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Prevalence of *Brugia malayi* in dogs in lymphatic filariosis endemic areas of Kerala#

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Abstract

The present study was envisaged to determine the prevalence of Brugia malayi in dogs in lymphatic filariosis endemic areas of Kerala. A total of 300 samples, consisting of 100 dog blood samples each from the endemic areas of Alappuzha, Ernakulam and Thrissur districts were screened. Out of 300 dogs screened by wet film technique, 56 (18.67 per cent) were positive for microfilariosis. Giemsa staining of blood smears revealed that 15 (five per cent) had sheathed microfilariae and 41 (13.67 per cent) had unsheathed microfilariae. All the samples found positive for sheathed microfilariae were subjected to histochemical differentiation for species identification. The occurrence of B. malayi in dog blood samples from Alappuzha, Ernakulam and Thrissur districts based on histochemical staining were one per cent, 1.33 per cent and 2.67 per cent, respectively. The overall occurrence of B. malayi was five per cent in the three districts. The present study confirmed the presence of B. malayi, a zoonotic pathogen, in dogs in Kerala.

Key words: *Brugia malayi, dog, histochemical staining*

Lymphatic filariosis (LF) is a leading cause of permanent and long-term disability in man and is ranked third in terms of disability adjusted life year (DALY) after malaria and tuberculosis. Caused by nematodes of the super family *Filarioidea* the infection occurs through prolonged exposure to bites of several species of mosquitoes belonging to the genera *Culex*, *Mansonia*,

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Aedes and *Anopheles* (Nuchprayoon *et al*., 2006). According to WHO, *Wuchereria bancrofti* is responsible for over 90 per cent of human infections globally and the remaining 10 per cent of infection is contributed by *Brugia malayi. Wuchereria bancrofti*, periodic strain of *B. malayi* and *Brugia timori* exclusively infect humans, whereas the subperiodic strain of *B. malayi* and *Brugia pahangi* are zoonotic (Tan *et al*., 2011). In humans, filariosis caused by *Brugia* species is commonly manifested as episodes of fever, lymphangitis and lymphadenitis. On the other hand, filarial infections in humans that have *W. bancrofti* as causative agent are presented in more severe form with fewer, acute episodes. Swelling and disfigurement of the limbs, lymphedema, hydrocele, chyluria, chylous diarrhea and chylorrhagia are some of the key indications and symptoms associated with filariosis in animals (Mathiarasan *et al.,* 2021). All canine filariae have the potential to infect humans and hence remain important from a public health perspective. Control of zoonotic filariae in canine and feline reservoirs would be of great public health importance and could contribute to a decrease in human filariosis cases (Rishniw *et al.,* 2006).

In Kerala, brugian filariosis caused by *B. malayi* is increasingly prevalent in dogs (Ambily *et al*., 2011). Identification of the parasite in dogs in lymphatic filariosis endemic areas would indicate the zoonotic threat. Hence, the present study focused on the detection and differentiation of *B. malayi* among dogs in lymphatic filariosis endemic areas of Kerala.

Materials and methods

Collection of canine blood samples

A total of 300 dogs above six months of age presented to the Government Veterinary Hospitals and Animal Birth Control centres of the State Animal Husbandry Department from endemic areas of Alappuzha, Ernakulam and Thrissur districts were screened by wet blood film examination during day hours between 9.00 a.m. to 4.00 p.m. (Table 1). Wet film positive dogs were selected for the study.

All the animals in the present study were screened for microfilariae by wet film examination and further the blood smears were examined after Giemsa staining.

Histochemical staining

Approximately three millilitres of blood was drawn from the cephalic vein of the animals and allowed to clot. The serum fraction was centrifuged for five minutes at 3000 rpm. The supernatant fluid was discarded leaving a drop in the bottom of the tube. A drop of this fluid containing the resuspended sediment was used for preparation of smears. Smears prepared from the sediment were air dried, fixed in absolute chilled acetone for one minute, further air dried and kept at -20ºC for histochemical staining for the detection of acid phosphatase activity as reported by Chalifoux and Hunt (1971). The smears were stained with Acid Phosphatase Leukocyte Kit (Far diagnostics, Italy) as per manufacturer's procedure. This kit utilises Naphthol AS-BI phosphatase as the substrate and pararosanilin as the chromogen. Identification of microfilariae was done according to Chalifoux and Hunt (1971) and Kobasa *et al.* (2004).

The data obtained were subjected to statistical analysis using the SPSS version 24.0. To determine the relation of different parameters (age, breed, gender) on occurrence of *Brugia* spp., Fisher's Exact Test and *Chi*-square test were performed.

Results and discussion

Wet film examination

Out of the three districts where the study was conducted, the occurrence of microfilariae based on wet film examination was found to be highest in Thrissur district (8.67 %) followed by Ernakulam (6.67 %) and Alappuzha district (3.33 %). The overall occurrence of microfilariae based on wet film examination across the three districts were 18.67 per cent. It was similar to the results of Loymek *et al.* (2021), who reported an occurrence of microfilariae in 16.2 per cent blood samples of dogs collected from Chanthaburi province of Thailand.

The findings of the present study are lower than the study of Ravindran *et al.* (2014)

Table 1. Canine blood samples collected from different sources

Sl. No.	Sources	Samples collected
1	District Veterinary Centre, Alappuzha	100
2	District Veterinary Centre, Ernakulam	50
3	Animal Birth Control Centre, Kakkanad	50
4	Animal Birth Control Centre, Chavakadu	40
5	Animal Birth Control Centre, Paravattani	25
6	Oyster Animal Health Care Hospital, Chowannur	15
7	Government Veterinary Hospital, Chavakadu	20
	Total	**300**

in Alappuzha district (31.17 per cent). Mak *et al.* (1980) reported a higher occurrence of microfilariae (57.4 per cent) in dogs in Peninsular Malaysia. Much higher rates of microfilariosis, 68.8 per cent was reported by Mallawarachchi *et al.* (2018) in dogs in Sri Lanka with human subperiodic brugian filariosis. A study by Ambily *et al.* (2011) conducted at Thrissur district, Kerala revealed that out of 100 dogs with symptoms of filariosis, circulating microfilariae were detected in 80 cases. This higher occurrence of canine microfilariosis might be due to the fact that the selected study population were having symptoms. However, Radhika (1997) and Sabu *et al*. (2005) reported a lower occurrence *ie.*, 7.59 per cent and seven per cent, respectively in blood samples of dogs from Thrissur district. The lower occurrence could also be attributed to proper health management of the animals.

Giemsa staining of blood smears

In Giemsa-stained smears, sheathed microfilariae were identified based on the morphological peculiarities. It appeared with bluish violet coloured body and a pink coloured sheath extending beyond both the anterior and posterior ends (Fig. 1).

Out of the 56 wet film positive samples in three districts, 15 sheathed and 41 unsheathed microfilariae were detected by Giemsa staining. The overall occurrence of sheathed and unsheathed microfilariae based on Giemsa staining were five per cent and 13.67 per cent, respectively, across the three districts (Table 2). No association was noticed between the occurrences of sheathed and unsheathed microfilariae in different districts by statistical analysis.

Fig. 1. Sheathed microfilariae on Giemsa staining

The occurrence of sheathed microfilariae in dogs in Alappuzha district was found to be higher in the present study compared to the findings of Ravindran *et al*. (2014), in which they estimated it to be 2.44 per cent. The occurrence observed in the present study was higher than the reports of Chirayath *et al.* (2017), who observed a lower occurrence (1.8 per cent) of sheathed microfilariae in dogs in Thrissur district.

Rathnayke *et al*. (2022) reported a high occurrence (20.6 per cent) of sheathed microfilariae in dogs in endemic and non endemic LF areas of Sri Lanka. In Sri Lanka, the high rates of zoonotic filarial infections strongly implicated dogs as potential reservoirs for human brugian filariosis.

Higher occurrence of sheathed microfilariae (16 per cent) in dogs with clinical symptoms suggestive of filariosis was reported by Ambily *et al.* (2011) in Thrissur district, Kerala. This lower occurrence (five per cent) of canine sheathed microfilariae in the present study might be the effect of proper chemoprophylaxis with antifilarial agents (ivermectin) in animals (Radhika,1997). However, the occurrence of sheathed microfilariae in dogs in the present

Table 2. Occurrence of canine microfilariae in different districts on Giemsa staining

Sl. No	District	Samples analysed	Giemsa staining					
			Sheathed samples		P Value	Unsheathed samples		P Value
			No.	%		No.	%	
1	Alappuzha	100	3	1.00	0.332 ns	7	2.33	0.055 ns
2	Ernakulam	100	4	1.33		16	5.33	
3	Thrissur	100	8	2.67		18	6.00	
Total		**300**	**15**	**5.00**		**41**	**13.67**	

ns:nonsignificant (p<0.05)

Table 3. Age wise occurrence of canine microfilariae in different districts by Giemsa staining

Sl. No.	District	Age (Year)	Samples analysed	Sheathed microfilariae		P value	Unsheathed microfilariae		P value
				No.	%		No.	%	
1	Alappuzha	<2	56	1	1.0	0.648 ns	2	2.0	0.166 ns
		2-4	36	2	2.0		5	5.0	
		>4	8	0	0.0		0	0.0	
		Total	**100**	**3**	**3.0**		**7**	**7.0**	
2	Ernakulam	<2	26	0	0.0	0.181 ns	3	3.00	0.237 ns
		2-4	69	3	3.0		13	13.00	
		>4	5	1	1.0		0	0.00	
		Total	**100**	**4**	**4.0**		**16**	**16.0**	
3	Thrissur	<2	32	2	2.0	1.00 ns	7	7.0	0.263 ns
		2-4	66	6	6.0		10	10.0	
		>4	2	0	0.0		1	1.0	
		Total	**100**	**8**	**8.0**		**18**	**18.00**	
Total			**300**	**15**	**5.00**	**0.478 ns**	**41**	**13.67**	**0.131 ns**

ns:nonsignificant (p<0.05)

study could emphasize the potential risk for human brugian filariosis in endemic areas.

Age wise occurrence of canine microfilariae in different districts by Giemsa staining

Dogs examined for the presence of microfilariae in Alappuzha, Ernakulam and Thrissur districts were categorised into three age groups, *viz*., less than two years, two to four years and more than four years of age.

No significant difference in occurrence of sheathed and unsheathed microfilariae in different age groups was observed. The present finding was in accordance with Ambily *et al*. (2011) and Ravindran *et al.* (2014). Valid conclusions regarding the association between susceptibility to microfilariae and age of dogs can be drawn by further extending the study to a larger population for an extended period of time.

Breed wise occurrence of canine microfilariae in different districts by Giemsa staining

Dogs examined for the presence of microfilariae in Alappuzha, Ernakulam and Thrissur districts were categorised into two breed groups, *viz.*, nondescript and exotic breeds.

Out of 41 of dogs positive for unsheathed microfilariae 34 belonged to nondescript followed by seven dogs from exotic breeds (Table 4). A significant difference in the occurrence of unsheathed microfilariae between different dog breed groups in different districts was observed. The results of the present study are contrary to the findings of Ambily *et al.* (2011) and Ravindran *et al.* (2014), where they reported a higher occurrence of sheathed and unsheathed microfilariae in exotic breeds. This could possibly be due to an

Table 4. Breed wise occurrence of canine microfilariae in different districts by Giemsa staining

Sl. No.	District	Breed	Samples analysed	Sheathed microfilariae		P Value	Unsheathed microfilariae		P Value
				No.	%		No.	%	
1	Alappuzha	Nondescript	40	1	1.0	1.00 ns	2	2.0	1.00 ns
		Exotic	60	2	2.0		5	5.0	
		Total	**100**	**3**	**3.0**		**7**	**7.0**	
2	Ernakulam	Nondescript	76	2	2.0	0.570 ns	16	16.0	0.011*
		Exotic	24	2	2.0		0	0.0	
		Total	**100**	**4**	**4.0**		**16**	**16.0**	
3	Thrissur	Nondescript	71	7	7.0	0.432 ns	15	15.0	0.26 ns
		Exotic	29	1	1.0		3	3.0	
		Total	**100**	**8**	**8.0**		**18**	**18.0**	
Total			**300**	**15**	**5.0**	**0.722ns**	**41**	**13.67**	**0.003***

ns:nonsignificant, *Significant (p<0.05)

Table 5. Gender wise occurrence of canine microfilariae in different districts by Giemsa staining

Sl. No	District	Gender	Samples analysed	Sheathed microfilariae		P Value	Unsheathed microfilariae		P Value
				No.	%		No.	%	
1	Alappuzha	Male	54	2	2.0	1.00 ns	6	6.0	0.12 ns
		Female	46	1	1.0		1	1.0	
		Total	**100**	**3**	**3.0**		**7**	**7.0**	
2	Ernakulam	Male	64	3	3.0	1.00 ns	13	13.0	0.158 ns
		Female	36	1	1.0		3	3.00	
		Total	**100**	**4**	**4.0**		**16**	**16.0**	
3	Thrissur	Male	58	5	5.0	0.717 ns	14	14.0	0.07 ns
		Female	42	3	3.0		4	4.0	
		Total	**100**	**8**	**8.0**		**18**	**18.0**	
Total			**300**	**15**	**5.00**	**1.00 ns**	**41**	**13.67**	**0.003***

ns:nonsignificant, *Significant (p<0.05)

increased exposure to mosquito vectors and also less medical attention and care received by nondescript population compared to other exotic breeds. The nondescript population are found to offer resistance to the clinical effects and this further increases the chances of spread of the infection. These findings show the necessity of regular screening of nondescript dogs in endemic areas.

Gender wise occurrence of canine microfilariae in different districts by Giemsa staining

Animals screened for the presence of microfilariae in blood samples were categorized based on the gender, as male and female (Table 5).

Ambily *et al.* (2011) and Ravindran *et al*. (2014) reported a higher occurrence of microfilariae in male dogs compared to female dogs. In study, there was a difference in the occurrence of unsheathed microfilariae between genders in different districts. The role of gender in the occurrence of sheathed microfilariosis needs further research.

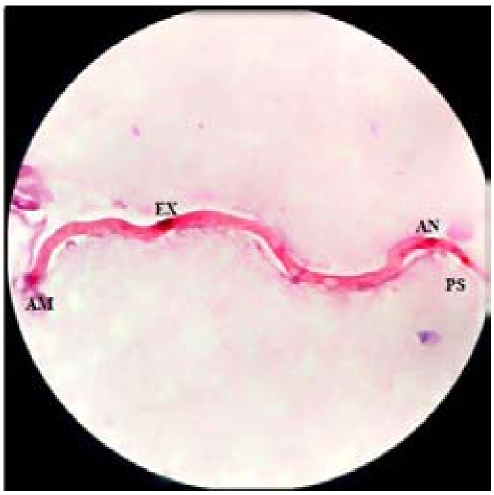
Species identification by histochemical differentiation

All samples found to be positive for sheathed microfilariae on Giemsa staining

Table 6. Occurrence of *B. malayi* in canines in different districts by histochemical staining

Sl. No.	Districts	Samples analysed	*B. malayi*		P Value
			No.	%	
1	Alappuzha	100	3	1.00	0.684^{ns}
2	Ernakulam	100	4	1.33	
3	Thrissur	100	8	2.67	
Total		**300**	**15**	**5.00**	

ns:nonsignificant ($p<0.05$)



AM - Amphid, EX - Excretory pore, AN - Anal pore, PS - Phasmid

Fig. 2. *Brugia malayi* on histochemical staining

were positive for histochemical staining with four-point staining pattern (Fig. 2). The four points (amphids, excretory pore, anal pore and phasmid) in the microfilarie had taken bright red colour, indicating the acid phosphatase activity in these four areas (Sadarama *et al*., 2019). This four-point staining pattern could be observed in all the 15 positive cases of sheathed microfilariae. The percentage occurrence of *B. malayi* in canine blood samples from Alappuzha, Ernakulam and Thrissur districts were one per cent, 1.33 per cent and 2.67 per cent, respectively. The overall occurrence of *B. malayi* based on histochemical staining was five per cent in different districts (Table 6).

This occurrence was higher than that reported by Ravindran *et al.* (2014), who reported that out of 164 dogs, six tested positive for *B. malayi* (3.66 per cent) by histochemical reaction. Higher percentage of *B. malayi* infection (15 per cent) in dogs was also reported by Ambily *et al.* (2011) based on histochemical staining in Thrissur district.

The presence of microfilariae in the peripheral blood of dogs in the present study was observed in the day time between 9.00 a.m. and 4.00 p.m., which indicated the presence of subperiodic strain of *B. malayi,* the zoonotic pathogen. So, the implementation of proper treatment and control measures are essential to curtail the potential risk of filariosis in animals as well as to reduce its public health risk.

Conclusion

The present study revealed a five per cent occurrence of subperiodic form of *B. malayi,* a zoonotic pathogen, in clinically asymptomatic dogs in Kerala. The occurrence of canine brugian infections in this study is indicative of the extent of the health risk and the importance of treating the animal reservoir. However, untreated animals may serve as a source of new infection and hence, the study indicated that in addition to chemotherapy and vector control, the control of infection in reservoir host is very much essential for the effective implementation of human filariosis control programme. Further studies on assessing the vector potentiality in different mosquito species present in different geographic regions of Kerala are required. The one health approach, with a collaboration between diverse disciplines, is recommended to address the complex issues associated with the control of zoonotic filariosis.

Conflict of interest

The authors declare that they have no conflict of interest.

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