Analysis of Body-Length Distribution of *Dirofilaria immitis* in the Cardio-Pulmonary Blood Vessel of Naturally Infected Dogs in Shiga Prefecture, Japan

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ABSTRACT. Body-length frequencies of *Dirofilaria immitis* collected from the cardio-pulmonary blood vessel of the dogs were studied in Shiga Prefecture. Japan, in October 1983 and in January and May 1984. The frequencies were not distributed normally because they included the populations of shorter-bodied worms, probably the growing younger worms, whereas the distributions of the body length of worms collected in July 1983 were proved to fit the single normal distribution. From the results of body-length distribution analysis, the worms collected in different seasons were each composed of 1 to 3 groups with different mean body-lengths, standard deviations and composition rates and succesive changes were observed in the distribution patterns. According to the analysis of population structures, the infective season was estimated to range from April or May to October or November in 1983 from the respective ages of shorter-bodied worm groups. The annual rates of first infection and re-infection in 1983 determined by the population of shorter-bodied females collected in January were 48% and 93% respectively and the number of newly infecting females per dog ranged from 1 to 27 with a geometric mean of 8.5. The numbers of newly infecting female worms were distributed following the negative binomial and Poisson distributions in newly infected and already infected dogs respectively.—KEY WORDS: body-length, *Dirofilaria immitis*, distribution, dog, Japan.

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Immature *Dirofilaria immitis* which has migrated into the cardio-pulmonary artery grows rapidly, and female and male worms reach nearly maximal body length 8 and 5 months after infection respectively [3, 4]. The shorter-bodied worms of developing stage are frequently found in the cardio-pulmonary blood vessel in autumn and winter seasons [14] and the structure of their population is assured corresponding to infection patterns of *D. immitis* in the latest infective season. In the present study, the body-length distributions of worms of cardio-pulmonary stage were analysed in different seasons to know the structure and dynamics of worm population which consisted of full-grown old worms and newly infected young ones.

MATERIALS AND METHODS

D. immitis worms were collected from the cardio-pulmonary blood vessel of dogs more than one year of estimated age at the Animal Conservation and Control Center in Shiga Prefecture, Japan in July and October 1983 and in January and May 1984. The heart and lungs were removed after euthanasia, and the worms were carefully collected from the right ventricle and pulmonary arteries and fixed with hot 5% formalin in 0.8% sodium chloride solution. The worms were sexually differentiated macro- and microscopically and the number of worms was counted in each dog. The formalin-fixed worms were measured for body length by an ordinary plastic measure. The method of Cassie [1] was mainly employed for analysis of body-length distribution and Ito *et al.* [2] was also consulted when needed.

RESULTS

A total of 215 dogs were examined for *D. immitis* of cardio-pulmonary stage and 3451 worms were collected from 135 positive dogs. The number of worms in positive dogs ranged from 1 to 143, 1 to 181, 3 to 199 and 1 to 74 in July and October 1983 and January and May 1984 respectively, and the variance/mean ratio was significantly greater than unity ($p<0.001$), this indicating that the underlying probability distribution of worms/dog was over-dispersed. Accordingly, the geometric means were calculated at 12.1, 10.1, 18.6 and 13.1 in July and October 1983 and in January and May 1984, respectively to stabilize variances for statistical comparison. No significant difference was observed between the thus converted numbers of male and

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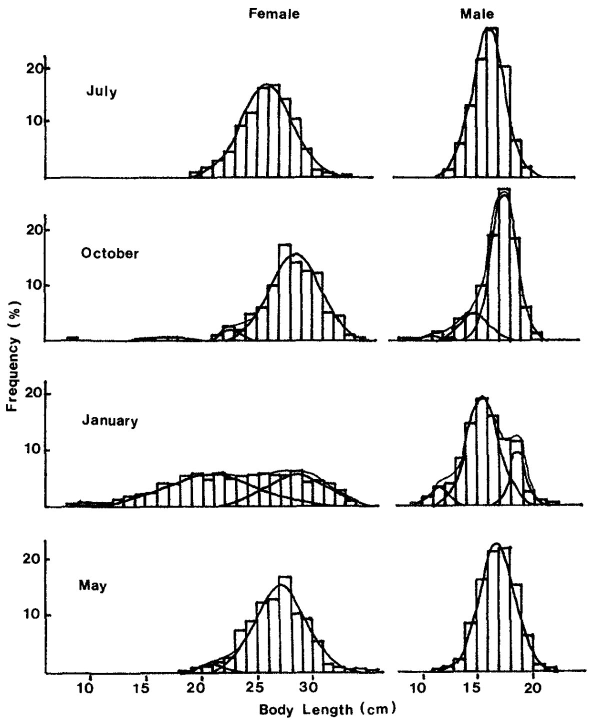

Fig. 1. Body-length distribution of *D. immitis*. Histograms are shown as frequencies in percentage of the total worms collected and measured. Fitted polymodal and monomodal normal distributions are shown by curves.

female worms ($p<0.01$).

The body-length distributions are shown in Fig. 1 by histograms. The distributions of male worms collected in October and January and females collected in October, January and May were skewed and did not fit the normal distribution at less than 5% of probability although the body-length distributions of males collected in July and May and females collected in July were proved to fit the normal distribution judged by Chi-square criterion. The distribution of body length were polymodal by estimating the respective means and standard deviations after divided into each component at inflexion points on the probability graph. The theoretical distributions calculated were fitted to the observed frequencies as shown in Fig. 1 and the fitness is shown in Table 1. All the composit distributions of calculated components well fitted the distributions observed according to small Chi-square values and the large probabilities judged by Chi-square test. It is likely from these results that the worms obtained in the cardio-pulmonary blood vessel of naturally infected dogs in different seasons are composed of 1 to 3 groups with different mean body-lengths, standard deviations and composition rates.

Table 1. The fitness of the polymodal or monomodal frequency distribution of body length of *D. immitis* collected from the cardio-pulmonary blood vessel of dogs in different seasons

Sex	Month	No. of Worms	Composition Rate (%)	Body Length Mean	Body Length S.D.	Fitness Chi-square	Fitness D.F.	Fitness Probability
Male								
	July	n=372						
		1) 372	100	16.1	1.4	7.380	5	0.20–0.30
	Oct.	n=246						
		1) 7	3	10.5	1.2			
		2)	17	15.0	1.3	2.438	2	0.20–0.30
		3) 197	80	17.5	1.2			
	Jan.	n=456						
		1) 36	8	11.5	0.8			
		2) 342	75	15.5	1.5	1.399	3	0.70–0.80
		3) 78	17	18.5	0.7			
	May	n=385						
		1) 385	100	16.9	1.7	1.731	8	0.98–0.99
Female								
	July	n=400						
		1) 400	100	25.9	2.4	9.230	11	0.30–0.50
	Oct.	n=254						
		1) 5	2	15.5	1.6			
		2) 13	5	22.5	0.9	7.188	7	0.30–0.50
		3) 236	93	28.5	2.3			
	Jan.	n=463						
		1) 278	60	20.5	4.2	9.934	17	0.90–0.95
		2) 185	40	28.5	2.8			
	May	n=427						
		1) 17	4	20.5	1.3	8.849	11	0.50–0.70
		2) 410	96	27.0	2.4			

Succesive changes were observed in the histograms and calculated distributions of body-length, as shown in Fig. 1. The shorter-bodied female (mean = 15.5 and 22.5 cm) and male (mean = 10.5 and 15.0 cm) worms appeared in October 1983. The females increased in number up to more than a half of total female worms, and the components of different body sizes overlapped in the distribution of males in January 1984. A small component of the shorter-bodied females (mean = 20.5 cm) remained until May 1984.

The biggest component of shorter-bodied females (mean = 20.5 cm) was observed in January 1984 when 50 dogs were examined. The discriminant point between shorter and longer-bodied components of theoretical distributions and the success rate at the point were 25.3 cm and 87.3% respectively. The number of shorter-bodied females in individual animals, which were determined on the discriminant point. Shorter-bodied females were collected from 31 out of 32 dogs positive for female worms. The seventeen dogs (48.6% out of 35 dogs negative for longer-bodied females) were parasitized only by shorter-bodied females and 14 dogs were parasitized by both shorter- and longer-bodied females.

The number distribution of shorter-bodied females in the animals examined in January was tested for the fitness to the theoretical distribution.

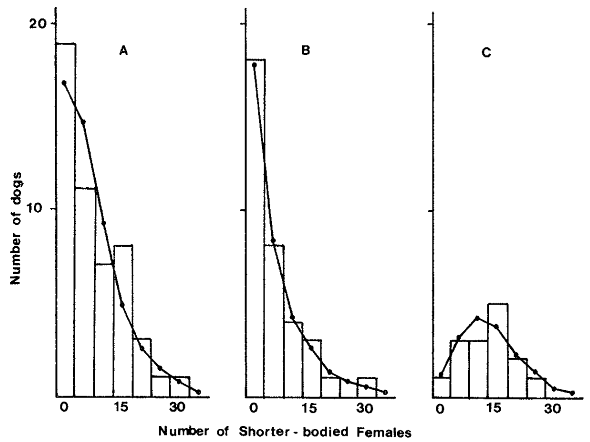

Fig. 2. Distributions of the shorter-bodied females of *D. immitis* collected in January 1984. Fitted distributions are shown by curves with dots and lines. A: All the dogs positive for the shorter-bodied females, negative binomial distribution d.f.=4 Chi-square=3.602. B: The dogs negative for longer-bodied females, negative binomial distribution, d.f.=4, Chi-square=1.263. C: The dogs poisitive for longer-bodied females. Poisson distribution, d.f.=4, Chi-square=1.459.

As a result, the distribution was proved to fit well the negative binomial although the frequency observed was lower in the range from 1 to 10 females and higher in that from 11 to 15 females than the theoretical distribution (Fig. 2-A). The negative binomial distribution also well fitted the distribution in the group of dogs which were negative for longer-bodied females (Fig. 2-B). On the other hand, the distribution observed in another group of dogs, which were parasitized by both shorter- and longer-bodied females, was likely to be Poisson's though the sample size was not large enough (Fig. 2-C).

DISCUSSION

Immature or apparently shorter-bodied *D. immitis* may be recognized easily to be in developing stage. However it is difficult to discriminate between the populations of younger- and older-worms collected in the field because of the presence of worms of various levels of growth and development. The poly- and mono-modal frequency distribution analysis has been applied to the studies on population structures of snails [8], mosquitoes [7, 9] and fishes [1]. In the present study, similar analysing method was applied to the body-length distribution of *D. immitis* with the result that the worm population recovered in different seasons was composed of 1 to 3 groups of shorter- and longer-bodied worms. It can be said that the shorter-bodied components in histograms correspond to growing younger populations which infected dogs in the latest season of mosquito activity in 1983. The size distribution of full-grown worms with the maximal body length corresponds to the monomodal distributions of females in July and of males in July and May and the longest-bodied component of each polymodal distribution, which have the values close to the mean body-length and standard deviation in each sex. Although the shorter-bodied components overlapped with the longer-bodied components of the polymodal distribution in the present survey, both distributions may be more clearly or completely separated, if worms are sampled between October and January or in other areas where the infective season is shorter.

The dynamics and intensity of *D. immitis* infection have been studied mainly by means of the survey on prevalence in dogs [3, 11–13] or that on intermediate host mosquitoes [12, 13]. Katamine *et* *al.* [3] estimated the infective period in a year by the period during which immature worms were detected from dogs by his continuous survey on significant number of dogs through the year. The infective season is also estimated by the development of infective larvae, the activity of mosquitoes, and temperature [10–13]. Annual infection rates of *D. immitis* were usually estimated by the prevalence in naturally infected dogs of known age. It was reported that 38% and 30% of young dogs of known age, which passed a single infective season in Tokyo [10] and Nagasaki [13], were infected with the average number of 11.1 and 5.1 worms respectively during the first infective season.

In the present study, the infective season was estimated from the shorter-bodied components referring to the growth in body length of *D. immitis* in dogs detailed by earlier investigators [4–6]. The shorter-bodied female and male components first appeared in October, consisting of worms of less than 5 to 6 months of estimated age which infected dogs in April to May. Another group of shorter-bodied females remained in May, consisting of the worms 6 to 7 months of age which infected in October to November. Accordingly it is suggested that the infective season in the year 1983 started in April or May and ended in October or November.

The annual populations of worms in both newly and already infected dogs were estimated from the calculated component of shorter-bodied females in January which consisted mostly of the worms having infected in the infective season of 1983. Both annual infection rate and average number of worms were greater than those reported in previous surveys in Tokyo and Nagasaki [10, 13]. Almost a half of the dogs were thought to be infected newly and most of the already infected dogs were re-infected during the infective season in 1983. These high annual infection rates coincide with high prevalence of *D. immitis* in Shiga [14].

Ohishi [10] indicated that there was no difference in average number of annually infecting worms between newly infected dogs and already infected dogs. However, different patterns of annual infection were observed in the worm number distributions which followed the negative binomial and Poisson distributions in the newly infected dogs and the already infected dogs respectively. This observation suggests that the already infected dogs provide the infection source for both other dogs and themselves and play an important role to form infection foci.

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