects dogs all around the world is dermatophytosis, a skin disease caused by a group of fungi called dermatophytes. It is understand that dermatophytes can be found in both the tropics and non-tropics. The aim of this review was to explore the prevalence and causative agents of dermatophytosis in canine in different regions. Articles on canine dermatophytosis from Indonesia and a few other non-tropical countries were collected. The data obtained were tabulated and discussed descriptively. The results showed that Indonesia 27/60 (45%) had almost two-fold higher prevalence of canine dermatophytosis compared to the non-tropical countries 287/1,027 (27.95%) considered. *Microsporum canis* was the main causative species found in canine dermatophytosis positive in both the tropics and non-tropics, followed by *Trichophyton* sp. Following that, *Microsporum gypseum* was among the common species found to infect dogs in the non-tropical countries. Other dermatophyte species found in the non-tropics were *Microsporum audouinii* and *Microsporum nanum*. Results obtained from this retrospective study give an illustration on the difference in prevalence and causative agents of canine dermatophytosis in tropical and non-tropical countries.

Keywords: Dermatophytes; dogs; non-tropics; tropics; prevalence

Introduction

Dermatophytosis (ringworm) is a superficial cutaneous fungal infection caused by a group of fungi called dermatophytes that not only invade the cornified layers (stratum corneum) of the skin, but also other keratinized structures like hair and nails of both animals and human (Outerbridge 2006; Quin et al., 2011; Tainwala and Sharma 2011; Weese and Fulford 2011). It is one of the common skin diseases suffered by dogs all around the world and dog patients usually present signs of itchiness in addition to having the classical ring-shaped skin lesions which can be alopecic, erythemic, and can become scaly and crusty (Ganguly and Sharma 2017; Wibisono and Putriningsih 2017). The three main genera of dermatophytes consist of *Microsporum* and *Trichophyton* that affect animals, and *Epidermophyton* that typically affects humans (Mcvey et al., 2013; Paryuni et al., 2020). In general, dermatophytes can be found worldwide but the occurrence of dermatophytosis and the preferred hosts for each dermatophyte species varies according to geographical locations (Moriello et al., 2017; Lagowski et al., 2019). Variations in climate may affect the epidemiology of dermatophytosis too (Maraki and Mavromanolaki 2016).

During rainy seasons, the incidence of canine dermatophytes in India was recorded to

be higher and few studies reported *Microsporum canis* as the predominant dermatophyte species isolated from the dogs while several others isolated *Microsporum gypseum* (Gangil et al., 2012; Singathia et al., 2014; Murmu et al., 2015; Narang et al., 2018; Parmar et al., 2018; Singh et al., 2018). A 16.8% of positive samples from dog specimens were reported by a study in Romania, while the prevalence of dermatophytosis in dogs in Iran was found to be 21.6% with *M. canis* being the predominant species, followed by *Trichophyton mentagrophytes* as well as *M. gypseum* (Yahyaraeyat et al., 2009; Nichita and Marcu 2010). Dermatophytosis cases in dogs in Russia were usually diagnosed in the autumn, with 4.5% incidence (Savinov et al., 2020). A study in Bulgaria suggested that climatic changes had an impact on local cases of canine dermatophytosis as the percentage of positive samples obtained were also higher during autumn in October and November (Michaylov et al., 2004). On the other hand, a higher incidence of *M. gypseum* infections in dogs during summer while *M. canis* in autumn and winter were reported by a study in Italy (Mancianti et al., 2002).

It was observed that the number of people having dogs as companion animals had been increasing over the years (Murray et al., 2005). Therefore, it is important for people to learn more about canine dermatophytosis, starting from the prevalence and causative species, as it is a zoonotic disease (Weese and Fulford 2011). Dermatophytosis in dogs is one of the most common cases detected by veterinarians in Indonesia but the possibility of the causative dermatophyte species being the same or different from other part of the world is still unclear. This study aims to find out the prevalence and the causative agents of canine dermatophytosis in Indonesia, a tropical country that lies on the equator, compared to non-tropical countries such as Iraq (a subtropical country in Western Asia), Turkey (a country that is situated on the border of Western Asia and Southeast Europe), and Ukraine (a country in Eastern Europe), which are all at the north of the equator, by comparing the canine dermatophytosis data obtained from articles collected from those countries that are located at different geographical regions.

Materials and Methods

This was a retrospective study involving main articles from Indonesia, Iraq, Turkey, and Ukraine found through google scholar, pubmed, sciencedirect, and researchgate (Seker and Dogan,2011; Adzima et al., 2013; Indarjulianto et al., 2014; Minnat and Khalaf (2019; Ponomarenko et al., 2019).

The reported data on the prevalence of dermatophytosis in canine and the dermatophyte species prevalent in dogs positive with dermatophytes were sorted out accordingly and tabulated in Microsoft Excel.

Results and Discussion

The prevalence of dermatophytosis in canine in Indonesia 27/60 (45%) was higher than that of Turkey 37/198 (18.69%) and Ukraine 147/664 (22.14%), while lower than that of Iraq 103/165 (62.42%). Overall, the prevalence of dermatophytosis in canine in the tropics 27/60 (45%) was higher than that of the non-tropics 287/1027 (27.95%) (Table 1).

In the tropical Indonesia, 17 (62.96%) of the 27 dogs positive with dermatophytes were

Table 1. Prevalence of dermatophytosis in canine in Indonesia (tropics) and the non-tropics

Country	Authors	No. of samples (dogs showing skin lesion), N	No. of samples positive dermatophytes, n (%)
Tropics			
Indonesia	Adzima et al., (2013)	10	10 (100%)
	Indarjulianto et al., (2014)	50	17 (34%)
	Total	60	27 (45%)
Non-tropics			
Iraq	Minnat and Khalaf (2019)	165	103 (62.42%)
Turkey	Seker and Dogan (2011)	198	37 (18.69%)
Ukraine	Ponomarenko et al., (2019)	664	147 (22.14%)
	Total	1027	287 (27.95%)

infected by *M. canis* while 10 (37.04%) other were infected by *Trichophyton* sp. In the non-tropics, 216 (75.26%) of the 287 dogs positive with dermatophytes were infected by *M. canis*, 50 (17.42%) by *Trichophyton* sp., 12 (4.18%) by *M. gypseum*, 7 (2.44%) by *M. audouinii*, and 2 (0.70%) by *M. nanum* (Table 2). The *Trichophyton* species in Indonesia was not identified but in the non-tropics, the *Trichophyton* sp. were identified as *T. mentagrophytes*, *T. rubrum*, and *T. terrestre* based on the data collected from the articles, where 9.76% of the dogs positive with dermatophytes were infected by *T. mentagrophytes*, 4.18% by *T. rubrum* while 3.48 % were infected by *T. terrestre*.

Tropical countries are countries with tropical climate as they lie across the equator (0°) and within the zone between the Tropic of Cancer and the Tropic of Capricorn which are parallel of latitude at 23° North and South respectively, meaning they get enough sunlight and are warm with an average temperature of above 20°C all year round (Oliver 2005; Morgan 2011). Non-tropical countries are those that lie outside the tropical zone and they can be in the subtropical zone, the temperate zone, or the frigid polar zone. The average temperature in the subtropical usually ranges from 10°C to above 20°C and the one in the temperate zone can be from below 10°C to above 20°C. Both zones have average temperature that varies depending on the seasonal months. The frigid polar zone on the other hand has an average temperature of below 10°C all year round (Oliver 2005).

In general, dermatophytes strive in places with warm and humid environment (Weese and Fulford 2011). However, an anomaly in Table 1 was that the prevalence of canine dermatophytosis in Iraq, one of the non-tropical countries, was shown to be higher than that of Indonesia, a tropical country. This may be due to the reason where most of the sample dogs of the study conducted in Iraq were long-haired dogs and one of the factors that have significant influence on the prevalence of dermatophytosis in dogs is hair coat. While the hair length of dogs were not discussed in the other four articles used as sample source for this study, the study by Minnat and Khalaf (2019) itself reported that dogs with long hair coat had significantly higher percentage of dermatophyte infection than dogs with short hair coat. Animals with long hair coat may be predisposed to dermatophytosis as long hair coat may protect the fungal spores from mechanical removal during licking, thus allowing the spores to remain within the hair more effectively and grow on the underlying skin (Nitta et al., 2016). A study that reported the dog breed, Yorkshire Terrier as the most common breed infected by dermatophytosis, hypothesized that the long hair coat was able to create an optimal micro-environment beneath the coat with ideal temperature and humidity

Table 2. Prevalence of dermatophyte species in canine with dermatophytosis in Indonesia (tropics) and the non-tropics.

Country	Authors	No. of samples positive dermatophytes, n	*M. canis*	*M. audouinii*	*M. gypseum*	*M. nanum*	*Trichophyton* sp.
Tropics							
Indonesia	Adzima et al., (2013)	10	NA	NA	NA	NA	10 (100%)
	Indarjulianto et al., (2014)	17	17 (100%)	NA	NA	NA	NA
	Total	27	17 (62.96%)	NA	NA	NA	10 (37.04%)
Non-tropics							
Iraq	Minnat and Khalaf (2019)	103	73 (70.87%)	7 (6.80%)	3 (2.91 %)	NA	20 (19.42%)
Turkey	Seker and Dogan (2011)	37	17 (45.95%)	NA	4 (10.81%)	2 (5.40%)	14 (37.84%)
Ukraine	Ponomarenko et al., (2019)	147	126 (85.71%)	NA	5 (3.40%)	NA	16 (10.88%)
	Total	287	216 (75.26%)	7 (2.44%)	12 (4.18%)	2 (0.70%)	50 (17.42%)

Note: NA: Data not available.

that allow fungal growth (Brilhante et al., 2003). Therefore, the anomaly where the occurrence of canine dermatophytosis in Iraq was higher than that of Indonesia may be explained by higher number of long-haired dogs was collected as samples for the study of canine dermatophytosis. This situation can be further explained by Minnat and Khalaf (2019) itself where German Shepherd, a breed with long hair coat and also a breed that made up majority of Iraqi police dogs (Tamimi and Wali 2019), made up a larger portion of the sample dogs. Removing this breed from the study will lower the prevalence of canine dermatophytosis in Iraq but still give a higher result if compared to Indonesia. This may be associated with the higher prevalence of ringworm infection in human patients in Iraq compared to Indonesia, where a study in Iraq isolated dermatophytes from 73.64% of the human patients while a study in Indonesia only reported 4.56% of dermatophytosis cases in a total of 417 cases in the medical record data (Hindy and Abiess 2019; Pravitasari et al., 2019), and it was reported that anthropophilic dermatophyte species can infect animals too when there is a contact (Kaplan and Georg 1957; Kano et al., 2010; Ganguly et al., 2017).

Despite the anomaly, overall, Indonesia had almost two-fold higher prevalence of canine dermatophytosis compared to the non-tropical countries. Indonesia has a tropical climate which is warm all year round with an average temperature of 28C and a relative humidity between 70 to 90% (Burton 2006). The results obtained demonstrated that dermatophytosis tends to be more common in warm and humid places like the tropics. For instance, studies on the prevalence of dermatophytosis in canine in other places or countries with tropical climate like Anand in Gujarat, India and Nigeria also showed a higher incidence of dermatophytosis in canine, with a result of 33.33% and 49.5% respectively (Nweze 2011; Parmar et al., 2018).

The predominant species found in canine with dermatophytosis in both the tropics and non-tropics was *M. canis*. *M. canis* is grouped as zoophilic dermatophyte species which prefer animals, especially cats and dogs as the preferred hosts and can be found worldwide (Quinn et al., 2011; Moriello et al., 2017; Lagowski et al., 2019). A study in Mexico isolated *M. canis* from 72.70% of dogs infected by dermatophytes (Torres–Guerrero et al., 2016). Several studies from India detected *M. canis* as the main causative species in dogs positive with dermatophytosis with a percentage as high as 61.1% (Murmu et al., 2015; Debnath et al., 2016; Narang et al., 2018; Singh et al., 2018). One study even reported that *M. canis* was found in all the dogs positive with dermatophyte infection (Parmar et al., 2018). *M. canis* is very common. Despite being a zoophilic species, it has zoonotic potential. *M. canis* was reported to be isolated from human patients with ringworm not only by studies in Indonesia, but also those from Iraq,Turkey, and other countries like Kuwait and Serbia (Akcaglar et al., 2011; Kadhim 2018; Karyadini et al., 2018; Hindy and Abiess 2019; AL-Khikani 2020). In Europe, it was also reported that *M. canis* was the common causative agent for tinea capitis (scalp ringworm), especially in children (Gorgievska-Sukarovska et al., 2017).

Trichophyton sp. made up the second large percentage of species found in canine with dermatophytosis in both the tropics and non-tropics. One study in India reported similar result where *Trichophyton* sp. was the most frequent fungi isolated from dogs with dermatophytosis after *M. canis* (Narang et al., 2018). The *Trichophyton* species in the non-tropics were reported to be *T. mentagrophytes*, *T. rubrum* and *T. terrestre*. *T. mentagrophytes* is one of the most common causative species in canine dermatophytosis. Like *M. canis*, this *Trichophyton* species is grouped in the zoophilic group which prefer animals as the preferred hosts and can be found worldwide (Quinn et al., 2011; Moriello et al., 2017; Lagowski et al., 2019). Therefore, *T. mentagrophytes* may also be the *Trichophyton* species isolated by the studies in Indonesia as this species is among the common dermatophyte species found in dogs (Nweze 2011; Weese and Fulford 2011; Gangil et al., 2012; Singh et al., 2018). On the other hand, *T. rubrum* is grouped as anthropophilic while *T. terrestre* is geophilic (Samanta 2015). These species however have the ability to infect animals under certain conditions or circumstances. *T. rubrum* can be found worldwide and there were studies that reported *T. rubrum* were the species

most commonly isolated from human patients with ringworm (Kadhim 2018; Parmar et al., 2018; Ryan et al., 2020). Dog patients can therefore be infected by *T. rubrum* through a direct or indirect contact with infected humans (Kano et al., 2010; Ganguly et al., 2017). The small percentage of isolated *T. terrestre* suggested that transmission of the geophilic *T. terrestre* occurs only when the dogs come in contact with contaminated soil as stated in some literature (Samanta 2015; Torres–Guerrero et al., 2016).

Following *M. canis* and *Trichophyton* sp., *M. gypseum* was among the common species found to infect dogs in all three non-tropical countries even though the percentage is low. Similar to *T. terrestre*, *M. gypseum* is grouped as geophilic dermatophyte species where the preferred habitat is the soil and can only infect the animals after direct contact (Quinn et al., 2011). A few studies even reported *M. gypseum* as the second most common species isolated from dog patients (Murmu et al., 2015; Debnath et al., 2016; Neves et al., 2018). Other dermatophyte species found in the non-tropics were *M. audouinii* and *M. nanum*. *M. audouinii* was reported as one of the causative species of canine dermatophytosis in Iraq. It is an anthropophilic species that typically affects children of age five to nine-year-old and seldom affects animals (Birchard and Sherding 2006; Sacheli et al., 2020). A case report that isolated *M. audouinii* from a ten-week-old boxer puppy suspected that the infection was transmitted through contact with two of the owner's children that had tinea capitis (scalp ringworm) some time before acquiring the dog (Kaplan and Georg 1957). Therefore, transmission of the *M. audouinii* isolated from canine patients in the study in Iraq was most likely from an infected human. Being both a geophilic and zoophilic species, *M. nanum* preferred hosts are pigs and the occurrence of the infection can be found worldwide (Birchard and Sherding 2006; Quin et al., 2011; Moriello et al., 2017). A small number of *M. nanum* were isolated from dogs with dermatophytosis in the study in Turkey. This was supported by a case study in Brazil that isolated this dermatophyte species from a 7-month-old female Dogo Argentino and the transmission was suspected to be direct contact of the dog with wild boars as this breed of dogs are usually used for wild boar hunting (Valandro et al., 2017).

The total sample size of 60 and 1027 dogs from the studies in both the tropics and non-tropics respectively were enough to provide a valid result of the study as the sample size typically used by researchers intending to use statistical analysis on their data in a study is at least 30 individuals (Cohen et al., 2011). However, some literature suggests a substantially larger number as a greater sample size provides greater power, providing a more precise and reliable estimation (Cameron 2012; Petrie and Watson 2013; Martínez–Abraín 2014). Besides that, if it were possible to have an equal sample size for both categories, the result obtained might give a more accurate representation of the comparison as groups of the same sample size give a greater statistical power. For this retrospective study, the sample articles collected were from different years of publication. There could be a less promising finding when comparing results of canine dermatophytosis studies that were performed in separate years as climate change happens over the years due to global warming, thus each country's climate condition appears to differ every year (BBC, 2020). These changes may affect the growth of dermatophytes as one of the factors that will lead to further changes in dermatophytosis epidemiology is changes in climatic conditions (Maraki and Mavromanolaki 2016). However, the term where the sample articles collected from each country were those that were published in the same year is difficult to satisfy. Therefore, for this study, articles that were published in the past ten years were used as the source of materials to ensure that the data collected were recent enough to provide up-to-date information.

Based on the articles, the samples in Indonesia were obtained from Banda Aceh and Yogyakarta, the one in Iraq were from Baghdad, those in Turkey were from Ankara and Izmir, and the one in Ukraine were from Kharkiv, that is, only one or two out of the many provinces in each countries. Consequently, the results of the studies themselves were not sufficiently complete to represent the countries as a whole, as they represented only about 2 to 6% of the total provinces of the countries mentioned. This is because the distribution of the dog population in each province may be

different. The dog population may vary based on their ownership states, that is, they are either owned dogs or unowned dogs, which in turns will influence their freedom in mobility. Other factors influencing distribution of dog population size across the provinces include human attitude and behaviour affected by religion and culture, reproductive capacity of the dog population, and zoonotic diseases which lead to controlling of the dog populations (International Companion Animal Management Coalition 2008). In Lombok Island, Indonesia, for example, the research on owned and unowned dog population estimation revealed that the trend of dog ownership differs between ethnic groups. In addition, it was recorded that the size of the dog population varies between rural and urban sites, with a greater number of unowned dogs found in urban sites (Mustiana et al., 2015). Zoonotic diseases, particularly those endemic in the province, affect dog population due to the control programs implemented by local and central governments to manage the dog population (International Companion Animal Management Coalition 2008). Those programs or methods used in managing dog population usually include culling, sheltering, and fertility control programs which would affect the population's reproductive capacity (Smith et al., 2019).

The climatic conditions between each province may also be different depending on its topography and this may as well affect the outcome of the studies in the articles themselves because as mentioned before, these changes may affect the growth of dermatophytes. Indonesia is a tropical country with wet and dry seasons, an average temperature of 28°C, a relative humidity of 70 to 90%, and yet, differences exist between the islands and within the island itself. Compared to Yogyakarta in central Java, Banda Aceh in the province of Aceh on the northern end of Sumatra has lower rainfall per year, hence getting lower humidity. Within island, the highlands in western Java have greater rainfall compared to the north-east coast of Java. One example that can be used to describe this is that Bogor has twice as many days of rainfall per year in contrast to Jakarta which is just 60 km up north (Burton 2006). Furthermore, in central Indonesia, the seasonal variations are largely due to monsoons (Lee 2015). This means that, depending on the seasons, the humidity for each province can vary. Yogyakarta in Special Region of Yogyakarta, for example, may have higher humidity compared to Surabaya in eastern Java because it has a higher rainfall count compared to Surabaya in eastern Java (Burton 2006). A study in India reported a prevalence as high as 51.1% of dermatophyte infection in canine during pre-monsoon and/or monsoon months (Singathia et al., 2014). This indicates that the variation in humidity between the provinces in Indonesia due to monsoons or rainy seasons might just play a role in the incidence of canine dermatophytosis. Iraq is a subtropical country where the lowlands and deserts are hot and dry with a long summer and short winter, while the north-eastern has an opposed climate, with longer winters and more rainfall (Rowell 2012). Based on geography, Turkey is located in the Mediterranean with a temperate climate. The complex nature of the landscape with mountains running parallel to the coasts contributes to the considerable differences in climatic conditions between regions. Western Turkey experiences a mild Mediterranean climate with hot, moderately dry summers and cool, wet winters while Eastern Turkey experiences long, cold winter with heavy snow (Sensoy et al., 2016). Ukraine on the other hand, has four agro-climatological zones where it is humid in the northwest, warm in the east and central, semi-arid in the north, and arid in the south (Food and Agriculture Organization of the United Nations 2015). These variations in the climatic conditions between provinces matter and can affect the occurrence of dermatophytosis in canine, demonstrated by a study in Minas Gerais, a state dominated with tropical climate in Brazil, showed that dermatophytes were isolated in 32.5% of the dogs while another study in São Paulo, a state with subtropical climate in Brazil, showed 29.8% (Beraldo et al., 2014; Neves et al., 2018). Therefore, the results of the studies themselves only represented a small part of the countries but not all as the prevalence of canine dermatohytosis and the dermatophyte species that infect the dogs varies according to geographical locations and climatic conditions. The available articles on canine dermatophytosis which can be used as samples are also limited in the respective countries

mentioned. Thus, the findings of this retrospective study only give an idea on how the prevalence and causative species of canine dermatophytosis may differ in the tropics and non-tropics.

Although the articles on canine dermatophytosis available to be used as sample sources are limited and the articles obtained as samples for this study were only confined to certain parts of the countries, the result obtained managed to look at how the prevalence and causative agents of canine dermatophytosis can be different in tropical and non-tropical countries.. Further study that include more sample source from different provinces in respective countries is needed to provide a more accurate and solid comparison.

Conclusion

The incidence of canine dermatophytosis and the causative species for dermatophytosis in canine is different according to geographical locations and climatic conditions. Indonesia had an almost two-fold higher prevalence of canine dermatophytosis compared to the non-tropical countries and the main dermatophyte species found to infect dog patients in both the tropics and non-tropics were *M. canis*, followed by *Trichophyton* sp. Other dermatophyte species that infect dogs in non-tropical countries were *M. gypseum*, *M. audouinii*, and *M. nanum*.

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Detection of *Bovine Herpes Virus* –I Infection Causing Infectious Bovine Rhinotracheitis in Imported Cattle: Serology and Molecular Method

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Abstract

Infectious Bovine Rhinotracheitis (IBR) is a disease in cattle caused by *Bovine Herpesvirus 1* (BHV-1). IBR disease in cattle is spread worldwide, is highly infectious, causing respiratory, reproductive, and neurological disorders. This disease is also economically disadvantageous in international trade. Traffic of cattle from abroad is still quite high, with a prevalence rate in the country of origin, Australia, of 15-96%. Subclinical infections often occur and also cause latent infections, so antigen and antibody detection is required to determine the animal's health status. This study aims to detect BHV-1 antibodies and antigens in imported cattle. Samples were taken from 25 imported cattle that entered through the port of Tanjung Intan Cilacap with two samplings. The first and second collection was carried out with an interval of 1 week. Samples taken included serum samples and nasal swabs from cows showing clinical symptoms of IBR. The sample is then tested with *Enzimes-Linked Immunosorbent Assay* (ELISA) antibodies and *real-time polymerase chain reaction* (PCR) 5% did not detect BHV-1 antigen. Samples with seronegative results, 88% detected BHV-1 antigen. The conclusion of this study was that 40% of the samples detected BHV-1 based on serology and real time PCR.

Keywords: *Bovine Herpesvirus*; Elisa; Real-Time PCR

Introduction

Infectious Bovine Rhinotracheitis (IBR) is an infectious and contagious disease in domestic cattle and wild cattle caused by a virus*Bovine Herpes Virus Type-1* (BHV-1). BHV-1 was first identified in dairy cows in California, USA, in 1953, but this virus remains a globally important pathogen and has a significant impact on health and well-being (Raaperi *et al*., 2014). BHV-1 can cause lifelong latent infection and can reactivate when an animal is stressed or exposed to corticosteroid drugs (Zhu *et al*., 2017).

According to *Office International des Epizooties* (OIE), IBR disease is one of the main problems in cattle farming, which has the potential to endanger international trade. Cattle traffic from abroad is still quite high. Indonesia is currently still importing cattle from Australia. The prevalence rate in Australia is 15-96% (AFFA., 2000). According to Sudarisman (2003), in Indonesia positive reactions to IBR serology do not only occur in imported animals but also in native Indonesian livestock. Serologically, IBR disease has existed in dairy cattle, beef cattle, and buffalo from several provinces in Indonesia with a prevalence of 5-72.9% (Sarosa, 1985). According to Naipospos (2014), all of UPT Breeding within the Directorate General of Health and Human Services (except BPTUHPT Bali Cattle) showed positive antibody titers ranging from 4-76%.

One of the duties of the Agricultural Quarantine Agency is to prevent the entry and spread of Quarantine Animal Pests (HPHK). Quarantine measures for importing beef cattle from abroad to

the territory of the Republic of Indonesia must be carried out through strict handling and inspection of large ruminants. IBR disease cannot be detected from clinical symptoms alone. The diagnosis of IBR disease has been developed in various ways, namely by virus isolation and identification, serological tests, immunoassay examinations, and detection of genetic material through molecular biology techniques. Diagnosis of BHV-1 infection can be difficult for many reasons. Subclinical disease is common, and BHV-1 can cause latent infection.

The ELISA test is a fast, inexpensive, and particular way to detect antibodies to BHV-1 (Riegel*et al.,*1987). The weakness of this test is that new antibodies can be seen through serological tests approximately 12 days after infection (Rodistits*et al.,*2006). The PCR test can confirm the diagnosis in the early stages of the disease, where the antigen has entered the body but no antibody titers have yet been formed. Animals in a state of latent infection can show positive antibodies (OIE, 2018). It is necessary to carry out serological and molecular tests to ensure the presence of antibodies and BHV-1 antigen in the sample.

This study was conducted to detect BHV-1 antibodies and antigens in the imported cattle. It was hoped that reports on the presence of IBR in imported beef cattle can be used as policy material in importing cattle.

Materials and methods

This research was approved and declared to meet ethical requirements for research on animals by the Research Ethics Commission of the Faculty of Veterinary Medicine, Gadjah Mada University, Yogyakarta, with number 0079/EC-FKH/Int./2020.

Sample

Samples were taken from 25 imported cattle that entered through the port of Tanjung Intan Cilacap with two samplings. The first and second collection was carried out with an interval of 1 week. Samples taken included serum samples and nasal swabs from cows showing clinical symptoms, namely runny nose, conjunctivitis, emaciation, and lethargy.

Materials and tools

The material used is the IBR ELISA Kit (IDEXX), which consists of a microplate that has been coated with inactive BHV-1 antigen, positive control, negative control, conjugate, diluent (2% BSA PBS), tetramethylbenzidine (TMB) substrate, reaction stop solution. (H solution$_2SO_4$), washing solution (0.05% Tween-PBS). Materials for real-time PCR include the QIAamp DNA Mini Kit (Qiagen Cat. No. 51304) consisting of QIAamp Mini Spin Columns, Collection Tubes (2 ml), Buffer AL, Buffer ATL, Buffer AW1, Buffer AW2, Buffer AE, QIAGEN® Protease, Solvent Protease, Proteinase K and*Selection Guide*. SensiFast Probe Lo-Rox Kit_terdiri dari RNase Free Water, 2X Sensifast Probe Mix, Primer Forward gBF 20 µM, Primer Reverse gBR 20 µM, dan Probe IBR 5 µM. Ethanol 96-100%,

Primer dan Probe yang digunakan sebagai berikut :Primer gB-F: 5'-TGT-GGA-CCT-AAA-CCT-CAC GGT-3' (position 57499–57519 GenBank®, accession AJ004801), Primer gB-R: 5'-GTA-GTC-GAG CAG-ACC-CGT-GTC-3' (position 57595–57575 GenBank®, accession AJ004801), TaqMan Probe: 5'- FAM-AGG-ACC-GCG-AGT-TCT-TGC-CGC-TAMRA-3' (position 57525–57545 GenBank®, accession AJ004801) . The equipment used in this study included micropipettes, analytical balances, centrifuges, incubators, ELISA readers (ELISA readers), and *Biosafety Cabinet Class II. PCR Work station,* Thermocyler, Heating Block, and PCR tube 0,2 ml

Time and Place of Research

The research was conducted from June 2020 to January 2021. Samples were taken in the form of serum and nasal swabs from imported male and female cattle that entered through the Quarantine Station.

Cilacap Class I Agriculture in June 2020. Serum sampling was carried out two times with an interval of 1 week. ELISA and Real-Time PCR tests were carried out at the Wates Veterinary Center.

Testing with ELISA

The ELISA test for IBR used in this study was the IBR gE ELISA Kit (IDEXX). All reagents and samples were removed at room temperature. The

washing buffer was prepared with a 1:10 dilution. 75 µl buffer diluent was added to all wells. Sample serum, positive and negative control serum, as much as 25 µl were added according to the layout. The microplate was covered and incubated for 60 minutes at 37°C^{O}C. The microplate was washed three times. 100 µl of conjugate was filled into the microplate, then closed and set for 60 minutes at 37^{O}C. The microplate was rewashed 3 times. 100 µl of TMB substrate was loaded into the microplate, then covered, incubated for 15 minutes at room temperature, and placed in a dark place. The stop solution was filled in as much as 100 µl., then read using an ELISA reader (ELISA reader) at a wavelength (λ) 450 nm. The ELISA result assessment is obtained from the formula below:

S/P = (sample OD – negative control OD), (OD control positive – OD control negative)

PCR Real-Time Testing

Sample Preparation. Sample preparation was carried out in the BSC *Class* II. Specimen forms nose, which is suspected to contain the BHV-1 virus. Sample *swab* in viral transport media was vortexed, then the *swab* pressed against the tube wall and *swab* then thrown away. The swab sample in transport medium sterile viral/PBS solution was centrifuged at 1,000 rpm for 10 minutes; then, the supernatant was taken. **DNA extraction according** to the QIAamp DNA Mini Kit DNA Extraction Protocol (QIAGEN, Cat. No. 51304). **Setup*Wash Buffer* 1 (WB 1).** 25 ml of 96-100% ethanol was added to 19 ml*Wash Buffer1* (WB1), available in the kit. WB1, added to ethanol, is stored at room temperature.

Preparation*Lysate.* A mixture of lysis buffer and proteinase *K* prepared according to the amount of sample to be extracted into a 2-ml tube (if the model is below 10) or 15-ml (if the example above 10) the composition is Lysis Buffer 200 µl plus*Proteinase K* 25 µl for one-time reaction/ sample.

The tube contains a mixture of lysis buffer and and*Proteinase K* transferred to space 2 µl Xeno DNA control was added to a box containing a variety of Lysis Buffer and proteinase *K.* Or add as much Xeno DNA as the number of samples (eg ten samples = 20 µl Xeno DNA), directly into a tube containing a mixture of Lysis Buffer and*Proteinase K*, then an aliquot of 227 µl mixed solution (Lysis Buffer +*Proteinase K* + Xeno DNA) as many as the number of samples to be extracted. 200 µl of each prepared piece was added to the solution mixture above. Positive and negative controls were designed by adding 200 µl IBR positive control cells and 200 µl IBR negative control cells. Incubation was carried out at 56ºC for 10 minutes, then immediately proceeded to the Binding and Washing stages.

Binding and Washing Stage

Ethanol 96-100% A total of 200 µl was added to the tube*Lysate* to obtain a final concentration of 37% ethanol, then closed the lid and mixed by vortexing for 15 seconds. The tube is centrifuged briefly to remove the melt from the inside of the cap. The mixture (including the residue) is carefully infused *QIAamp spin column* (in a 2 mL collection tube) without wetting the rim, then centrifuged at 8000 rpm for 1 minute. The QIA spin column was placed in a clean 2 mL collection tube, and the filtrate tube was discarded. The QIAamp spin column was opened, and AW1 500 µL Buffer was added without wetting the rim, then centrifuged at 8000 rpm for 1 minute. The QIA spin column was placed in a clean 2 mL collection tube, and the filtrate tube was discarded. The QIAamp spin column was opened, and 500 µL AW2 Buffer was added without wetting the rim, then centrifuged at full speed (13,000 rpm) for 3 minutes. The QIA spin column is placed in a clean 2 mL collection tube, and discard the tube containing the filtrate and centrifuged at full speed (13,000 rpm) for 1 minute, then proceeded to step*elution*.

Level elution. Place the QIAamp spin column in a 1.5 mL microcentrifugation tube and discard the filtrate collection tube. The QIAamp spin column is then opened and 50 µL AE Buffer or distilled water was added, incubated at room temperature for 1 minute, then centrifuged at 8000 rpm for 1 minute. This step was repeated again, then the DNA was stored in a -20 freezer or direct use.

Master Mix Reagent Preparation

Master mix using **SensiFast Probe Lo-Rox Kit** with the composition of 1 reaction: RNase Free Water 0.8 µl, 2X Sensifast Probe Mix 10.0

µl, Primer Forward gBF 20 µM 0.4 µl, Primer Reverse gBR 20 µM 0.4 µl, Probe IBR 5 µM 0.4 µl. Each reaction (1 tube of optical PCR/optical 96-well plate) was carried out in aliquots of 12 µl, and then 8 µl of each DNA template was added for the sample tested, positive control cells, and negative control cells. A positive control can use positive control cells or IBR/BHV-1 DNA control. Close the PCR tube or seal plate with an optical adhesive cover and spin on a benchtop centrifuge with a 96-well plate adapter to lower all reagents to the bottom of the well at 1,500 rpm for 1 minute.

Realtime PCR reaction.

The tube or 96-well plate is run in a real-time PCR thermocycler with the following reaction conditions: 1 X: 50^{O}C for 2 minutes; 1 X: 95^{O}C for 5 minutes; 45X (cycle); 95^{O}C for 15 seconds; 60^{O}C for 45 seconds. The results were analyzed using the software available on the machine.

Interpretation of IBR/BHV-1 Real-Time PCR Results

The threshold setting for real-time PCR IBR/BHV-1 is done before determining the interpretation. The cycle threshold (Ct) is set automatically (Auto Ct) to reduce operator subjectivity. The detection limit for Real-time PCR IBR/BHV-1 based on the adjustment of the Ct value of the IBR/BHV-1 DNA control can be concluded as follows: Positive (Ct<40), Dubius (40<Ct<45), Negative (CT = 45).

Data analysis

Detection results by ELISA test and real-time PCR in this study were analyzed descriptively.

Table 1. Test Results with Antibody ELISA and real-time PCR

No	Kode Sample	Hasil ELISA I	Hasil ELISA II	Hasil Real-Time PCR
1	590	Positive	Positive	Positive
2	631	Positive	Positive	Negative
3	787	Positive	Positive	Positive
4	907	Positive	Positive	Positive
5	989	Positive	Positive	Positive
6	1019	Positive	Positive	Positive
7	1094	Positive	Positive	Positive
8	1162	Positive	Positive	Negative
9	1170	Positive	Positive	Positive
10	1285	Positive	Positive	Negative
11	1321	Positive	Positive	Negative
12	1342	Negative	Negative	Positive
13	1396	Positive	Positive	Positive
14	1429	Positive	Positive	Negative
15	1432	Positive	Positive	Positive
16	1433	Negative	Negative	Positive
17	1607	Negative	Negative	Positive
18	1659	Negative	Negative	Positive
19	1690	Negative	Negative	Positive
20	1707	Negative	Negative	Negative
21	1720	Negative	Negative	Positive
22	1821	Positive	Positive	Positive
23	1869	Negative	Negative	Positive
24	2063	Positive	Positive	Negative
25	2381	Negative	Negative	Positive
Result Prosentage : Positive (+)		64 %	64 %	72 %
	Negative(-)	36 %	36 %	28 %

Results and Discussion

The results of the ELISA test from the first and second collection of 25 serum samples obtained seropositive results for 16 samples and nine samples for seronegative. Meanwhile, 25 nasal swab samples examined by Real-Time PCR obtained 18 positive and seven negative results (Table 2).

ELISA test results based on the table above showed 64% seropositive and 36% seronegative. The high IBR seropositivity in the examined samples is directly proportional to the high prevalence rate in the country of origin, Australia, which is 15-96% (AFFA., 2000). According to Sudarisman (2003), positive reactions to IBR serology do not only occur in imported animals but also in native Indonesian livestock. Serologically, this disease has existed in dairy cattle, beef cattle, and buffaloes from several provinces in Indonesia, with a prevalence of 5-72.9% (Sarosa, 1985). In fact, according to Naipospos (2014), all UPT Breeding within the Directorate General of Health and Human Services (except BPTUHPT Bali Cattle) showed positive antibody titers (range 4-76%).

The samples examined in this study came from cattle that were not vaccinated because the import entry document did not state that the cattle had been vaccinated in the country of origin. Antibody

detected can occur because the cow has been exposed to the IBR virus through natural infection during maintenance/ fattening in the stable, and latent infection can also occur. The immune response can be detected 12 days after the animal is infected with BHV-1 and lasts for approximately 14 months. The increase in immune titers continues to increase up to 10 months after infection (Rodistits*et al.,*2006). This immune response can last a lifetime, although it may be below the detection limits of some tests after several years (Mechor*et al*., 1987). BHV-1 can persist in infected animals in a latent state in sensory neurons, for example, in the trigeminal or sacral ganglia. Viruses can be reactivated and cause viruses shedding (re-excretion) without showing clinical symptoms. And animals have positive antibodies. (OIE, 2018). There are several ways that can activate and accelerate the virus reactivation process in a latent state, including if the cattle are stressed, the cages are too crowded, transportation, and administration of corticosteroids (Muylkens*et al*., 2007).

Imported cattle can come from several feedlots in the area of origin, so that if there is an infection on the ship, transmission will easily occur. Direct transmission through breathing of the BHV-1 virus is very easy from one livestock to another or from one group to another. The virus can be spread through nasal secretions or droplets containing the virus (Mars *et al*., 2000).

New infection and re-excretion of virus from cows with latent infection can occur during the cow's journey of approximately ten days on board. With conditions of poor density and air circulation in the ship, cows become stressed and exhausted during transportation. The incubation period after infection occurs for 2-3 days, followed by discharge from the nose, drooling, fever, lack of appetite, and depression. Within a few days, the discharge from the nose and eyes turned mucopurulent. Most infections are very mild or subclinical (Van Oirschot et *al*., 1993). A comparison of OD/titer values in the ELISA I test and ELISA II test showed that ten samples experienced an increase in OD, and 15 samples experienced a decrease in OD. An increase in antibody titer indicates infection or reactivation. Serum antibody titer decreased; it can be assumed that the disease is latent (Turin et al. 1999). According to OIE (2018), A seroconversion from negative to positive or fourfold or a higher increase in antibody titers is considered to prove acute infection

Method of *real-time polymerase chain reaction* (PCR) in this study aimed to detect the presence of genetic material of the glycoprotein C (gC) marker gene from the BHV-1 virus. The real-time PCR method is an alternative test that is very precise for the identification of the BHV-1 virus in the above samples. Real-time PCR results are expected to be read faster with a higher level of sensitivity and specificity than conventional IBR PCR. PCR results targeting the gC region of BHV-1 are in line with several previous studies (Afshar and Eaglesome, 1990; Van Engelenburg et al., 1993). The GC code for the nucleotide sequence is shown to be present in all BHV-1 strains and is

highly specific. Glycoprotein C is a glycoprotein on the surface of the main envelope that plays a role in viral virulence. Glycoprotein C is also required for viral entry by binding to target cell heparin sulfate receptors (Van Engelenburg et al., 1993)

Real-Time PCR testing has several advantages over conventional PCR methods. Real-Time PCR testing uses only one pair of primers, which provides sensitivity close to or equal to the nested PCR method, with a much lower risk of contamination. Target amplification and detection are carried out simultaneously. There is no post-amplification PCR product handling, which significantly reduces the risk of contamination (Anonymous., 2008). Based on the Real-Time PCR test, 72% positive results and 28% negative results were obtained from 25 samples. Limit of detection for IBR/BHV-1 Real-time PCR based on adjustment of IBR/BHV-1 DNA control Ct values. From several optimization tests, the PCR results can be interpreted as follows: Positive (Ct<40), Dubius (40<Ct<45), and Negative (Ct=45).

Comparison of the ELISA test and real-time PCR showed that ten samples detected antibodies in the serum samples and also the BHV-1 antigen obtained from a bovine nose swab sample. At the same time, 1 sample did not show the presence of both. This is possibly due to BHV-1 infection with previous exposure to the ten samples while there was no infection and no prior exposure to 1 sample. Eight seronegative samples showed the presence of BHV-1 antigen on the nasal swab, which indicated the possibility of a new infection or the beginning of an infection so that the immune response had not appeared. Other studies have also reported detecting BHV-1 in seronegative cow samples. Dekaet *al.*(2005) examined 24 semen samples, 12 each from seropositive bulls and seronegative., Rocha *et al*. (1998) also reported the detection of BHV-1 DNA by PCR from seronegative cows. So, there is not always a correlation between antibody status and virus excretion because BHV-1 DNA detection by PCR was also obtained from seronegative cows. Six seropositive samples did not show BHV-1 virus from the nasal swab samples. This may be due to prior exposure and may be related to the fact that the herpes virus may persist for life or Latent infection with periodic reactivation and shedding of the virus may occur intermittently or continuously (Rola*et al.,*2005)

Conclusion

Based on the ELISA test and real-time PCR showed that 40% reaction to BHV-1 antibodies and antigen from serum and nasal swabs samples of imported beef cattle.

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Effect of Dry Period Time on Milk Production and Health in Dairy Cows

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Abstract

The dry period is when the cow is not milking before calving, traditionally around 6 to 8 weeks. The primary purpose of the dry period is to treat mastitis, accelerate the regeneration of mammary gland cells and increase milk production in the following lactation period. However, recent research studies have shown that a dry period of 6 to 8 weeks is still controversial in increasing maximal milk production after delivery. Until now, the most optimal dry period is still a subject of debate related to production efficiency and the health of the mother cattle. This paper aimed to review the results of research on the dry period, which is considered the most optimal for the health of cows and increasing milk production in the subsequent lactation period.

Keywords: controversial; dairy cows ; dry period; milk production

Introduction

The dry period is defined as the period when the cow is no longer breastfeeding but is experiencing changes in nutrition, metabolism, and mammary glands (Dingwell *et al.,* 2001; Kok *et al.,* 2019). The dry period also be defined as when the cow is not milked for approximately 6 to 8 weeks until the calf is born (Arnold and Becker, 1936; Knight, 1998; Bachman and Schairer, 2003). The dry period is intended to give the dairy cows rest to recover their health after the milking period. However, recent studies have shown that a dry period of 6 to 8 weeks has received many objections from researchers (Santschi *et al.,* 2011; Steeneveld *et al.,* 2013). According to Sawa *et al.* (2015), the optimal dry period to increase milk production is 51-70 days. Boujenane's research (2019) shows that a dry period of 40-80 days results in optimal milk production in Holstein cattle. Until now, the dry period considered the most optimal, is still a matter of debate related to the efficiency of production and the health of cows (Klein and Woodward, 1943; Smith and Legates, 1962; Bachman and Schairer, 2003).

Research on dry periods and their effects on milk production have been conducted with varying results (Wood, 1977; Dias *et al.,* 1982; Sorensen and Enevoldsen, 1991; Watters *et al.,* 2008). Klein and Woodward (1943) stated that an initial dry period would decrease milk production but significantly increase milk production in later lactation periods. The results of this study were also supported by Coppock et al. (1974), who stated that a dry period of 60 days was able to maximize milk production in the subsequent lactation period. According to Sawa *et al.* (2013), the dry period before delivery is needed to regenerate the mammary glands so that milk production is expected to be maximized during the subsequent lactation. This opinion was supported by several other researchers who state that the dry period of dairy cows is a critical phase of the lactation cycle and helps increase milk production for the next lactation period (Andersen *et al.,* 2005; Pezeshki *et al.,* 2010) as well as reproductive health for the next period (Andersen *et al.,* 2005; Pezeshki *et al.,* 2010) and reproductive health for the next period (Kuhn *et al.,* 2005; Beever, 2006). The results showed that

in the first three weeks after drying, cattle have a high risk of experiencing physiological changes, reproductive tract disorders, and mastitis (Green *et al.,* 2002; Pantoja *et al.,* 2009) and susceptible to exposure to bacteria from the environment (Capuco *et al.,* 1997)

Over time, reports from several researchers state that shortening the dry period can improve energy balance (EB), fertility, and metabolic status (Rastani *et al.,* 2005; Andersen *et al.,* 2005; Watters *et al.,* 2009; De Feu *et al.,* 2009). On the contrary, several studies have shown that shortening the dry period can reduce milk production and quality and reproductive health problems in the later lactation period (Annen *et al.,* 2004; Pezeskhi *et al.,* 2007; Mantovani *et al.,* 2010). Although there is still much debate, it is believed that the dry period in dairy cows is still one of the methods to increase milk production in the following lactation. This brief review aims to examine the effect of the dry period on dairy cows concerning increased milk production and quality, which is produced in the subsequent lactation period. The writing team hopes that this article can add insight to dairy farmer practitioners in Indonesia.

Dry period and its effect on milk production

The dry period is vital in calf birth preparation, milk production, and dairy cow health (van Knegsel *et al.,* 2013). Several factors influence the dry period method in cattle to achieve optimal results before entering the next lactation period. Factors that can influence milk production (Sorensen and Enevoldsen, 1991), parity (Funk *et al.,* 1987; Kuhn *et al.,* 2006), season (Fabris *et al.,* 2019), body condition of the cow (Singh *et al.,* 2020), spontaneous termination of lactation () and other livestock-specific factors (de Vries *et al.,* 2015). One of the benefits of the dry period, which is believed to be very influential, is increasing milk production during the subsequent lactation period. One of the benefits of the dry period, which is believed to be very influential, is increasing milk production during the subsequent lactation period. The increase in milk production is because this period allows the mammary glands to go through the average involution period and to ensure that the number of mammary cells will develop generally during early lactation (Van Knegsel *et al.,* 2013).

Research on the treatment of dry periods in dairy cows has long been carried out in order to obtain maximum yield and quality of milk (Woodward and Dawson, 1926; Arnold and Becker, 1936; Smith and Legates, 1962; Keown and Everett, 1986; Makuza and McDaniel; 1996). Several theories have been proposed to explain the importance of the dry period at the end of lactation, namely restoring the body to its original condition, regenerating the mammary glands, increasing milk production and minimizing metabolic disorders, and reducing the incidence of postpartum mastitis (Smith *et al.,* 1967; Kok *et al.,* 2019; Bradley and Green, 2001; Watters *et al.,* 2008; Van Knegsel *et al.,* 2013; Grewal *et al.,* 2018). According to Capuco *et al.* (1997), mammary cell regeneration will be faster during dry periods than when cows are still being milked until calving. Therefore the dry period will result in a large concentration of new mammary cells at delivery which explains the high peak milk production in the following lactation after the dry period (Van Knegsel *et al.,* 2013). Furthermore, according to Smith *et al.* (1985) and Burvenich *et al.* (2007), the dry period is also crucial for controlling intramammary infection (IMI) because there is a strong suspicion that many cases of clinical coliform mastitis that occur during early lactation originate at the end of the dry period.

Until now, there seems to be no agreement on the most optimal dry period for maximum milk production in the following lactation. However, ample research shows that an extended dry period of around 6-8 weeks will positively impact milk production in later lactation. On the other hand, a short dry period will harm milk production (Bachman, 2002; Hoseyni *et al.,* 2017). However, the differences in the results of these studies are not acceptable. The results of Watters *et al.* (2008) showed different results because the reduction of the dry period from 55 days to 34 days did not affect the health status of the animals, including milk production and colostrum quality. Table 1 below presents the research results related to the dry period with the result of increasing milk production and quality during the subsequent lactation period.

Table 1. Relationship between dry period and increased milk production and quality in the subsequent lactation period.

Cow	Dry Periods (day)	Milk production and quality	reference
Holstein	60-69	Increased milk production, percentage of fat content,	Funk et al., 1987
Jersey	70	Increased milk production	Sorensen and Enevoldsen, 1991
Holstein	60	Increased milk production, the percentage of fat and protein content	Kuhn *et al.,* 2006
holstein	34 - 55	There is no increase in milk production and colostrum quality	Waters *et al.,* 2008
Holstein	40-60	Increased milk production	Hussein, 2009
Holstein	40-60	Increased milk production	Hernandez *et al.,* 2012
Holstein	40-60	Increased milk production, the percentage of fat and protein content	Sawa *et al.,* 2012
Holstein	51-70	Increased milk production, the percentage of fat and protein content	Hossein-Zadeh and Mohit, 2013
Holstein	41-80	Increased milk production percentage of fat content	Boujenane, 2019

Table 1 shows that the optimal duration of the dry period can vary between cows. However, the recommended optimum time to increase milk production in the subsequent lactation is 45-60 days (Dias *et al.,* 1982; Bachman and Schairer, 2003). Table 1 also shows that almost all of the dry period between 45-60 days shows an increase in milk production in the following lactation period. Short dry periods of less than 45 days were not recommended because milk production will decrease in subsequent lactations (Rastani *et al.,* 2005; Church *et al.,* 2008) and insufficient time for the udder to involute (Collier *et al.,* 2012). In contrast, according to (Capuco *et al.,* 1997), mammary gland involution in dairy cows generally ends on day 25 of the dry period, and significant tissue proliferation occurs at that time. Thus a dry period of 30 days may be sufficient time for tissue involution and regeneration under suitable conditions. Different results were also obtained from the research report by van Knegsel *et al.* (2013), which stated that a short dry period (28-35 days) showed an increase in protein levels of 0.06% compared to a dry period of 56-64 days. Therefore, the time of the dry period until now remains an interesting research topic to find an ideal time to obtain maximum milk production. Although there are still a lot of controversies with the dry period length, from table 1, it can conclude that until now, the optimal dry period for increasing milk production in the following lactation is between 45-60 days.

Variations in the optimal duration of the dry period in cattle can also be caused by various conditions such as cattle genetics, type of cattle, animal physiological conditions, feed intake, nutritional quality, season, climate differences, rearing management, and pathogenic environmental bacteria (Smith *et al.,* 1985). ; Capuco *et al.,* 1997; Pezeshki *et al.,* 2010; Grewal *et al.,* 2018: Boujane, 2019; Kok *et al.,* 2019; Fabris *et al.,* 2019; Singh *et al.,* 2020). Therefore, more in-depth research is still needed to understand the dry period treatment in dairy cows to obtain maximum results and minimize the risk of unwanted health problems. Table 1 shows that a dry period of 50-60 days will improve BCS values (Hoseyni *et al.,* 2017). The improvement in the BCS value is likely due to a change in the cow's diet associated with a decrease in the provision of grain in the feed, accompanied by an increase in hay or high-fiber forage. According to Dingwell et al. (2001), adapting dry rations with high fiber content decreased dry matter intake and increased rumination time during dry periods. Furthermore, at the end of the dry period, there is a decrease in rumen volume due to the growth of the fetus, and its development will be complete during this phase.

Relationship between dry periods and cow health

Research on dry periods concerning cows' health has been done a lot. Many research results

still show contradictions; even so, researchers must look at the various aspects and methods used in the research to be more objective in assessing the results. In addition to showing positive results (Swanson and Poffenbarger, 1979), many dry-period treatments also show adverse effects on dairy cows in various aspects following the lactation period (Watters *et al.,* 2008). Dry periods are closely related to health, including mastitis (Natzke *et al.,* 1975; Bradley and Green, 2001), decreased fertility (Beever, 2006), calf health (Keown *et al.,* 1986), colostrum quality (Pritchett *et al.,* 1991) and milk quality or composition. The results of several studies have shown that cows without dry treatment can experience a decrease in milk production by 11-25% (Remond et al., 1992). Bachman and Schairer (2003) research showed the same results; there was a decrease in milk production of 5-6% during subsequent lactation when the dry period was shortened to 30 days. However, many positive effects are obtained by treating the dry period in cattle, although it still requires long research to determine the ideal dry period. Table 2 shows the period's effect on the cows' health.

Table 2 shows the results of the study where the dry period was reduced to a range of 20-40 days. The research results in table 2 also show various kinds of results, which are sometimes contradictory. Some research results show a decrease in milk production, but some show no change or even an increase in milk production. The shortening of the dry period also showed no adverse effect on the general health of the cattle (Pezeshki *et al.,* 2008). In contrast, Fraser *et al.* (1997) stated that a short dry period or even no dry period could affect the health of metabolism, udder health, and cattle fertility. Nonetheless, research by O'Hara *et al.* (2019) showed no significant difference in fertility between cows during the dry period of 4 weeks compared to 8 weeks. Furthermore, O'Hara *et al*. (2019) stated that cows diagnosed with postpartum mastitis had a 3-fold increase in the dry period of 4 weeks compared to 8 weeks.

According to some researchers, shorter dry periods will reduce the frequency of dietary changes, which can reduce stress. Reducing dietary changes in dry periods is thought to increase the survival of the rumen microbial flora population, which is desirable to optimize the work of the rumen microflora (Goff and Horst, 1997; Penner *et al.,* 2011; Jolicoeur *et al.,* 2014). The study results in table 2 also show that the dry period of 28 days

Table 2 shows the dry period's effect on the cows' health.

Cow	Dry Periods (day)	Effect	Reference
Holstein	30	Decreased milk production	Van Knegsel *et al.,* 2014
Murrah buffalo	16-30	Decreased milk production, fat content and total solids,	Reddy *et al.,* 2019
Holstein	35	Decreased milk production	Pezeshki *et al*., 2007
Holstein	28	Decreased milk production, no changes in somatic cells and milk composition, low Body Condition Score (BCS).	Hoseyni *et al.,* 2017
Holstein	28	There is no negative effect on health status and reproductive efficiency	Pezeshki *et al.,* 2008
Holstein	28	There is no effect on milk production	Annen *et al.,* 2003
Holstein	40	There is no effect on milk production	Shoshani *et al.,* 2014
Holstein	30	Prone to mastitis	Natzke *et al.,* 1994
Holstein	30-39	Decreased milk production	Makuza and McDaniel 1996
Holstein	34	Decreased milk production	Bachman, 2002
Holstein	30-39	Decreased milk production	Keown and Everett 1986
Holstein	32	Decrease in the number of somatic cells	Klusmeyer *et al.,* 2009
Holstein	30	mastitis	Church *et al.,* 2008
Swedish Red dan Swedish Holstein	28	Increased incidence of mastitis, low colostrum volume	O'Hara *et al.,* 2019

did not show any changes in the number of somatic cells. According to Lipkens *et al.* (2019), in healthy cows, the dry period shows a lower SCC in the subsequent lactation period compared to cows that experience pain during the dry period. Bradley *et al.* (2015) added the importance of management factors for controlling udder health during dry periods so that it was expected to reduce SSC rates during the subsequent lactation period. Even so, some researchers still think that the impact of the dry period on udder health observations based on SSC is still ambiguous because it shows different research results. According to Andersen *et al.* (2005), the dry period does not affect the number of SSC. On the contrary, Klusmeyer *et al.* (2009) showed increased SSC.

In table 2, it can see that short dry periods also increase the risk of mastitis. Nonetheless, the short dry period is still being debated today. According to Van Hoeij *et al.* (2016), the effect of prolonged dry periods on udder health is closely related to parity, milk production level, and udder health. According to Pantoja *et al.* (2009), intramammary infection during the dry period will increase the risk of clinical mastitis at the start of the next lactation. Therefore, dry period management is crucial in minimizing or preventing mastitis during dry periods (Eberhart, 1986). One of the management of the dry period to prevent mastitis is by giving antibiotics during the dry period (Janosi and Huszenicza, 2001; Hillerton *et al.,* 2017). Even though antibiotics are a strategy for treating mastitis in dry periods, we must be aware of the negative impacts. Most antibiotics used in the dairy farming industry are applied to control mastitis, especially in dry periods (Bradley and Green, 2000; Bradley and Green, 2001). However, excessive and uncontrolled use will lead to increased resistance.

Conclusion

The most optimal dry period is still a matter of debate related to production efficiency and the health of the mother cattle. Until now, research is still being carried out on the dry period that is most optimal for milk production produced and the cows' health in the subsequent lactation.

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