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ORIGINAL ARTICLE

Comparison of efficacy of ivermectin and diethylcarbamazine against naturally infected *Brugia malayi* microfilaria in dogs

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Abstract Filarial parasites like *Brugia pahangi* and *Brugia malayi* can infect dogs. Adults of *Brugia* genus resides in the lymphatic system and microfilariae, in blood. There are increasing reports of detection of *B. malayi* microfilariae in dogs. A study was undertaken to compare the efficacy of repeated oral dosing of ivermectin (IVT) and diethylcarbamazine (DEC), individually and in combination against naturally infected *B. malayi* microfilariae in dogs. The species of the microfilariae was confirmed by acid phosphatase staining and polymerase chain reaction. The three treatment groups were 200 mcg/kg body weight IVT daily for 14 days (I), 6.6 mg/kg body weight DEC daily for 14 days (II) and IVT and DEC together in the same dose for a period of 5 days (III). Microfilarial status of the peripheral blood was assessed on the 0th, 7th, 14th and 21st day. Haematological parameters were measured on day zero and on the 21st day. Though, all the three treatment groups showed a reduction in the microfilarial concentration through the study period, complete absence of detectable microfilaremia was not noticed in any of the three groups by 21st day. Among the haematological parameters, statistically significant difference was observed in the post-treatment means of haemoglobin levels of group III when compared with group II. Since group III regime (IVT + DEC) was shorter and just as effective as the longer ones, it is considered superior to the other two.

Keywords *Brugia malayi* · Dog · Microfilaricidal · Ivermectin · Diethylcarbamazine

Introduction

A wide range of filarial parasites are being detected in animals. Often these agents have a zoonotic potential. Filariasis due to *Brugia malayi* accounts to 5% of the human filariasis in India (Suma 2013). Among different states, Kerala is endemic for human brugian filariosis. *B. malayi* is reported from dogs in Kerala resulting in a wide range of manifestations from amicrofilaremia to lymphedema (Ambily et al. 2011; Chirayath et al. 2017a, b). Albendazole and diethylcarbamazine are the microfilaricidal drugs used worldwide as part of the Global Elimination of Lymphatic Filariosis Program (GELFP) with the objective of reducing microfilariae levels below the transmission rate by Mass Drug Administration (MDA), in areas where the disease is endemic. Successful use of ivermectin and DEC in brugian filariosis has been reported in felines and Indian leaf nose monkey. DEC has been used successfully to treat microfilaremia caused by *Dirofilaria immitis* in canines but there exists a paucity of literature for the use of these drugs against microfilariae of *Brugia* spp. Hence the current case study was undertaken to compare the efficacy of these agents in canines.

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Materials and methods

Identification of positive cases

A total of 3000 dogs above the age of 4 months presented to the University Veterinary Hospital Kokkalai and Teaching Veterinary Clinical Complex, Mannuthy, Thrissur, Kerala, India, for various complaints, general check-up or vaccination were screened by wet blood film examination for moving microfilariae during 8:00 am to 4:30 pm. Thick blood smears prepared from dogs positive for wet film examination from ear pinna, for moving microfilariae were subjected to Giemsa staining. Blood smears from dogs positive for sheathed microfilariae by Giemsa staining were subjected to acid phosphatase staining according to the procedure described by Chalifoux and Hunt (1971). Eighteen dogs with microfilarial staining pattern by acid phosphatase method as that of *B. malayi* were selected for the study. Clinical signs were recorded for the positive cases of brugian filariosis.

Species of the microfilariae were confirmed by polymerase chain reaction (PCR). Genomic DNA was extracted from whole blood and PCR was conducted using primers for amplification of *Hha* 1 repeat sequences (Xie et al. 1994). PCR products obtained were sequenced analyzed using Basic Local Alignment Search Tool (BLAST) and submitted to GenBank.

Treatment protocol

Positive cases with brugian microfilaria were randomly divided into three groups with 6 animals in each group. The drug regimen was started from the subsequent day onwards.

Group I—Ivermectin @ 200 µg/kg body weight orally for 2 weeks
Group II—Diethylcarbamazine @ 6.6 mg/kg body weight orally for 2 weeks
Group III—Ivermectin (IVT) @ 200 µg/kg body weight with diethylcarbamazine (DEC) @ 6.6 mg/kg body weight orally for 5 days

Cases which were heavily positive on wet film examination were given prednisolone @ 1 mg/kg body weight orally for 3 days from the start of the chemotherapy to prevent a possible post-treatment inflammatory reaction activated during the process of killing of microfilariae.

Treatment response

Response to treatment was assessed based on the microflilaremic status of peripheral blood along with improvement in the haematological parameters.

Microfilaremic status

The number of microfilariae in the thick blood smear prepared from 25 µl of peripheral blood was counted on the 0th, 7th, 14th and 21st day.

Haemtological parameters

Haematological parameters were assessed on the 0th and 21st day by collecting whole blood (2 ml) in EDTA vials for evaluation of the total erythrocyte count (TEC), volume of packed red cells (VPRC), haemoglobin (Hb) concentration, total leukocyte count (TLC), differential leukocyte count (DLC) and platelet count using automatic animal blood cell counter.

Statistical analysis

Haematological parameters were analyzed using paired *t* test. The results in between the groups was assessed using one was analysis of co-variance (ANOVA). The microfilarial counts on weekly basis in the three groups were assessed using repeated measures ANOVA for Day 0, 7, 14 and 21. The analysis was done using the statistical package, Statistical Package for Social Sciences (SPSS) version 24.0 (Fig. 1).

Results

Identification of positive cases

Brugia malayi microfilariae were identified by the demonstration of metabolic activity at four points (amphids, phasmids, anal and excretory pore) by precipitation

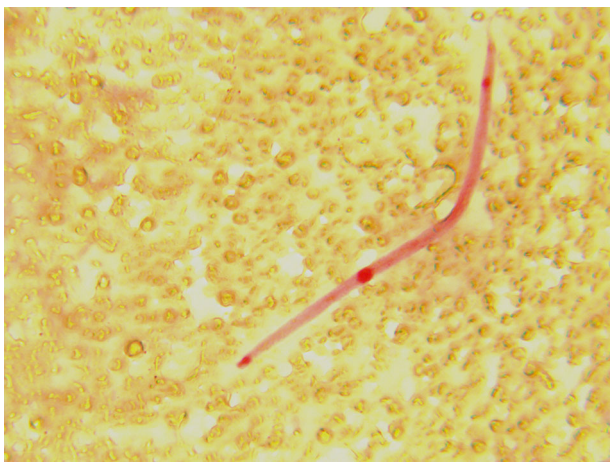

Fig. 1 Acid phosphatase staining of *Brugia malayi* microfilaria showing staining at amphid, phasmid, anal pore and excretory pore

of red azo dye (Plate 1) after acid phosphatase staining (Chirayath et al. 2015, 2017b).

Polymerase chain reaction with *Hha*1 primers gave an amplified PCR product of 322 bp. The product obtained was sequenced and analyzed using Basic Local Alignment Search Tool (BLAST). The product revealed 99% homology with *B. malayi* isolate. Nucleotide sequence data reported in this paper are available in the GenBank™ under the accession number: *MG063782*.

Out of the 3000 dogs screened, seventy were positive for brugain filariosis. Of the 70 cases, 27 cases were asymptomatic. The clinical symptoms could be divided into generalised signs (anorexia, pyrexia and congested mucous membrane), gastrointestinal signs (vomiting), dermatological signs (alopecia and dermatitis), lymphatic signs (lymphadenopathy, lymphangitis, limb edema, scrotal edema and lameness) and ophthalmological signs (lacrimation and corneal opacity). Pyrexia was the most common clinical finding and was seen in 40% of the cases. Apart from the generalised signs, lymphadenopathy was most commonly observed (31.42%) followed by limb edema (22.85%).

Evaluation of treatment groups bases on microfilaremic count

The microfilaremic counts in individual cases and the mean microfilaremic counts in the three groups are shown in Tables 1, 2, 3 and 4. Though, all the three treatment groups showed a reduction in the microfilarial concentration during the weekly assessment, complete absence of detectable microfilaremia was not noticed in any of the three groups till the 21st day. By the 21st day the average microfilaraemic count had dropped to 1.333 in group I and II, while it was 1.167 in group III.

Evaluation of treatment based on haematological parameters

The mean TEC, VPRC, Hb, TLC, DLC and platelet groups are shown in Tables 5, 6, 7, 8 and 9 respectively. The mean TEC values of the first two groups on day zero and 21st day when compared to the standard values revealed a mild anaemia. Though there was an increase in the TEC values on the 21st day, the difference was not statistically significant. Both the pre and post-treatment mean values of VPRC in all the three groups were below the standard normal range for dogs. A slight increase was noticed in VPRC from the pre-treatment to post-treatment values which were not statistically significant. The pre-treatment haemoglobin values were lower than the standard values in first and second group. The values had improved post-treatment in all the three groups. Statistically significant difference was observed in the post-treatment means of group III when compared with group II.

The average total leukocyte count of Group I and II were higher than the standard values whereas group III was similar to the standard range. The post-treatment total leukocyte values of all the three groups were within the normal range thus indicating an improvement in this parameter. However, the improvement was not statistically different. Both, the pre and post-treatment values were within the standard range for lymphocyte per cent. The pre-treatment granulocyte per cent was slightly higher than the higher range of the standard granulocyte per cent for all the three groups. The post-treatment values were in the standard range for group II and III. The pre-treatment average of platelets in group II was lower than that of the standard value. There was a mild increase in the platelet count in all the three groups post-treatment.

Discussion

In Ivermectin (2 weeks) treated group, gradual decrease in microfilaremia over the weeks was similar to the response noted previously in humans (Reddy et al. 2000) and animals (Murthy et al. 2004; Taweethavonsawat and Chungpivat 2013). Complete microfilaricidal action by the 21st day was detected only in one animal. This treatment was further supported (Phantana et al. 2002) where single oral or subcutaneous administration of ivermectin were used to reduce the microfilarial load in cats with *B. malayi* microfilariae.

In Diethyl carbamazine (2 weeks) treated group, rapid decrease of microfilarial counts were observed. Similar

Table 1 Microfilaraemic count on weekly assessments in Group I (ivermectin @ 200 μg/kg for 2 weeks)

Days	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Day 0	10	20	17	12	22	7
Day 7	7	9	9	8	17	4
Day 14	3	4	5	3	8	2
Day 21	0	2	2	1	2	1

Table 2 Microfilaraemic count on weekly assessments in Group II (diethylcarbamazine @ 6.6 mg/kg for 2 weeks)

Days	Case 7	Case 8	Case 9	Case 10	Case 11	Case 12
Day 0	16	9	7	18	11	19
Day 7	7	6	5	11	5	8
Day 14	0	2	4	5	4	3
Day 21	0	0	1	4	3	0

Table 3 Microfilaraemic count on weekly assessments in Group III (ivermectin @ 200 μg/kg with Diethylcarbamazine @ 6.6 mg/kg for 5 days)

Days	Case 13	Case 14	Case 15	Case 16	Case 17	Case 18
Day 0	27	11	10	15	9	23
Day 7	9	8	9	10	6	15
Day 14	4	6	5	7	4	8
Day 21	0	2	0	1	1	3

Table 4 Average microfilaraemic count in the different groups in the weekly assessments

Groups	Day 0	Day 7	Day 14	Day 21	*p* value
Group I	14.667^{a}	9.000^{b}	4.167^{c}	1.333^{d}	0.001*
Group II	13.333^{a}	7.000^{b}	3.000^{c}	1.333^{d}	0.001*
Group III	15.833^{a}	9.500^{a}	5.667^{b}	1.167^{c}	0.004*
p value	0.788^{ns}	0.408^{ns}	0.076^{ns}	0.968^{ns}	

*Significant at 1% level. *ns* not significant. There is no significant difference within the coloumns. Means with common superscript (a–d within rows for the three groups) do not vary significantly

Table 5 Total erythrocyte count of microfilaraemic dogs before and after treatment

Treatment groups	Total erythrocyte count (10^6/cmm) Mean ± SE	
	Zeroday	21st day
Gropu I	3.9550 ± 0.48099	4.9967 ± 0.34480
Group II	4.2917 ± 0.54626	5.2450 ± 0.52594
Group III	5.4133 ± 0.25565	5.7517 ± 0.29771
p value	0.084^{ns}	0.421^{ns}

ns not significant

Table 6 Volume of packed red cell of microfilaraemic dogs before and after treatment

Treatment groups	Volume of packed red cell (%)	
	Zero day	21st day
Group I	24.333 ± 2.4867^{b}	31.867 ± 2.9090
Group II	21.433 ± 2.8466^{b}	28.667 ± 2.3734
Group III	34.450 ± 1.6397^{a}	35.900 ± 2.0489
p value	0.004*	0.150^{ns}

Means with the same superscript do not vary significantly from each other

ns not significant

*Significant at 1% level

response is reported with DEC treatment in Indian leaf monkeys with Brugian microfilariae (Murthy et al. 2004). 12 days regimen of DEC followed in human patients also resulted in a rapid short term microfilarial suppression (Fernando et al. 2010). The variance seen in individuals, with some cases showing 100% microfilaricidal action whereas only 72–85% clearance in other cases, similar to previous reports (Stolk et al. 2005), where high variance in the efficacy of the DEC treatment was reported.

When iveremectin was combined with DEC for 5 days, there was drastic reduction in the microfilarial count when compared to the day of presentation. Complete microfilaricidal action was seen in two animals of the group by the 21st day and this finding was comparable to reported results (Chansiri et al. 2005), where single dose of ivermectin and DEC was used in cats infected with *B. malayi.* They reported that complete clearance of the microfilaria

Table 7 Haemoglobinconcentration of microfilaraemic dogs before and after treatment

Treatment groups	Haemoglobin (g/dl)	
	Zero day	21st day
Group I	9.833 ± 0.9962	11.600 ± 0.7132[a,b]
Group II	9.900 ± 1.2285	10.867 ± 0.9656[b]
Group III	12.867 ± 0.6731	14.067 ± 0.8849[a]
p value	0.078[ns]	0.047

Means with the same superscript in a column do not vary significantly from each other

ns not significant

was dependent on the initial microfilaria load and animals with lower burden showed faster clearance. Further studies could investigate long term microfilaricidal action in dogs using single dose administration with a combination. Shorter duration of therapy would increase owner compliance and reduces the cost of the treatment.

Mild anemia, leukocytosis and thrombopaenia were noted in microfilaraemic animals. These finding were similar to that seen in previous studies conducted (Ambily 2009; Chirayath 2013; Thomas 2016). Anaemia, thrombocytosis and leukocytosis in animals infected with *Dipetalonema reconditum* have been previously reported (Hashem and Badawy 2008) and this was similar to the results of the haematological assessment in the current study. They attributed the anemia to the haemolytic activity of the microfilariae.

There was no statistically significant improvement in the haematological parameters of any of the groups after treatment. Amongst the six haematological parameters assessed between the three groups, statistically significant difference was noted only for the haemoglobin values. Group III (IVT + DEC) showed a statistical increase in the haemoglobin values on the 21st day when compared with group II.

Conclusion

There was no significant difference in the weekly assessment of the microfilaraemic status in between the three treatment groups. A significant difference was noted in the microfilaraemic counts within the groups. The microfilaraemic count was found to be zero in individual cases in all the three groups. None of the treatments resulted in complete absence of detectable microfilaraemia by 21st day. Mild anemia, leukocytosis and thrombopaenia were noted in microfilaremic animals. Amongst the six haematological parameters assessed between the three groups, statistically significant difference was noted only for the haemoglobin

Table 8 Total leukocyte count and differential leukocyte count

Parameters	TLC (10^3/cmm)		Lym %		Mon %		Gra %	
	0th day	21st day	0th day	21st day	0th day	21st day	0th day	21st day
Group I (IVM)	18.733 ± 3.6673[ns]	15.400 ± 1.2772[ns]	18.017 ± 2.0560[ns]	17.267 ± 3.6718[ns]	4.683 ± 0.5400[ns]	5.117 ± 0.5799[ns]	78.800 ± 3.1620[ns]	75.717 ± 3.3394[ns]
Group II (DEC)	19.533 ± 4.1758[ns]	13.883 ± 1.8398[ns]	18.767 ± 1.5594[ns]	16.950 ± 1.3703[ns]	6.133 ± 0.5858[ns]	5.300 ± 0.4851[ns]	76.800 ± 2.4963[ns]	73.483 ± 2.1409[ns]
Group III (IVM + DEC)	12.300 ± 1.6164[ns]	11.233 ± 1.4405[ns]	19.183 ± 2.7910[ns]	13.767 ± 1.8057[ns]	5.267 ± 0.8452[ns]	5.423 ± 1.0452[ns]	75.550 ± 3.4595[ns]	73.133 ± 4.4059[ns]
p value	0.275	0.187	0.930	0.560	0.333	0.348	0.755	0.847

Values with ns do not vary significantly from each other

Table 9 Platelet count of microfilaraemic dogs before and after treatment

Platelet	0th day	21st day
Group I	181.167 ± 44.7150	201.500 ± 42.0379
Group II	142.167 ± 31.5145	168.667 ± 19.8287
Group III	208.333 ± 20.8865	247.333 ± 15.3355
p value	0.402^{ns}	0.176^{ns}

values on the 21st day. Group III showed a statistical increase in the haemoglobin values on the 21st day when compared with group II.

Animals of group I (Ivermectin) and group II (DEC) were administered microfilaricidal drug for a period of 2 weeks whereas group III (Ivermectin + DEC) were given microfilaricidal drug for only 5 days. The results of group III showed no statistical difference when compared with the other two groups except for the increase in haemoglobin when compared to group II. However since this treatment regime was shorter and just as effective as the longer ones, it was considered superior to the other two.

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Compliance with ethical standards

Ethical statement There is no separate funding for this research project. The project is formulated following the guidelines of Institutional Animal Ethics Committee and got approval from faculty Research Committee with code number Ad/9/96/MVM/2015/CM. It is certified that all biomaterials required for the research were collected and the treatments were assigned, with prior written consent from the owners of the animals. There is no conflict of interest for the research findings. The article is a part of thesis submitted to Kerala Veterinary and Animal Sciences and submitted with consent from all authors.

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