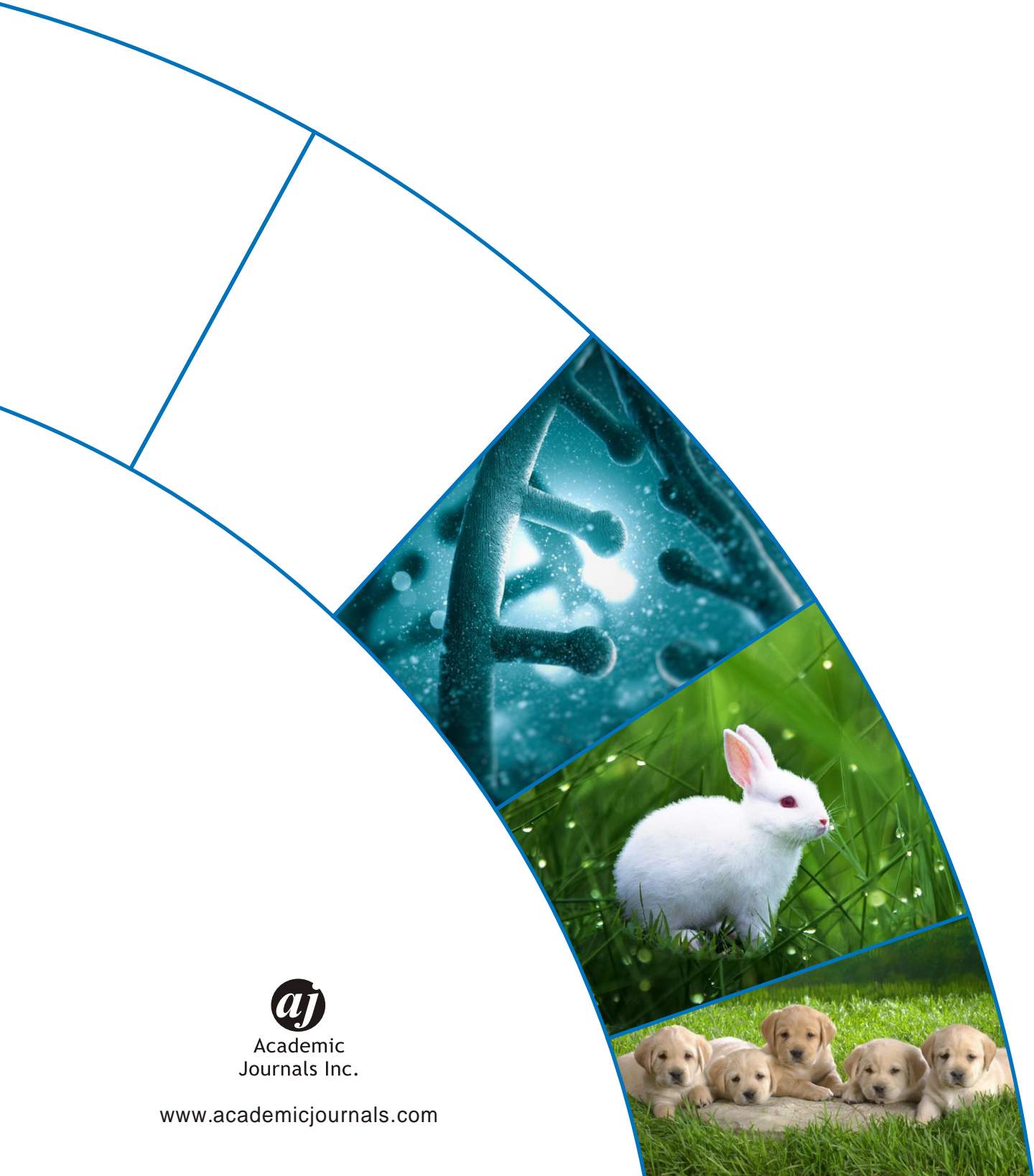


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## Research Article

# Molecular Evidence of Filariasis Transmission Through Cats and Dogs in West Sumatra

Tri Wijayanti, Bina Ikawati, Corry L.J. Sianturi, Bondan F. Wahyudi, Jarohman Raharjo, Dewi Marbawati and Zumrotus Sholichah

Banjarnegara Health Research and Development Unit, National Institute of Health Research and Development, Ministry of Health, Banjarnegara, Jawa Tengah, Indonesia

## Abstract

**Background and Objective:** West Sumatra Province is ranked as the 11th highest filariasis case in Indonesia in 2013. Pasaman Barat and Pesisir Selatan have passed Transmission Assessment Survey (TAS)-1 on filaria elimination program. Evaluation for mass drug administration in Pasaman Barat has proved that microfilariae from *Brugia malayi* are still found in humans. This study investigated microfilariae in cats, dogs and primates in Pasaman Barat and Pesisir Selatan to determine their potential reservoir status. **Materials and Methods:** A cross-sectional study was carried out in Pasaman Barat and Pesisir Selatan Districts in 2017. Total samples of 202 reservoir animals were used in this study, namely house cats (*Felis catus*), dogs (*Canis familiaris*) and *Macaca fascicularis*. Reservoir blood samples were taken by a veterinarian on savena or femoralis vein and then examined by microscopic. Polymerase Chain Reaction (PCR) had been done to cross-check positive *B. malayi* samples. **Results:** Microscopic examination showed that 6 cats in Pasaman Barat were positive for *B. malayi* and confirmed by PCR and 7 dogs were positive *Dirofilaria* sp. All of the reservoirs in Pesisir Selatan were negative for *B. malayi*, but there were 19 dogs positive *Dirofilaria* sp. **Conclusion:** This study proved that cats can be the source of *B. malayi* transmission to humans in Pasaman Barat. The animal can be a potential source of transmission of *Dirofilaria* sp. from dogs to humans in both districts. Alertness in cats and dogs as the reservoir of *B. malayi* and dirofilariasis transmission needs to be raised.

**Key words:** *Brugia malayi*, *Dirofilaria* sp., Pasaman Barat, Pesisir Selatan, reservoir

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**Corresponding Author:** Bina Ikawati, Banjarnegara Health Research and Development Unit, National Institute of Health Research and Development, Ministry of Health, Banjarnegara, Jawa Tengah, Indonesia

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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Filariasis is an infectious disease caused by roundworms of Filarioidea superfamily. Filariasis in human caused by *Wuchereria bancrofti*, *Brugia malayi*, *Loa loa*, *Onchocerca volvulus* and *Dirofilaria* spp<sup>1</sup>. Mass drug administration (MDA) is one of the programs to eliminate Filariasis in humans. National coverage of MDA in Indonesia was 71.1%<sup>2</sup>. West Sumatra Province is ranked as the 11th highest filariasis cases in Indonesia in 2013 with 225 cases. Pasaman Barat District is the highest filaria endemic district in West Sumatra Province with a prevalence of 12.40 per 100,000 population (49 cases) in 2014<sup>3</sup>. The other filaria endemic district in West Sumatra is Pesisir Selatan. Pasaman Barat and Pesisir Selatan have passed Transmission Assessment Survey (TAS)-1 on filaria elimination program.

Belitung District Indonesia is one of the districts that completed five rounds of MDA in 2010 and declare in achieving elimination Filariasis in 2017 after TAS-3. The survey in 2019 found that microfilaria (Mf) density in a human was high, screening of 311 and 360 individuals in Lasar and Suak Gual villages showed Mf prevalence of 5.1 and 2.2%. *Mansonia* spp. existence and long-tailed macaques (*Macaca fascicularis*) are prevalent in this district and detected *Brugia* spp. DNA indicates a possible role in zoonotic transmission in this district<sup>4,5</sup>. Based on the condition in Belitung District, it is necessary to confirm filaria worms in reservoir animals (dog, cat, monkey) for filaria elimination program, because *B. malayi* is zoonotic. This study investigated microfilariae in cats, dogs and primates in Pasaman Barat and Pesisir Selatan to determine their potential reservoir status.

## MATERIALS AND METHODS

**Study area:** The location of the study was based on the results of the Transmission Assessment Survey (TAS)-1 held by Filariasis Sub-Directorate in 2016 in the sentinel and spot area of filariasis (each district has 2 locations). The study in Pasaman Barat District was conducted in Jorong Katiagan, Nagari Katiagan, Kinali Sub District and Jorong Koto Sawah, Nagari Ujung Gading, Lembah Melintang Sub District in 2017. The results of the TAS-1 at Pasaman Barat District showed a positive child who lives in Jorong (Sub Village) Koto Sawah, Nagari (Village) Ujung Gading. The second place was Nagari Katiagan, Kinali District. This nagari is a filariasis endemic area with Mf rate in 2011 was 18.5% and designated as a sentinel region. Where as in Pesisir Selatan District, research was conducted in the sentinel area namely Kambang Timur Nagari

in Lengayang Sub District and Koto Nan Duo IV Koto Hilie Nagari, Batang Kapas Sub District as a spot area.

**Research protocol:** Reservoirs (cats, dogs, monkeys/*Macaca fascicularis*) survey have been carried out by using traps for Macaca. The monkey surveys were assisted by residents using traps made from bamboo and wood on the Pesisir Selatan and traps in the form of iron baskets in Pasaman Barat. The number of samples were assumed by the prevalence of Mf rate *B. malayi* in canine was 7% from previous research<sup>5</sup> and deviation standard = 0.05, so the target of all reservoir was 100 animals in each district. Total samples of 202 reservoir animals were used in this study, namely house cats (*Felis catus*), dogs (*Canis familiaris*) and *Macaca fascicularis*.

**Blood sampling:** The blood samples were taken at least 1 cc from the saphenous and cephalic vein (cat/dog) or femoral vein (silvered leaf monkey/Macaca) by a veterinarian at night. The samples blood was provided for thick blood samples for microscopic examination and PCR test. The PCR kit (Fermentas Pvt Ltd.) used Hha1 primer that amplifying fragment 322 bp of *Brugia malayi*<sup>6</sup>. The forward and reverse primer sequences for *B. malayi* were (Hha1 F 5'-GCG CAT AAA TTC ATC AGC3', Hha1 R 5'-GCG CAA AAC TTA ATT ACA AAA GC3'), respectively<sup>6</sup>.

Thick blood preparations (60 µL) were stained with Giemsa and examined microscopically at the Parasitology Laboratory in Banjarnegara Health Research and Development Unit. The remaining blood was preserved in Whatman filter paper to be further examined by the PCR (Polymerase Chain Reaction) method at the Center for Research and Development of Biomedical and Basic Technology of Health Research and Development in Jakarta. The Giemsa-stained process and examination method of blood samples are the same as the process carried out in humans.

## RESULTS

The results of the reservoirs survey in Pasaman Barat District in the Ujung Gading Nagari there were 55 animals caught (dogs and cats), while in the Katiagan Nagari the caught animals were 47 (dogs, cats and monkeys/*Macaca fascicularis*). Ujung Gading is not a suitable habitat for Macaca and langurs because it is surrounded by palm plantations and rice fields with no forest left, so there was no primate taken as the sample in this area. The survey conducted in Katiagan, Pasaman Barat did not succeed in finding langurs. The only primate taken as the sample was the villager's pet. Results of the reservoirs survey in Pasaman Barat District based on sex showed in Table 1.

Table 1: Results of microscopic examination for microfilariae on reservoirs in Pasaman Barat District

Location	Kind of reservoir	Sex				Total positive	Total sample
		Male	Positive	Female	Positive		
Ujung Gading	Cat	17	0	22	0	0	39
	Dog	14	5 ( <i>Dirofilaria</i> sp.)	2	0	5 ( <i>Dirofilaria</i> sp.)	16
	(a)	31	5	24	0	5	55
Katiagan	Cat	10	1 ( <i>B. malayi</i> )	20	5 ( <i>B. malayi</i> )	6 ( <i>B. malayi</i> )	30
	Dog	12	1 ( <i>Dirofilaria</i> sp.)	4	1 ( <i>Dirofilaria</i> sp.)	2 ( <i>Dirofilaria</i> sp.)	16
	<i>Macaca fascicularis</i>	0	0	1	0	0	1
(b)		22	2	25	6	8	47
Total (a+b)		55	7	49	6	13	102

Table 2: Results of microscopic examination for microfilariae at reservoirs in Pesisir Selatan District

Location	Kind of reservoir	Sex				Total positive	Total sample
		Male	Positive	Female	Positive		
Kambang Timur	Cats	9	0	8	0	0	17
	Dogs	18	7 ( <i>Dirofilaria</i> sp.)	14	4 ( <i>Dirofilaria</i> sp.)	11 ( <i>Dirofilaria</i> sp.)	32
	<i>Macaca fascicularis</i>	1	0	0	0	0	1
(a)		28	7	22	4	11	50
Koto Nan Duo IV	Cats	11	0	9	0	0	20
Koto Hilie	Dogs	24	5 ( <i>Dirofilaria</i> sp.)	6	3 ( <i>Dirofilaria</i> sp.)	8 ( <i>Dirofilaria</i> sp.)	30
(b)		35	5	15	3	8	50
Total (a+b)		63	12	37	7	19 ( <i>Dirofilaria</i> sp.)	100

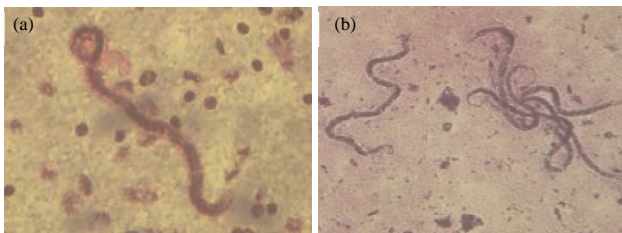


Fig. 1(a-b): Microscopic observation of microfilariae, (a) *Brugia malayi* on cats and (b) *Dirofilaria* sp., on dogs

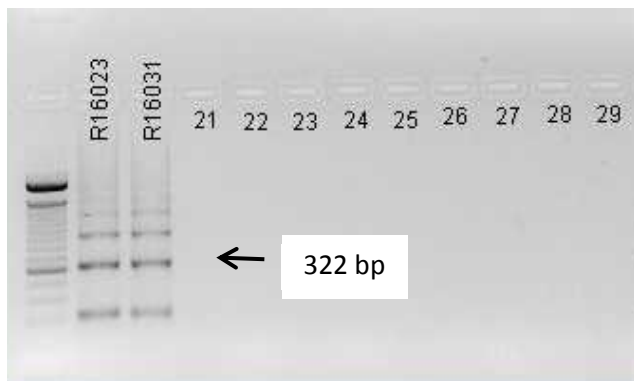


Fig. 2: PCR amplified product of Hha1 gene *Brugia malayi* on cats in Pasaman Barat District (R16023 and R16031)

The microscopic examination of thick blood samples of reservoirs in Ujung Gading was negative for *B. malayi*, but 5 dogs were positive for *Dirofilaria* sp. While in Katiagan,

6 cats were positive for *B. malayi* and 2 dogs were positive for *Dirofilaria* sp. All cats and dogs in Katiagan were dominantly pets, even though there was the stray animal that live in settlements.

Reservoir survey in Pesisir Selatan District in Nagari Kambang Timur, there were 50 animals caught (cats, dogs and *Macaca fascicularis*) while in Nagari Koto Nan Duo IV Koto Hilie there were 50 animals caught (cats and dogs). Location of the survey in Nagari Kambang Timur near hills as an ecosystem of *Macaca fascicularis*. Location of the survey in Nagari Koto Nan Duo IV Koto Hilie hills area as an ecosystem of *Macaca fascicularis* quite far. Results of the reservoir survey and microscopic examination for microfilariae in Pesisir Selatan District showed in Table 2.

Table 2 showed there weren't microfilariae *B. malayi* in reservoirs taken from Pesisir Selatan District but there were 19 dogs positive for microfilariae *Dirofilaria* sp.

All reservoir blood samples which were positive for *B. malayi* by microscopic examination were confirmed by PCR. Microscopic observation of *Brugia malayi* found in cats and *Dirofilaria* sp., that found in dogs shown in Fig. 1a and b.

Microscopic examination in Fig. 1 showed two distinct nuclei at the tip of the tail, cephalic space 2:1, microfilariae with the pink-stained sheath in Giemsa preparations, so this microfilariae was identified as *B. malayi*. All sample positive microscopic *B. malayi* by PCR presence at 322 bp PCR product, was shown in Fig. 2.

## DISCUSSION

The presence of cats that are positive for *B. malayi* in Pasaman Barat showed that reservoir animals, especially cats, play an important role in the transmission of filariasis to humans. This was related to the interaction between cats and humans in everyday life. Therefore, it is necessary to watch out for the transmission of filaria from cats to humans. Animals that played the role of reservoir for Filariasis besides cats were dogs, apes and Macaca<sup>7-9</sup>. Dogs and cats are commonly human pets and even though it is not proprietary they are found around human and are likely to be key reservoirs of pathogens that infect persons in the same environment<sup>9</sup>.

Cats were positive for *B. malayi* in Katiagan, Pasaman Barat District was 20% (6 cats) through microscopic examination and confirmed by PCR. The entire cats positive for *B. malayi* in Pasaman Barat were domestic cats. This proved that *B. malayi* filariasis is zoonotic, as it was found in animals and humans<sup>10</sup>. In the other country, Sri Lanka, reservoirs of Brugian filariasis are dogs and cats. Prevalence of *Brugia* spp., in dogs (54.4%) and cats (34.3%). In this country, *B. malayi* reemerged and appears to be a novel genetic variant. Vectors and infectious zoonotic reservoirs are present in abundance. This matter could well pose a threat to the Filariasis elimination status in Sri Lanka<sup>11,12</sup>.

To eliminate filariasis transmission, the cats that were positive for *B. malayi* microfilariae in Pasaman Barat must also be treated. The results of this survey were delivered to the department related (Pasaman Barat Food Crops, Horticulture and Animal Husbandry Services) to provide treatment of filariasis mainly in domestic cats. In Narathiwat Province (*B. malayi* endemic area), Thailand, one strategy to eliminate filariasis was annual Ivermectin treatment among cats that commenced in 2003 resulting in a decline of mf prevalence among cats from 8.0% in 1995 to 0.8% in 2015<sup>13</sup>. Another research showed that treatment of dogs with *B. malayi* by the combination of ivermectin (IVT) 200 µg kg<sup>-1</sup> body weight and DEC 6.6 mg kg<sup>-1</sup> body weight for 5 days was shorter and just as effective as the 200 µg kg<sup>-1</sup> body weight IVT daily for 14 days and 6.6 mg kg<sup>-1</sup> body weight DEC daily for 14 days<sup>14</sup>. Giving Filariasis treatment to cats can be done on domestic cats but it will be hard to do on wild cats. Cats as reservoirs of *Brugia malayi* especially in Pasaman Barat District should get attention to anticipate reemerging Filariasis in humans.

In addition to cats that were positive for *B. malayi*, dogs in Pasaman Barat and Pesisir Selatan were also positive for *Dirofilaria* sp. *Dirofilaria repens* and *D. immitis* are the most common nematode worms found in the tropics area. Animal ownership, especially dogs in Pasaman Barat and Pesisir

Selatan, which are used to accompany residents to hunt in the forest or work on their fields, need attention. The high population of dogs in the two districts is because it helps hunt pigs, which are well organized in the PORBI (a pig hunters organization). Hunting pigs is local culture, the folk game that has been handed down for generations and developed into sports in the Minangkabau of West Sumatra. A large number of free-released animals at the time of the survey showed human closeness to animals, which could potentially spread zoonotic diseases, both endoparasites and ectoparasites. In addition to a large number of wild animals, there is still a low awareness among pet owners (such as dog and cat owners) to vaccinate their animals, it is possible to transmit diseases between animals or from animals to humans and vice versa.

A similar study showed the prevalence of dirofilariasis in local dogs (30 individuals) from 5 villages in Lhoknga Aceh Besar District was 10%. *Dirofilaria* worms are rarely reported in humans in Indonesia, but several countries have reported cases *Dirofilaria* in humans<sup>15</sup>. France reported the presence of *D. immitis* in women aged 48 in 2012<sup>16</sup>. An Argentine study also reported the same type of *Dirofilaria* that infected humans and dogs both microscopically and molecularly, which was identified as *D. immitis* in 2012. Similarly, women from the San Juan Province were infected with male *D. immitis*<sup>17</sup>.

*Dirofilaria repens* has been reported to infect humans for a long time which indicated that dirofilariasis is an emerging zoonosis. In Lithuania, *D. repens* was diagnosed in humans in the period 2011-2018 with the type of attack three ocular and six subcutaneous<sup>18</sup>. Ocular *Dirofilaria repens* also infected 8 patients in Greece from 2000-2012<sup>19</sup>. Risk factors for dirofilariasis in humans increase due to climate change and extensive vector distribution. *Dirofilaria repens* has a broad geographical distribution including in Africa, Asia and Europe, as a cold-temperature region.

Dirofilariasis has now been confirmed to infect animals and humans in cold-temperature areas such as the Southeastern part of Finland (Europe) in 70 year old women who have a history of not travelling abroad<sup>20</sup>. Based on the disease caused in Europe and America, *D. immitis* has another name commonly referred to as Canine heartworm, in humans, it usually causes "Human pulmonary dirofilariasis (HPD), rarely found in the extrapulmonary and subcutaneous, whereas *D. repens* has another name "formerly dirofilaria conjunctiva" which usually causes dirofilariasis in the subcutaneous or ocular dirofilariasis, rarely results from dirofilariasis pulmonary. Until 2012, there were 1,782 cases of dirofilariasis in humans in America and Europe, consisting of 372 cases of *D. immitis* which caused HPD and 1,410 cases of *D. repens*, the majority of them in Europe including at least 24 cases in travelers<sup>21</sup>. This

indicated that dirofilariasis is an emerging parasitic disease in dogs and humans in the United States and Europe. Global warming increases disease transmission through vectors, enzootic distribution and the prevalence of microfilariae in dogs in non-endemic areas that are tourist areas.

Literature review about *D. repens* on humans from the total of 20 (90.91%) in Eropa and two (9.09%) from Asia reported there were 59.09% males, 36.36% females and, in one 4.55% case, sex was not reported. A total of 11 (50.00%) cases had subcutaneous dirofilariasis, six (27.27%) had ocular dirofilariasis, with single cases (4.55% each) of genital, mammary, lymphatic and a combination of subcutaneous and pulmonary dirofilariasis described by Pupić-Bakrač *et al.*<sup>22</sup>. Previous research showed that *Dirofilaria repens* infection was detected in a 39 years old woman from Kuchesfahan district of Gilan Province, Northern Iran with a suspected case of cutaneous fascioliasis. These infections have manifested as an itching and highly erythematous subcutaneous tender nodule on her right thigh<sup>23</sup>.

Most cases of dirofilariasis in humans are different from malignant processes associated with pulmonary, abdominal and urogenital lesions which often require invasive procedures, so to prevent dirofilariasis in humans is more emphasized in chemoprophylaxis dirofilariasis in animals (dogs) and further increases the examination and treatment of dogs (animals) in areas that have a high prevalence of dirofilariasis. This can be done by further enhancing epidemiological surveys and expanding epizootic areas in wild and domestic animals, as well as further enhancing microfilariae surveys of mosquitoes<sup>21</sup>.

Research in 2017 in Doha, Qatar, held 150 animal owners, although 83% of them were vaccinated every year and almost all (95.3%) were given dry food but the examination showed 51% of them were found endoparasites and ectoparasites in their favourite animals<sup>24</sup>. Amounts 8.7% of pet owners in the study reported having been infected with zoonosis, comprise with 10 people with dermatophytosis, cat scratch 2 people and diseases caused by ticks/ectoparasites in one person<sup>24</sup>.

There is still a lack of attention from animal owners and the scarcity of routine inspection of animals against dirofilariasis in Indonesia, one of which is due to the limited available diagnostic tools. The most common method for diagnosing *D. immitis* in dogs is an examination of microfilariae and/or specific antigen examination. Examination of microfilariae has a very low sensitivity because of the occult infection ie infection without the presence of microfilariae in peripheral blood. The *D. immitis* antigen detection test currently available is only able to detect female worm antigens<sup>25</sup>. The limitation of this study was on the little number of monkeys that had been caught although in

observation population of monkeys including *Macaca* has seen a lot in survey location in Pasaman Barat. Research that focuses on monkeys as a reservoir for Filariasis or *Dirofilaria* could be done especially in Pasaman Barat.

## CONCLUSION

Dogs in the Pesisir Selatan and Pasaman Barat found positive *Dirofilaria* sp., which were 19 from 62 (14.5%) in the Pesisir Selatan and 7 from 32 (21.9%) in Pasaman Barat. *Brugia malayi* filariasis in cats was found in Pasaman Barat with 6 of 69 (8.7%) cats examined. Two long-tailed monkeys (*Macaca fascicularis*) examined from the two districts were entirely negative for microfilariae *B. malayi*. There needs to be awareness of filaria transmission from cats and dogs to humans, both *B. malayi* and *Dirofilaria*.

## SIGNIFICANCE STATEMENT

Reservoir detected as filaria reservoir in survey location was the first time. *Brugia malayi* in domestic animals (cats) and dirofilaria in domestic animals (cats and dogs). This study will help the researchers, governments and other organizations in order if there is no progress in filariasis condition despite many efforts had been done to the community to eliminate filariasis, to check on domestic reservoirs (cats, dogs or monkeys). These findings call attention for also handling animals in filariasis dan dirofilariasis transmission.

## ETHICAL CLEARANCE

This research has ethical approval from the Ethics Commission of the National Institute of Health Research and Development Center, Indonesia Ministry of Health, number: LB.02.01/2/KE.167/2017 and LIPI number B-1430/IPH.I/S.02.04/V/2017. Approval was obtained from relevant Provincial Directors of animal production and health and divisional veterinary officers. Informed written consent was obtained from the owners of domestic cats and dogs.

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