

STUDIES ON THE PATHOGENICITY OF *ANCYLOSTOMA CEYLANICUM* I. BLOOD LOSS IN EXPERIMENTAL DOGS

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INTRODUCTION

Ancylostoma ceylanicum was first named and described by Looss in 1911 as a hookworm of a civet cat in Ceylon (Looss, 1911). Two years later, this species of hookworm was reported as a human parasite in prisoners from the Mymensing jail who had been transferred to Berhampore (Lane, 1913). Since then it has been reported in dogs, cats and man from several parts of the world (Leiper, 1915; Chandler, 1925; Yoshida *et al.*, 1968; Velasquez and Cabrera, 1968; Areekul *et al.*, 1970a). Experimental infection in volunteers revealed that *A. ceylanicum* from dogs could establish infection in humans by either oral or cutaneous routes (Areekul *et al.*, 1970b; Yoshida *et al.*, 1971). Since there has been a high infection rate of *A. ceylanicum* in dogs and cats in some areas and a high prevalence of natural infection in man in various countries (Anten and Zuidema, 1964; Velasquez and Cabrera, 1968; Yoshida *et al.*, 1968; 1973; Areekul *et al.*, 1970a; Chowdhury and Schad, 1972; Zuidema *et al.*, 1972), this species of hookworm is now thought to be medically important and a significant cause of ancylostomiasis in man (Areekul *et al.*, 1970a). The pathogenesis of *A. ceylanicum* in various hosts has not been well established. The present work was therefore undertaken in order to estimate the gastrointestinal blood loss in dogs experimentally infected with *A. ceylanicum* using ⁵¹Cr-labelled red cells.

MATERIALS AND METHODS

Blood loss through the gastrointestinal tract was determined in 13 dogs. The animals were brought from the Bangkok Rabies Control Centre and were caged individually. One or two courses of Ancylo (0.1 ml per pound of body weight) were administered subcutaneously until the stools were negative for hookworm eggs for at least 3 weeks, as determined by the concentration method of Ritchie (1948). They were then infected with filariform larvae of *A. ceylanicum* by the oral (a group of 7 dogs) or cutaneous route (a group of 6 dogs). The faeces were examined by the concentration method for hookworm eggs, twice weekly in the first week and then every other day in the second week throughout the experiment. Eggs in the stool were counted by Stoll's method (Stoll, 1961). Blood loss was estimated using ⁵¹Cr-labelled red cell by a method previously described (Areekul *et al.*, 1970c). Measurement of blood loss was carried out daily throughout the experimental period which usually lasted about 9 to 12 weeks after infection.

The animals were then sacrificed and the intestines were examined for hookworms. Blood loss per worm per day was determined by subtracting the blood loss before infection from the average blood loss during the last 3 weeks and dividing the result by the number of hookworms.

Haemoglobin concentration was determined by the cyanmethaemoglobin method, and haematocrit by centrifuging the blood at 10,000 g for 5 minutes.

RESULTS

The number of larvae administered, worm recovered, pre-patent period and egg output are shown in Table 1. There was a wide variation in the ratio of the number of larvae to worm recovered, i.e., ranging from 5/1 to 408/1 and 5/1 to 83/1 in dogs experimentally infected by oral and cutaneous routes respectively. There was no significant difference of the pre-patent period in these 2 groups, i.e., 11 days.

The mean values of egg production in the present study were 357 ova/gm faeces/worm (range 5-775) and 6,756 ova/day/worm (range 197-12,350) in dogs infected by the cutaneous route. The corresponding values in dogs infected by the oral route were 269 ova/gm faeces/worm (range 15-1,010) and 1,985 ova/worm/day (range 633-43,600).

The amount of blood loss through the gastrointestinal tract of 13 dogs before and after infection with *A. ceylanicum* by the oral and cutaneous routes and the daily blood loss per worm are shown in Table 2. The blood loss was first detected in the faeces, on the average, on day 11 after cutaneous and oral infection. The mean blood loss was found to be 0.033 ml/worm/day and 0.038 ml/worm/day in dogs infected by the cutaneous and oral routes, respectively. There seemed to be a direct relationship between the number of worm and blood loss through the gastrointestinal tracts in these dogs. However, blood loss expressed as ml per worm per day showed a reverse relationship with the total number of worms recovered. Changes of haemoglobin concentration and blood loss in the infected dogs are illustrated in Figs. 1 and 2.

Table 1

Number of larvae administered, worms recovered and egg output in dogs experimentally infected with *A. ceylanicum*.

No.	No. of larvae administered	No. of worms recovered			Ratio of larvae/worms	Days after infection	Pre-patent period	Ova in stool	
		Male	Female	Total				Ova/w/gm	Ova/w/d
Oral route									
D-7	11,600	927	1,293	2,220	5/1	15	15	-	-
D-9	15,100	7	30	37	408/1	80	12	135	3,980
D-11	9,400	32	44	76	124/1	73	14	218	8,350
D-13	16,300	106	178	284	57/1	59	11	1,010	43,600
D-15	9,200	1,277	2,030	3,307	3/1	60	12	170	7,790
D-16	2,400	43	128	171	14/1	58	8	64	3,488
D-17	2,400	24	69	93	26/1	59	8	15	633
Cutaneous route									
D-4	9,300	11	101	112	83/1	77	11	5	197
D-6	11,600	977	1,386	2,363	5/1	56	12	662	9,615
D-8	9,300	939	755	1,694	6/1	31	10	41	996
D-10	9,400	124	281	405	23/1	72	10	301	12,350
D-12	16,300	547	912	1,459	11/1	39	15	775	10,620
D-14	16,300	3,658	3,620	7,278	2/1	18	11	-	-

Table 2

Blood loss caused by *A. ceylanicum* in experimental dogs.

No.	No. of worms recovered	Commencement of blood loss (day)	Blood loss (ml/day)			Blood loss (ml/worm/day)
			Before infection	Average blood loss	Due to worms	
Oral route						
D-7	2,220	9	2.13	60.90	58.80	0.0264
D-9	37	13	0.62	1.47	0.85	0.0229
D-11	76	16	0.39	2.72	2.33	0.0306
D-13	284	8	2.35	17.05	14.70	0.0517
D-15	3,307	10	0.32	117.67	117.35	0.0355
D-16	171	10	0.87	10.60	9.73	0.0569
D-17	93	9	0.24	3.84	3.60	0.0387
Cutaneous route						
D-4	112	13	0.18	1.98	1.80	0.0160
D-6	2,363	11	0.51	38.25	37.74	0.0159
D-8	1,694	10	0.85	61.93	61.08	0.0360
D-10	405	10	0.45	17.94	17.49	0.0431
D-12	1,459	11	2.39	88.10	85.71	0.0587
D-14	7,278	11	1.24	127.10	125.86	0.0170

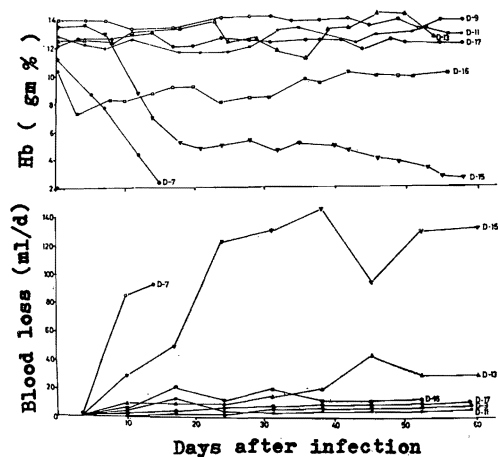


Fig. 1—The haemoglobin concentration and the blood loss (ml/day) in dogs infected with *A. ceylanicum* by oral route.

DISCUSSION

The mean pre-patent periods for *A. ceylanicum* in the present study were found to be 12 days (range 10-15 days) and 11 days

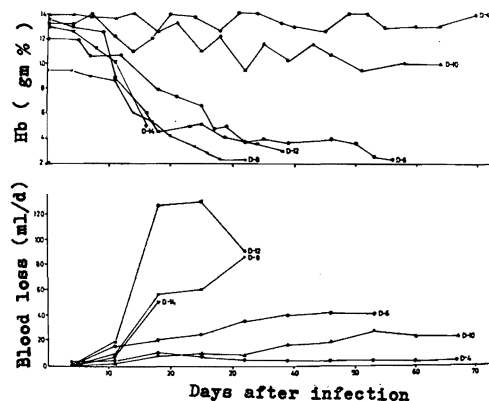


Fig. 2—The haemoglobin concentration and the blood loss (ml/day) in dogs infected with *A. ceylanicum* by cutaneous route.

(range 8-15 days) after infection by the cutaneous and the oral routes, respectively. Rep (1966) reported a similar result, i.e., 15-17 days in 3 dogs infected by the cutaneous route. The pre-patent periods in human volunteers experimentally infected with *A.*

ceylanicum were longer, i.e., 21-35 days and 18-26 days after infection by the cutaneous and oral routes, respectively (Maplestone, 1933; Wijers and Smit, 1966; Areekul *et al.*, 1970b; Haydon and Bearup, 1963; Yoshida *et al.*, 1971).

The mean values for egg production in the present study were found to be 309 ova/gm faeces/worm and 4,153 ova/day/worm. Studies in the human subjects showed that the egg output equalled 53 eggs/worm/gm faeces and 5,800 eggs/worm/day in a volunteer who harboured 52 female worms and 3,494 eggs/worm/day in 4 volunteers who harboured 2 to 60 female worms (Areekul *et al.*, 1970b; Yoshida *et al.*, 1971).

The daily blood loss caused by *A. ceylanicum* in dog in the present study was found to be 0.0345 ml/worm. This figure was slightly higher than 0.0137 ml/worm obtained from 2 dogs experimentally infected with *A. ceylanicum* during the pre-patent period, i.e., 2-3 weeks after infection (Rep, 1966). Rep *et al.*, (1968) reported the relationship between blood loss in 5 dogs and the sexual behaviour of *A. ceylanicum* but they did not state the amount of blood loss and the number of worms obtained from these dogs; thus the daily blood loss caused by each worm could not be calculated from their experiments.

A. ceylanicum is the smallest of the species of hookworm in man, dogs and cats (the other species are *N. americanus*, *A. duodenale*, *A. caninum*, and *A. braziliense*). Studies on the daily blood loss caused by *A. duodenale* and *N. americanus* in man were found to be 0.14-0.40 ml/worm and 0.01-0.09 ml/worm respectively (Roche *et al.*, 1957; Foy *et al.*, 1958; Gilles *et al.*, 1964; Areekul *et al.*, 1970c), while the blood loss by *A. caninum* in dogs were 0.037-0.124 ml/worm (Roche and Martinez-Torres 1960; Areekul *et al.*, 1975). All these findings suggest a direct relationship between the size of hookworm species and

the amount of daily blood loss caused by each species of hookworm except for *A. braziliense* which causes a relatively insignificant amount of blood loss, i.e., 0.0075 ml/worm/day (Miller, 1966; Areekul *et al.*, 1974).

As mentioned earlier, *A. ceylanicum* has been reported as a natural hookworm infection in man in various countries. A heavy infection rate has been reported recently in three Dutch marines who returned from Surinam. They harboured 104, 125 and 297 *A. ceylanicum*, respectively (Anten and Zuidema, 1964). The latter two patients were also found to be anaemic with haemoglobin values of 11.3 and 8.9 gm per cent respectively. Assuming that *A. ceylanicum* caused a blood loss of 0.03 ml/worm/day as estimated in the present study, the daily blood loss in these two patients would be 3.8 and 8.9 ml, respectively. This suggests that *A. ceylanicum* could establish a heavy infection in man and therefore could cause a considerable amount of blood loss from the intestinal wall. *A. ceylanicum* should therefore be considered as a significant clinical aetiology of hookworm disease in man.

SUMMARY

Blood loss caused by *A. ceylanicum* was determined in 13 dogs using ⁵¹Cr-labelled red cells. Blood loss was first detected in the faeces on the 10th to 13th day after cutaneous and 8th to 16th day after oral infection respectively. The mean blood loss was found to be 0.033 ml/worm/day and 0.038 ml/worm/day in these 2 groups of dogs respectively. There was a direct relationship between the number of worm and the amount of blood loss. The blood loss expressed as ml per worm per day showed a reverse relationship with the number of worms recovered. Considering a large amount of blood loss and the decrease in haematological values in these dogs, *A. ceylanicum* should be

considered as one of the hookworm species of medical importance.

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