

Endemicity *Brugia malayi* Status Post Transmission Assessment Survey in Indonesia-2017

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ABSTRACT

Since 2002 Indonesia has implemented the Mass Drug Administration (MDA) in regencies/cities that are endemic for lymphatic filariasis. The success of regencies/cities in MDA after 5 years of implementation can be known through the TAS (transmission assessment survey). In 2017 an evaluation study was conducted to determine the failure and success of elimination of filariasis from epidemiological aspects (host, agent, environment) in 12 regencies endemic to zoonotic malayi filariasis who have passed TAS-1, TAS-2, and TAS-3. Evaluation study was carried out using finger blood sampling methods for residents, blood collection in reservoirs (long-tailed monkeys, cats and dogs) and vector mosquito capture. Finger blood test results on residents in 12 regencies: 3 regencies that have passed TAS-1 still found positive microfilaria populations, namely: Pasaman Barat (0.9%), Kuantan Singingi (0.2%), and Hulu Sungai Utara (0, 2%). As well in the 3 regencies that have passed TAS-3, namely: Bangka Barat (0.6%), Belitung (1.2%), and Kotawaringin Barat (0.8%). Results of blood tests on the reservoir, were found to be positive for *B. malayi* in house cats in the regencies of Pasaman Barat, Pelalawan, and Kotawaringin Barat; long-tailed monkeys in Belitung and Kotawaringin Barat regencies; and dogs in Kotawaringin Barat regency. The mosquito vectors that were caught and tested positive DNA for microfilaria larvae based on PCR examination were *Culex vishnui*, *Culex quinquefasciatus*, *Mansonia dives*, *Mansonia uniformis*, *Anopheles karwari*, *Aedes aegypti*, *Aedes cancris*, *Aedes linneatus*, and *Armigeres kucingensis* in the Pesisir Selatan, Pasaman Barat, Kuantan Singingi, Bangka Barat, Belitung, and Hulu Sungai Utara regencies. From the results of the study, even though an area has passed the TAS, transmission of the disease still occurs because of the presence of microfilaria in the reservoir and filaria larvae in mosquitoes. It is recommended that regencies that have passed the TAS continue to carry out active surveillance of vulnerable populations exposed in endemic villages.

Keywords: TAS, filariasis endemicity, Mass Drug Administration (MDA)

1. INTRODUCTION

Indonesia is a tropical archipelago which is 1 of 81 endemic filariasis countries with 3 species causing lymphatic filariasis; *Brugia malayi*, *Wuchereria bancrofti*, and *Brugia timori*¹. Of the three causes of the filariasis, most are endemic areas of *B. malayi*, especially *B. zoonotic malayi*. Areas that are endemic of *B. malayi* zoonotic are generally located on two islands namely Sumatra and Kalimantan. On these two islands there are 210 cities/regencies, 118 recommended cities/regencies are endemic areas of filariasis².

In Indonesia, filariasis control efforts began in 1975 with microfilaria surveys carried out in 10 regencies in the provinces of West Sumatra, South Kalimantan and East Nusa Tenggara³. The ten regencies are known as endemic filariasis regencies with many cases of elephantiasis found. For almost 2 decades, the implementation of filariasis

control was only aimed at certain areas with high endemicity. In 2000 WHO declared global elimination of filariasis, and two years after the declaration the Government of Indonesia implemented an intensive program of eliminating filariasis in 236 regencies/cities endemic to filariasis.

Until the end of 2016, out of 236 regencies/cities that are endemic for filariasis, 55 regencies/ cities have mass drug administration (MDA) for 5 years. The remaining 181 regencies/cities will implement MDA until 2020, with a population of 76 million. Of the 55 regencies/cities that have implemented MDA, there are 12 regencies/cities endemic of *B. malayi* which in 2016 had been carried out the Transmission Assessment Survey (TAS). The results of the TAS in the 12 regencies are 6 regencies passed TAS-1, 2 regencies passed TAS-2, and 4 regencies passed TAS-3.

This article is a further analysis of the filariasis multicenter study conducted in 2017 in 23 cities/regencies in 14 provinces. The study was approved by the Health Research Ethics Commission of the National Institute of Health Research and Development in accordance with the letter of the Chairman of the Ethics Commission No. LB.02.01/2 KE.167/2017 dated May 4, 2017. From this article, we will find out the status of endemicity in endemic areas of *Brugia malayi* zoonotic after the implementation of TAS. Is transmission still happening even though the implementation of mass treatment was completed 6-13 years ago.

2. METHOD

The study was conducted in 12 endemic regencies of *B. malayi* zoonotic that had implemented TAS. The regencies are Labuhan Batu and Nias (North Sumatra Province); Agam, Limapuluh Kota, Pasaman Barat, and Pesisir Selatan (West Sumatra Province); Pelalawan and Kuantan Singingi (Riau Province); Bangka Barat and Belitung (Bangka Belitung Province); Kotawaringin Barat (Central Kalimantan Province); and Hulu Sungai Utara (South Kalimantan Province). Type of the research is cross-sectional. Population is the residents of villages where primary school children live who tested positive for antibodies in the regencies who passed the TAS in 2016. If the regencies that passed the TAS did not have children who tested positive for antibodies, then the population is villagers who live in the sentinel or spot areas. Respondents are villagers who live ≥ 5 years. Samples were chosen at random by using the formula Stanley Lemeshow ⁴ as follows: $n = Z^2 \cdot \frac{P(1-P)}{d^2}$. n = number of samples Z^2 $1-\alpha/2$ = 1.960 (95% confidence level), P = 0.28, d = 0.05. Based on the formula, the number of samples in each village is 310 respondents or 620 respondents in each regency. A total of 620 respondents were interviewed at home during the daytime, while finger blood was taken at night in a designated place (the house of the village head or village hall or mosque). Before finger blood sampling, a clinical examination is conducted on the respondent by a medical doctor. Reservoir animals (cats/dogs/long-tailed monkeys/leaf monkeys) whose blood will be taken are the respondent's pets. It also set a trap to get long-tailed monkeys/leaf monkeys in the forest around the study location village. The criteria for inclusion of finger blood sampling in the population are over the age of 5 years and are willing to take part in the study by signing an informed consent sheet by the subject concerned. Exclusion criteria for finger blood sampling are children over the age of 5 who are sick, pregnant women, and sufferers of severe illness and/or chronic illness. Finger blood obtained from human respondents as much as 60 μ l thick blood preparations were made and examined under a microscope to determine the presence or absence of microfilariae. While blood preparations derived from reservoir animals taken from venous blood as much as 2.5 ml and then made thick blood preparations, then examined under a microscope. Microfilaria positive blood preparations were then

examined using the PCR method to confirm *Brugia malayi* species. Also catching mosquitoes in and around the homes of children who are positive TAS and/or chronic filariasis. The mosquitoes that are caught are examined to find out the species. To find out the positive-negative of mosquitoes containing filariasis larvae DNA was examined by PCR method.

3. RESULTS AND DISCUSSION

A total of 12 regencies were designated as study sites out of a total of 101 regencies/cities, which are endemic areas of *Brugia malayi* zoonotic. An area (regency/city) is determined as an endemic area based on the results of a finger blood survey if $>1\%$ of the population in a village is positive for microfilariae. Finger blood surveys were conducted long before the implementation of the MDA and this study was carried out. For regencies/cities indicated by endemic filariasis, a total of 5 rounds of MDA (5 consecutive years) are carried out. After completing 5 rounds, an evaluation is carried out among primary school children aged 6-7 years with a TAS. TAS results will indicate whether the transmission is still presence or not. If the positive result below 18 peoples (critical cut-off point) which mean the transmission of filariasis is low and the possibility of a small case increase. Meanwhile if the number of positive is equal to/more than 18 peoples, that is indicates transmission sustained. This critical cut-off point 18 is in accordance with WHO requirements ⁵.

Table 1 below implements the results of the TAS and the location of the study.

Table 1 TAS Results 2016 and Sites of Data Collection

No	Regencies	TAS Results (*)		Pass	Sites of Data Collection	
		Pos	Neg		First Site	Second Site
1	Nias	0	1.585	TAS 1	Lolofooso (*)	Telegeona'ai (*)
2	Agam	3	1.692	TAS 1	Pasir Tiku	Sungai Jaring
3	Pesisir Selatan	0	1.644	TAS 1	Koto Hilir (*)	Kampong Tamur (*)
4	Pasaman Barat	1	1.621	TAS 1	Ujung Gading	Katiangan (*)
5	Kuantan Singingi	11	1.772	TAS 1	Sukadama	Pulau Panjang Cereni
6	Hulu Sungai Utara	0	1.573	TAS 1	Pihuang (*)	Banjang (*)
7	Labuhan Batu	0	1.590	TAS 2	Padang Bulan (*)	Tebing Linggahara (*)
8	Pelalawan	17	1.559	TAS 2	Ukui	Sialang Bungkok
9	Limapuluh Kota	0	1.621	TAS 3	Mungo (*)	Labuah Gunung (*)
10	Bangka Barat	0	1.611	TAS 3	Air Gantong (*)	Tanjung Nini (*)
11	Belitung	0	1.590	TAS 3	Cenauk (*)	Kembiri (*)
12	Kotawaringin Barat	4	1.544	TAS 3	Sungai Bakau	Dawak

Note: (*) = sentinel/spot area.

TAS results data obtained from the Directorate General of Disease Control, Ministry of Health.

From Table 1 above, it appears that all regencies have passed the TAS. Passed TAS-1 in Nias, Agam, Pesisir Selatan, Pasaman Barat, Kuantan Singingi and Hulu Sungai Utara regencies; TAS-2 Labuhan Batu and Pelalawan regencies; and TAS-3 in Limapuluh Kota, Bangka Barat, Belitung and Kotawaringin Barat regencies. Even though they have passes, there are still regencies that have positive TAS elementary school children, namely Agam 3 children, West Pasaman 1 child, Kuantan Singingi 11 children, Pelalawan 17 children, and Kotawaringin Barat 4 children. In Table 1 above, 15 villages are the sentinel/spot villages and 9 villages are villages where TAS positive children live.

The following Table 2 below presents the characteristics of the research subjects in 12 research location regencies.

Table 2 Characteristics of Research Subjects In the Twelve Regencies Location of the Study Based on TAS

No	TAS Status Regency	Sex		Age (Year)			
		Man	Woman	Total	5-17	18-64	65 >=
I Pass TAS 1							
1	Nias	309	325	634	267	322	45
2	Agam	307	389	696	253	404	39
3	Pesisir Selatan	274	356	630	196	386	48
4	Pasaman Barat	327	354	681	249	400	32
5	Kuantan Singingi	295	379	674	212	437	25
6	Hulu Sungai Utara	285	339	624	170	428	26
	Sub Total I	1.797	2.142	3.939	1.347	2.377	215
II Pass TAS 2							
1	Labuhan Batu	283	351	634	214	392	28
2	Pelalawan	311	324	635	210	406	19
	Sub Total II	594	675	1.269	424	798	47
III Pass TAS 3							
1	Limapuluh Kota	292	350	642	180	391	71
2	Bangka Barat	284	378	662	234	409	19
3	Belitung	308	357	665	162	450	53
4	Kotawaringin Barat	285	336	621	162	440	19
	Sub Total III	1.169	1.421	2.590	738	1.690	162
	Total (I + II + III)	3.560	4.238	7.798	2.509	4.865	424

Status

From Table 2 above, it appears that the comparison of respondents by sex between men and women is 45%: 55% in the three regions of TAS status. In terms of age, most respondents were in the age range of 18-64 years.

The following Table 3 below presents the results of a microscope examination to detect the presence of microfilaria from the finger blood survey.

Table 3 Number and Percentge of Research Subjects Finger Blood Survey Examination Results Based on TAS and Regency Status

No	Regency	Positive		Negative	
		n	%	n	%
I	Pass TAS 1				
1	Nias	0	0,0	618	100,0
2	Agam	0	0,0	620	100,0
3	Pesisir Selatan	0	0,0	620	100,0
4	Pasaman Barat	6	0,9	625	99,1
5	Kuantan Singingi	1	0,2	631	99,8
6	Hulu Sungai Utara	1	0,2	619	99,8
II	Pass TAS 2				
7	Labuhan Batu	0	0,0	621	100,0
8	Pelalawan	0	0,0	621	100,0
III	Pass TAS 3				
9	Limapuluh Kota	0	0,0	620	100,0
10	Bangka Barat	4	0,6	622	99,4
11	Belitung	8	1,2	614	98,2
12	Kotawaringin Barat	5	0,8	615	99,2

Table 3 above shows from 12 regencies; 3 regencies that have passed TAS-1, namely: Pasaman Barat, Kuantan Singingi, and Hulu Sungai Utara, still found positive microfilaria populations. As well in the 3 regencies that have passed TAS-3, namely: Bangka Barat, Belitung, and Kotawaringin Barat, still found positive microfilaria populations.

The number of peoples that positive for microfilaria was 0.3% (25 peoples positive out of 7,746), the microfilaria rate was still below 1%. But If we compare by the regency, microfilaria rate in Belitung is 1.2%, Pasaman Barat 0.9%, Kotawaringin Barat 0.8% and Bangka Barat 0.6%. Belitung has passed TAS-3, meaning that 5 years after MDA the transmission is sustained. Three regencies (Agam, Pasaman Barat, and Pesisir Selatan) are regencies

that previously did not pass TAS-1 so that the MDA extension was carried out for 2 rounds. The failure of Pasaman Barat in implementing the first period of MDA (5 years) is related to the results of the study of Rita M. Dewi et al in 2015 with the high prevalence of antibodies in children aged 6-7 years (6.3% of 523 sample ⁶. For Pasaman Barat (passed TAS-1), Kotawaringin Barat (passed TAS-3), and Bangka Ba rat (passed TAS-3); although under 1% in 2016, it is possible the transmission is still present. The disease alertness is needed to prevent the increasing of microfilaria rate ^{7, 8, 9,10}. A multicenter study conducted in 11 countries, 10 of which showed a decrease in the prevalence of lymphatic filariasis below 2% after completing the five-round MDA ¹¹.

Of the 25 positives people, the number of parasites per blood sample is an average of 15 parasites (1-128 mf parasites). The highest parasitic density was found in 1 person in West Bangka (128 parasites/60 µl) and the lowest was in 1 person in the Hulu Sungai Utara (1 parasite/60 µl). The average parasitic density in Belitung and Pasaman Barat is the same, namely 20 parasites/60 µl, Bangka Barat 35 parasites/60 µl, and in Kotawaringin Barat 14 parasites/60 µl. ⁸ With an average density like that shows that the transmission of filariasis is still taking place in the regencies of Belitung, Bangka Barat, Pasaman Barat, and Kotawaringin Barat ^{7, 8, 9, 10}. As many as 22 positive people are in the range of the age group of 15-54 years, the remaining 3 people under the age of 15 years.

The results of examination of reservoir blood specimens to detect the presence of B. malayi microfilaria can be seen in Table 4 below.

Table 4 Result of Animal Reservoir Samples Confirmed by PCR Method by Regency

No	Regency	Cat		Long-tailed Monkey		Dog	
		Number of Specimens Examined	Number of Positive	Number of Specimens Examined	Number of Positive	Number of Specimens Examined	Number of Positive
I Pass TAS 1							
1	Nias	82	0 (0,0%)	1	0 (0,0%)	20	0 (0,0%)
2	Agam	71	0 (0,0%)	1	0 (0,0%)	31	0 (0,0%)
3	Pesisir Selatan	37	0 (0,0%)	1	0 (0,0%)	62	0 (0,0%)
4	Pasaman Barat	69	6 (8,6%)	1	0 (0,0%)	32	0 (0,0%)
5	Kuantan Singingi	84	0 (0,0%)	3	0 (0,0%)	16	0 (0,0%)
6	Hulu Sungai Utara	91	0 (0,0%)	5	0 (0,0%)	4	0 (0,0%)
II Pass TAS 2							
7	Labuhan Batu	93	0 (0,0%)	4	0 (0,0%)	5	0 (0,0%)
8	Pelalawan	86	3 (3,4%)	9	0 (0,0%)	5	0 (0,0%)
III Pass TAS 3							
9	Limapuluh Kota	36	0 (0,0%)	1	0 (0,0%)	66	0 (0,0%)
10	Bangka Barat	97	0 (0,0%)	1	0 (0,0%)	3	0 (0,0%)
11	Belitung	85	0 (0,0%)	30	1 (3,3%)	3	0 (0,0%)
12	Kotawaringin Barat	79	6 (7,6%)	5	1 (20,0%)	17	1 (5,8%)
Total		910	15 (1,6%)	62	2 (3,2%)	264	1 (0,3%)

From Table 4 above, a total 1,236 reservoir animals have been examined which consist of 910 Felis catus (house cats), 62 Macaca fascicularis (long-tailed monkeys) and 264 Canis familiaris (house dogs). There are two species of reservoir animals, namely Felis silvestris (forest cat) and Presbytis cristatus (leaf monkeys) were not able to be collected.

The results of sample examination by PCR method, seen in the regencies of Pasaman Barat, Pelalawan, and Kotawaringin Barat found DNA Brugia malayi in Felis catus (house cats), in Belitung and Kotawaringin Barat on

Macaca fascicularis (long-tailed monkeys), in Kotawaringin Barat on *Canis familiaris* (house dog).

In endemic areas of *Brugia malayi* zoonotic, both sub-periodic nocturnal and non periodic types, if a positive reservoir of *B. malayi* microfilaria is found, the animal has the opportunity to become a source of transmission to humans. In Table 4, out of 5 regencies (Pasaman Barat, Pelalawan, Bangka Barat, Belitung, and Kotawaringin Barat); *B. malayi* was found in house cats in 3 regencies (Pasaman Barat, Pelalawan and Kotawaringin Barat), long-tailed monkeys in 2 regencies (Belitung, and Kotawaringin Barat), and dogs in Kotawaringin Barat regency.

Felis catus (house cat) and *Canis familiaris* (house dog) are animals that are closely related to humans. The two types of animals that were sampled were the resident pets that were the subject of the survey. In house cats, it is assumed that the chance of transmission of filariasis from cats to humans, as well as from cats to cats is the same. In cats as reservoir animals there is no limit on the indicator of how large the mf-rate has the potential to be a problem for transmitting to humans, whereas in humans it has been determined that the mf-rate is > 1%. *F. catus* (house cat) and *Canis familiaris* (house dog) are human pets, so it is necessary to be aware of the possibility of *B. malayi* transmission from house cats or house dogs to residents in the three districts above (Pasaman Barat, Pelalawan, and Kotawaringin Barat).

Filariasis *malayi* infection in dogs is rarely reported even though it can happen. In Indonesia, *B. malayi* infection in dogs has not been widely publicized. However, Dicki Andiarsa et.al has published the case of *B. malayi* in dogs in Kotawaringin Barat which is also part of this multicenter study¹². And this is the only case of *malayi* filariasis in dogs in this study. In Chertala, India, with a histochemical staining pattern found 6 positive dogs similar to *B. malayi* among 164 dogs examined¹³. In Sri Lanka, Chandana HM et.al found *Brugia* spp in dogs (54.4%) and cats (34.3%)¹⁴.

Table 5 below presents the results of the PCR examination of the mosquitoes that were caught and detected the DNA of *B. malayi* larvae.

Table 5 PCR Examination Results on Mosquitoes Caught by Regency

No	Regency	Positive DNA <i>B. malayi</i> Larvae
1	Pesisir Selatan	<i>Culex vishnui</i>
		<i>Mansonia dives</i>
2	Pasaman Barat	<i>Culex vishnui</i>
		<i>Aedes canercomes</i>
		<i>Aedes aegypti</i>
3	Kuantan Singingi	<i>Culex vishnui</i>
4	Pelalawan	<i>Mansonia dives</i>
		<i>Culex quinquefasciatus</i>
5	Bangka Barat	<i>Anopheles karwari</i>
		<i>Aedes aegypti</i>
		<i>Armigeres kucingensis</i>
6	Belitung	<i>Culex vishnui</i>
		<i>Aedes linneatopenis</i>
7	Hulu Sungai Utara	<i>Mansonia uniformis</i>

The results of catching mosquitoes carried out in 12 regencies of the study site, there are 7 regencies whose

mosquitoes contain DNA of *B. malayi* larvae. Five other regencies: Nias, Labuhan Batu, Agam, Limapuluh Kota, and Kotawaringin Barat; no mosquitoes contain DNA of *B. malayi* larvae.

In this study, 100 species of mosquitoes were captured and identified from 15 genera⁷. In table 5, there were 9 species of mosquitoes that were caught, 8 species of which were new species namely: *Cx. sitiens*, *Cx. vishnui*, *Ae. vexans*, *Ae. cancricomes*, *Ae. aegypti*, *Ae. linneatopenis*, *An. karwari*, and *Ar. kucingensis*. The 8 species have not been known as filariasis transmitting vectors in Indonesia. The results of the PCR method were found to be positive for filarial larvae DNA, although microscopy was not performed to ascertain the presence of L3 larvae, the 8 species had the potential to become filariasis vectors. One of them is *Cx. vishnui* turned out to be a vector of filariasis in Bihar, India¹⁵.

Until 2005, there were 23 species of filariasis-transmitting mosquitoes in 23 provinces in Indonesia. The 23 species of mosquitoes are *Cx. quinquefasciatus*, *Cx. annulirostis*, *Cx. whitmorei*, *Cx. bitaeniorhynchus*, *Ma. uniformis*, *Ma. indiana*, *Ma. dives*, *Ma. bonneae*, *Ma. annulata*, *Ma. annulifera*, *An. nigerimus*, *An. subpictus*, *An. barbirostris*, *An. aconitus*, *An. vagus*, *An. dives*, *An. maculatus*, *An. farauti*, *An. koliensis*, *An. punctulatus*, *An. bancrofti*, *Ae. kochi* and *Ar. subaltabus*¹⁶. The existence of an additional 8 species, means that in Indonesia there are currently 31 species of mosquitoes that are potential transmitters of filariasis.

The following Table 6 is a compilation of Tables 1, 3, 4, and 5 to find out the presence or absence of *malayi* filariasis transmission in 12 regencies of the study location.

Table 6 Summary of Brugian Filaria Transmission in 12 Regencies

No	Regency	Host (Man)		Reservoar	Vektor	Conclusion
		TAS	SDJ			
Pass TAS 1						
1	Nias	--	--	--	--	Transmission does not exist
2	Agam	+	--	--	--	Transmission is less likely
3	Pesisir Selatan	--	--	--	+	Transmission is less likely
4	Pasaman Barat	+	+	+	+	Transmission is high possibility
5	Kuantan Singingi	+	+	--	+	Transmission is high possibility
6	Hulu Sungai Utara	--	+	--	+	Transmission is possible
Pass TAS 2						
7	Labuhan Batu	--	--	--	--	Transmission does not exist
8	Pelalawan	+	--	+	+	Transmission is high possibility
Pass TAS 3						
9	Limapuluh Kota	--	--	--	--	Transmission does not exist
10	Bangka Barat	--	+	--	+	Transmission is possible
11	Belitung	--	+	+	+	Transmission is high possibility
12	Kotawaringin Barat	+	+	+	--	Transmission is high possibility

Note: + = positive sample. -- = negative sampel.

Table 6 shows that in 3 regencies (Nias, Labuhan Batu, and Limapuluh Kota) there was no transmission of filariasis. Meanwhile, Agam and Pesisir Selatan regencies are less likely to have filariasis transmission. Hulu Sungai Utara and Bangka Barat are likely to have filariasis transmission. In 5 other regencies (Pasaman Barat, Kuantan Singingi, Pelalawan, Belitung, and Kotawaringin Barat) there is a high possibility of transmission of filariasis.

Filariasis transmission, especially *Brugia malayi* zoonotic, can occur if there are 5 main interrelated elements, namely the presence of infectious disease sources (humans and animals), parasites (filarial worms), infectious vectors (mosquitoes), vulnerable humans (hosts), and the environment (physical, biological, economic, social and cultural). In populations residing in endemic or non-endemic areas, if the five main transmission factors are met, then the possibility of exposure to microfilaria may occur. Residents who are exposed to microfilaria due to vector activity that carries microfilaria larvae, the source of infectious diseases that are in the settlement and its surroundings, and possible environmental conditions.

In Table 6, although there are only 3 factors that determine the transmission of filariasis (host, reservoir, vector), these three factors are very strong for transmission. Of the 6 regencies that have passed TAS-1, only Nias regency has no transmission. In Agam regency with the discovery of an elementary school child who tested positive for antibodies, the possibility of transmission of filariasis was very small. Transmission when the study was carried out did not occur. Children who tested positive for antibody were exposed 2-3 years before the study was carried out. In the Pesisir Selatan regency, although positive filarial larvae vectors are found, the possibility of filariasis transmission is also very small. However, it is necessary to be aware of the possibility of a reservoir that is a source of filariasis. In the Hulu Sungai Utara regency, transmission can occur. Especially in the study site, many leaf monkeys and long tail monkeys were found ¹⁰.

In Labuhan Batu regency, passing TAS-2, filariasis transmission is also absent. But in Pelalawan regency, although there are children who test positive for antibodies when TAS, reservoirs and mosquitoes are caught positive for microfilaria, transmission is likely.

The 4 regencies that have passed TAS-3, only Limapuluh Kota regency has no transmission. In Bangka Barat regency with the discovery of a positive population of microfilariae in the blood, and a positive vector of microfilariae larvae, transmission can occur. Belitung and Kotawaringin Barat regencies, the possibility of transmission will occur. Moreover, in these 3 regencies (Bangka Barat, Belitung, and Kotawaringin Barat) the population positive for microfilaria ranges from 0.6-1.2%. The effort to filariasis elimination with the application of additional MDA is one of the efforts that can cut off transmission of filariasis between residents ^{17,18}. The administration of ivermectin to reservoirs (cats) ¹⁹, environmental sanitation and personal hygiene ²⁰, active community participation and intensification of the campaign to control and prevent filariasis ²¹ are important factors for successful elimination of filariasis. In addition, other efforts such as collecting mosquitoes are needed to detect the presence or absence of DNA filariasis in vector mosquitoes ²².

4. CONCLUSION

1. In 12 endemic regencies of *B. malayi* zoonotic, mf rate above 1% is still found, even though MDA has finished and has implemented TAS-1, TAS-2 and TAS-3; there is even regency that have passed TAS-3.
2. From the capture of mosquitoes obtained 8 new mosquito species that are positive for DNA microfilaria larvae as potential infectious filariasis.
3. In some regencies found positive microfilaremia sufferers, reservoir animals were also positive of *B. malayi* microfilaria. So far in Indonesia only known as leaf monkeys and cats as *B. malayi* reservoirs. In this study also found in dogs and long-tailed monkeys.

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