EXPERIMENTAL STUDIES ON ANCYLOSTOMIASIS IN CATS

I. *ANCYLOSTOMA CANINUM* ERCOLANI, 1859 AND *A. TUBAEFORME* ZEDER, 1800 FOUND IN CATS IN JAPAN

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It has been reported that three species of hookworms, *Ancylostoma caninum* ERCOLANI, 1859, *Ancylostoma braziliense* GOMEZ de FARIA, 1910, and *Uncinaria stenocephala* RAILLIET, 1884, were found in cats. The latter two species are distributed in limited regions (not in Japan). They have so peculiar a form that they can be distinguished clearly. Therefore, the infestation with either of them is easily confirmed in cats.

However, about the infestation with *A. caninum* in cats, many ambiguities still remain. SCOTT¹⁵⁾ considered that the so-called cat strain and dog strain were morphological synonyms, though there had never been vacillation or exchange of normal hosts between the two strains. After SCOTT, a number of scholars differentiated the cat strain from the dog strain by their further studies on morphological aspects. They were, however, divergent in nomenclature of the specimen in question. For example, MÖNNIG⁵⁾ named the hookworm found in cats *Ancylostoma caninum* var. *longespiculata*. In Japan, NODA⁷⁻⁹⁾ obtained from cats the same hookworm as that found by MÖNNIG, and named it *Ancylostoma longespiculatum* MÖNNIG, 1938, regarding it as a different species. After these studies, BIOCCHI¹⁾ in 1954 named this hookworm *Ancylostoma tubaeforme* ZEDER, 1800, because he discovered the report of ZEDER, in which ZEDER named this hookworm *Strongylus tubaeformis*. Since then, that name has been accepted by BÖHM²⁾, ROHDE¹⁴⁾, FITZSIMMONS⁴⁾, and BURROWS³⁾.

As a result of such contradictory report of nomenclature, the problem on infestation of *A. caninum* in cats, itself, remains inevitably obscure. This ambiguity or obscurity is clarified by the authors' investigation of the morphological aspect of the hookworm in cats.

MATERIALS AND METHODS

A total of 136 cats were examined. They had been brought to the Veterinary Hospital, University of Tokyo, from Tokyo, Chiba, Kanagawa, and Hyogo Prefectures initially for some other experiments.

When obtained from the digestive tracts of these cats by detailed inspection, hookworms were immediately made transparent with lactophenol solution after fixation in 10% formalin solution. After this treatment, various parts of each specimen were exactly measured under the microscope.

Fifty eggs were collected randomly from the feces of each infected cat and examined by the floating method with saturated sodium chloride solution.

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To compare with these hookworms from cats, 40 worms (20 males and 20 females) and 50 eggs of *A. caninum* were collected from dogs and measured by the same method.

RESULTS

1. Conditions of infestation of cats with hookworms

The 136 cats were examined for conditions of infestation with hookworms. The results are presented in Tables 1 and 2.

Table 1. Incidence of Hookworms in Cats in Japan

District (Prefecture)	Number of cats examined	Number of cats infected	Rate of incidence (%)
Tokyo	40	4	10.0
Chiba	1	1	100.0
Kanagawa	9	1	11.1
Hyogo	86	5	5.8
Total	136	11	8.1

Table 2. Number of Hookworms Detected from Cats

Cat No.	Locality	Number of hookworms			Method of examination
		Male	Female	Total	
C- 1	University of Tokyo	2	3	5	Autopsy
C- 2	University of Tokyo	3	13*	16	Autopsy
C- 3	Bunkyo-ku, Tokyo	0	1**	1	Autopsy
C- 4	Itabashi-ku, Tokyo	1	1	2	Anthelmintic medication
C- 5	Kashiwa, Chiba	3	2	5	Autopsy
C- 6	Kawasaki, Kanagawa	1	0	1	Autopsy
C- 7	Kobe, Hyogo	1	3	4	Autopsy
C- 8	Kobe, Hyogo	0	1	1	Autopsy
C- 9	Kobe, Hyogo	1	2	3	Autopsy
C-10	Kobe, Hyogo	0	3	3	Autopsy
C-11	Kobe, Hyogo	4	9	13	Autopsy
Total		16	38	54	

* Containing 2 immature worms.

** Immature worm.

As shown in Table 1, hookworms were found in four cats from Tokyo, one from Chiba, one from Kanagawa, and five from Hyogo Prefecture. The percentage of the infested cat to the uninfested is 8.1 per cent.

As shown in Table 2, 51 mature and 3 immature hookworms (16 males and 38 females) were obtained from the eleven infested cats. The number of hookworms per cat was 16 at maximum, 1 at minimum, and 5 on the average.

From these results it was inferred that a considerable number of cats might suffer from infestation with hookworms, and that the worms might be distributed over considerably wide areas in Japan.

2. Comparisons of the measurements and forms of the presented hookworms

a. Comparisons of the divided specimens of hookworms from cats

By mere observation of the external form and the internal organs, the hookworms from cats could not be distinguished from those from dogs. By measuring various parts of the body, however, the hookworms from cats were divided into two groups, A and B. In Group A were classified 19 specimens harvested from two cats, C-1 and C-2

Table 3. Measurements of Hookworms from Cats and Dogs (mm)

		Hookworms from cats		A. caninum from dogs	
		Group A (C-1, C-2)	Group B (C-3~C-11)		
		Female 14, Male 5	Female 21, Male 11	Female 20, Male 20	
Body size	♀	10.0~12.4×0.33~0.45 (11.1) (0.40)	7.7~11.8×0.25~0.45 (9.9) (0.39)	11.5~15.0×0.49~0.55 (13.8) (0.52)	
	♂	6.6~8.7×0.30~0.34 (7.7) (0.31)	7.0~10.1×0.28~0.38 (8.3) (0.33)	7.5~11.0×0.35~0.43 (9.1) (0.39)	
Buccal capsule	♀	0.22~0.24×0.14~0.18 (0.23) (0.16)	0.18~0.23×0.13~0.16 (0.21) (0.14)	0.28~0.31×0.18~0.20 (0.29) (0.20)	
	♂	0.18~0.20×0.13~0.15 (0.19) (0.14)	0.15~0.19×0.08~0.15 (0.18) (0.12)	0.21~0.24×0.14~0.16 (0.23) (0.15)	
Esophagus	♀	0.94~1.19×0.17~0.21 (1.02) (0.19)	0.76~0.89×0.16~0.21 (0.84) (0.18)	1.08~1.22×0.20~0.23 (1.15) (0.22)	
	♂	0.76~0.94×0.14~0.17 (0.87) (0.15)	0.68~0.85×0.13~0.17 (0.78) (0.15)	0.90~1.10×0.16~0.19 (0.95) (0.18)	
Anterior end to excretory pore	♀	0.45~0.70 (0.62)	0.43~0.69 (0.57)	0.73~0.92 (0.86)	
	♂	0.45~0.60 (0.52)	0.45~0.64 (0.53)	0.55~0.80 (0.77)	
Anterior end to cervical papillae	♀	0.50~0.71 (0.66)	0.45~0.70 (0.60)	0.75~0.94 (0.90)	
	♂	0.48~0.60 (0.54)	0.48~0.68 (0.56)	0.61~0.85 (0.81)	
Thickness of cuticles (μ)	♀	6~14 (9)	13~40 (28)	10~20 (14)	
	♂	5~11 (9)	17~25 (22)	10~20 (15)	
Tail	♀	0.14~0.21 (0.18)	0.11~0.16 (0.14)	0.22~0.23 (0.22)	
Situation of vulva	♀	1.68~2.00 (1.87):1.00	1.60~2.66 (1.89):1.00	1.65~2.23 (1.84):1.00	
Spicules	♂	0.69~0.90 (0.81)	1.26~1.72 (1.42)	0.90~1.05 (0.97)	
Gubernaculum	♂	0.14~0.17 (0.15)	0.08~0.10 (0.09)	0.16~0.20 (0.18)	
Notches of spicules (μ)	♂	27~35 (32)	42~70 (52)	23~35 (27)	
Egg	size (μ)	60.0~85.0×32.5~43.5 (69.3) (38.3)	54.6~77.5×33.6~57.5 (62.9) (40.5)	62.57~7.5×40.0~49.0 (67.5) (44.3)	
	length: width	1.81:1.00	1.53:1.00	1.54:1.00	

Remark: In parentheses are shown average values.

(Table 2). In Group B were classified 32 specimens derived from nine cats, C-3 to C-11 (Table 2).

As presented in Table 3, measurements were made at the following points: (1) body size, (2) size of the buccal capsule, (3) size of the esophagus, (4) distances from the anterior end to the excretory pore and to the cervical papillae in the male and female, (5) ratio of the distance from the vulva to the anterior end to that from the vulva to the posterior end, (6) distance from the posterior end to the anus in the female, (7) lengths of the spicules and the gubernaculum, (8) the depth of the notches of the spicules, and (9) the thickness of the cuticle in the male and female.

The forms of the cervical papillae and the pre-bursal papillae are presented in Figs. 5, 6, 13, and 14.

The remarkable differences in measurement or in forms are summarized as follows.

Males

Spicules: 0.69~0.90 mm, averaging (av.) 0.81 mm, in Group A (Fig. 7) and 1.26~1.72 mm, av. 1.42 mm, in Group B (Fig. 8). Notches of spicules: 27~35 μ, av. 32 μ, in Group A (Fig. 11) and 42~70 μ, av. 52 μ, in Group B (Fig. 12). Gubernaculum: 0.14~0.17 mm, av. 0.15 mm, in Group A (Fig. 9) and 0.08~0.10 mm, av. 0.09 mm, in Group B (Fig. 10).

Females

Esophagus: 0.94~1.19 mm, av. 1.02 mm, in Group A and 0.76~0.89 mm, av. 0.84 mm, in Group B.

Males and Females

Cuticular layer of male: 5~11 μ , av. 9 μ , in Group A (thick at the excretory pore) and 17~25 μ , av. 22 μ , in Group B. Cuticular layer of female: 6~14 μ , av. 9 μ , in Group A and 13~40 μ , av. 28 μ , in Group B. Cervical papillae: Their point was acute in Group A (Fig. 5) and blunt in Group B (Fig. 6). Pre-bursal papillae: They were thick and swollen in the middle in Group A (Fig. 13) and slender and of the same width in Group B (Fig. 14).

b. Comparison between hookworms from cats and those from dogs

The external shape and internal organs were compared between the hookworms from cats (Groups A and B) and those from dogs (*A. caninum*). By the exact measurement of various parts of the body, considerable differences were observed in size between the hookworms of different origin.

As shown in Table 3, the average body size of Group A was small, and that of Group B was smaller than that of *A. caninum*. Corresponding to this reduction in body size, most of the other parts were smaller in size in Groups A and B than in *A. caninum*.

In spite of these differences in absolute length, the ratios among seven features on which the classification of Groups A and B was based were essentially the same in Group A as in *A. caninum*.

c. Comparison of egg form between Groups A and B and *A. caninum* from dogs

Table 3 gives the length and width of the egg in Groups A and B and *A. caninum* from dogs. It also presents the ratio of length to width of the egg. Figs. 15, 16, and 17 illustrate the forms of eggs in the three groups.

As shown in Fig. 16, the egg of Group A was more slender than that of Group B (Fig. 17) or that of *A. caninum* (Fig. 15). This observation was supported by the fact that the ratio of length to width (1.81:1.00) of the egg of Group A was higher than that of Group B (1.53:1.00) or *A. caninum* (1.54:1.00). Another characteristic of Group A was the length of the egg, which was longer than that of any other group.

When the absolute length and width of the egg were compared, they were shorter in Group B (av. 62.9 by 40.5 μ) than in *A. caninum* (av. 67.5 by 44.3 μ). The ratio of length to width of the egg, however, was essentially the same between Group B (1.53:1.00) and *A. caninum* (1.54:1.00).

DISCUSSION

1. Division of hookworms from cats

When seven parts of the body were examined, 51 hookworms derived from cats were different in many points. For example, some part of one worm was 1/2 the same part of another. When such differences were examined and compared, the worms were divided into two groups, A and B.

a. Identification of Group A worms

The body were shorter in absolute length in Group A than in *A. caninum*, but the ratios of length among the seven parts were essentially the same between Group A and *A. caninum*. The shorter absolute lengths in Group A worms derived from cats may be ascribed to the size of their host, the cat, which is usually smaller than the dog. Such a phenomenon has been reported before about other parasitic worms. In this manner, the hookworm of Group A could be distinguished from *A. caninum*.

Table 4. Review on Measurements of *A. tubaeforme* and *A. longespiculatum* (mm)

Species Source		<i>A. tubaeforme</i> Burrows, 1962	<i>A. longespiculatum</i> Noda, 1952	<i>A. caninum</i> var. <i>longespiculata</i> Mönnig, 1938
Body size	♀	12.0~15.0×0.38~0.43 (13.2) (0.41)	8.8~12.0×0.32~0.38 (10.4) (0.35)	10.2~11.5×0.39~0.45
	♂	9.5~11.0×0.30~0.35 (10.3) (0.32)	8.1~9.2×0.25~0.30 (8.8) (0.28)	10.0~10.2×0.29~0.34
Buccal capsule	♀	smaller than those of <i>A. caninum</i>	0.19~0.24×0.14~0.19 (0.21) (0.17)	
	♂		0.15~0.20×0.10~0.15 (0.18) (0.14)	
Esophagus	♀	0.81~0.95 (0.87)	0.72~0.95×0.14~0.21 (0.82) (0.18)	0.72~0.78 (0.78)
	♂		0.63~0.80×0.12~0.17 (0.73) (0.16)	
Anterior end to excretory pore	♀		0.53~0.80 (0.65)	0.62~0.67
	♂		0.57~0.65 (0.60)	0.54~0.62
Anterior end to cervical papillae	♀		0.55~0.82 (0.70)	0.65~0.67
	♂		0.58~0.68 (0.63)	0.59~0.65
Thickness of cuticle (μ)	♀♂	9.6 (near excretory pore)		
Tail	♀	0.16~0.23 (0.18)	0.12~0.16 (0.13)	0.13~0.16
Situation of vulva	♀		1.6~2.1 (1.8):1.00	
Spicules	♂	1.10~1.47 (1.29)	1.42~1.53 (1.48)	1.43~1.62
Gubernaculum	♂		0.073~0.094 (0.084)	
Notches of spicules (μ)	♂	48~69		
Egg size (μ)		55.0~75.7×34.4~44.7	54~58×41~43	

Remark: In parentheses are shown average values.

b. Identification of Group B worms

Table 4 shows the data on the hookworm of Group B obtained from literatures. It was impossible to differentiate this group with the data collected on *A. caninum* or Group A. There were differences in the method of gathering data between the present authors and the previous ones, but agreement was found in the following points: the lengths of the spicules and esophagus, and the depth of notches of the spicules with *A. tubaeforme*, and the lengths of the spicules, gubernaculum, esophagus, and tail of the female, the size of the buccal capsule, and the forms of the cervical papillae and pre-bursal papillae with *A. longespiculatum* or *A. caninum* var. *longespiculata*.

The hookworms which had the same organs as those of Group B were described by eight previous investigators. In 1800, ZEDER detected a hookworm from the cat and called it *Strongylus tubaeformis*^{1,3)}. In 1938, MÖNNIG⁵⁾ published a report on a hookworm derived from the cat and named it *A. caninum* var. *longespiculata*, because he regarded it as a variety of *A. caninum*. Then, in 1952, NODA⁷⁻⁹⁾ found the same hookworms in cats in Japan. He carefully referred to the study of MÖNNIG and proposed a change in nomenclature from *A. caninum* var. *longespiculata* to *A. longespiculatum*, as he regarded the former as an independent species.

While neither MÖNNIG nor NODA referred to the work of ZEDER, BIOCCEA¹⁾ introduced the original description of ZEDER in his report published in 1954. BIOCCEA called his specimen *A. tubaeforme*. Since then, this name has been accepted by BÖHM²⁾, ROHDE¹⁴⁾, FITZSIMMONS⁴⁾, and BURROWS³⁾.

As mentioned above, *A. longespiculatum* was identical with *A. tubaeforme* and this nomenclature has gained scientific reliability. Therefore, it seemed proper to adopt *A. tubaeforme* for the name of Group B. The authors decided to call the Group B worm

A. tubaeforme.2. Significance of *A. caninum* detected from cats

The first point is as follows. Since the time *A. tubaeforme* was recognized, only this species has been reported by several investigators. Accordingly, the infection of cats with *A. caninum* showed a tendency to be doubted. The authors reported first that both *A. caninum* and *A. tubaeforme* infected cats. As a result, hookworms detected from cats must be surely identified after careful examination of their forms. The infection of cats with *A. tubaeforme*, however, is more frequent than that of cats with *A. caninum*.

The former was found in nine cats from different communities in Tokyo, Chiba, Kanagawa, and Hyogo Prefectures, while the latter was reported in two cats from such an isolated community as the Veterinary Hospital, University of Tokyo. In this hospital, cats and dogs were kept in the same yard. Therefore, cats may have chances to get into contact with dogs and incur *A. caninum* infection from dogs very easily. Since *A. caninum* could not be found in any other region in Japan, the alleged infection of cats with this species in Japan was strongly supposed to be that with *A. tubaeforme*.

The second significance is that the characteristic forms of *A. tubaeforme* and *A. caninum* were clarified independently after *A. tubaeforme* had been compared in detail with both kinds of *A. caninum* from dogs and cats.

3. Form of eggs

An agreement in the form of eggs was found between *A. tubaeforme* (Group B) and *A. caninum* from dogs. Corresponding to the reduced size of its parent hookworm, the egg of *A. caninum* from cats (Group A) was more slender than that of *A. caninum* from dogs or *A. tubaeforme*. If this phenomenon is confirmed statistically, it will be utilized for the discrimination of *A. tubaeforme* and *A. caninum* in the stage of eggs. The number of samples collected, however, was not large enough for this purpose. Therefore, many eggs are now being gathered by artificial infection. The results of this continuing work will be published in future.

SUMMARY

As a result of examination of cats for hookworms and morphological studies on hookworms detected from cats and dogs in Japan, the following points were clarified.

1. Hookworms were found in 11, or 8.1 per cent, of cats examined. This result suggests that hookworm infestation may be considerably important for cats in Japan.

2. Two species, *Ancylostoma caninum* ERCOLANI, 1859 and *Ancylostoma tubaeforme* ZEDER, 1800, were obtained from the 11 cats. The former was from 2 cats at the Veterinary Hospital, University of Tokyo, and the latter from 9 cats kept in Tokyo, Chiba, Kanagawa, and Hyogo Prefectures. Consequently, it is thought that the two species are distributed in many parts of Japan, and that the former is found rarely and the latter mainly in cats.

3. *A. tubaeforme* and *A. caninum* from dogs and cats were compared. *A. tubaeforme* differed clearly from *A. caninum* in the following aspects: its longer spicules, shorter gubernaculum, thicker cuticle, and shorter esophagus of the female, peculiar form of the cervical papillae and prebursal papillae, and deeper notches of the spicules.

4. As a result of comparison of the measurements and bibliographical consideration, the authors think that *A. caninum* var. *longespiculata* and *Ancylostoma longespiculatum* MÖNNIG, 1938 may be synonyms of *A. tubaeforme*.

5. *A. caninum* derived from cats was smaller in body size, and its egg was more slender in form than *A. caninum* from dogs and *A. tubaeforme* and their eggs.

A part of this work was presented at the 91st meeting of the Association of Japanese Clinical Veterinarians in 1963. In this series of studies, mutual infection with *A. tubaeforme* and *A. caninum* from dogs was experimented with dogs, cats, and mice to elucidate the development and infective attitude of these two species. The results of these studies were partially presented at the 59th, 60th, and 61st semiannual meetings of the Japanese Society of Veterinary Science in 1965 and 1966.

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猫の鉤虫症に関する実験的研究

I. 本邦の猫に寄生した2種の鉤虫 *Ancylostoma caninum* ERCOLANI, 1859 および *A. tubaeforme* ZEDER, 1800 について

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猫に寄生する鉤虫については、本邦であまり知られていないが、すでに1800年に書かれた古い文献にその記載がある。しかし乍ら本鉤虫の種類の同定については、従来、明瞭を欠いた点が多かった。

すなわち SCOTT は、猫の鉤虫が、犬鉤虫と形態上の差が全くなく、両者は同種の *A. caninum* であるとし、Cat strain と Dog strain に区別されると考えた。その後 MÖNNIG は、猫の鉤虫と *A. caninum* との形態学上の相違点を明らかにした。また1954年になって BIOCCA は、すでに ZEDER が報告した古い記録の優先を尊重して、猫の鉤虫を *A. tubaeforme* ZEDER, 1800 と命名し、*A. caninum* と著明な形態上の差があることを立証した。

それ以降の多数の研究発表は、すべて BIOCCA の説に従い、猫に寄生した鉤虫が、いずれも *A. tubaeforme* であったと報告している。従って最近では、*A. caninum* の猫寄生説よりも、*A. tubaeforme* の寄生に注意が払われる傾向になって来た。

そこで著者らは、この問題を本邦の猫について検討するため、東京・神奈川・千葉・兵庫の各都県下で飼育された猫136頭を材料として、鉤虫の寄生状況を調査した。その結果、11頭の感染猫から、54隻の鉤虫を検出し得た。これらの鉤虫の細部構造を計測し、その形態を詳細に比較した結果、交接刺および副交接刺の長さ、交接刺末端切れ込みの深さ、頸部乳頭および交接囊前乳頭の形状、Cuticle 層の厚さ、雌虫の食道長などの個所に、著明な相違点を有する A 群と B 群の2種に区分することができた。A 群の形態は、犬から採集した *A. caninum* と一致し、また B 群は明らかに *A. tubaeforme* と同種であると同定した。

以上の通り、わが国における猫の鉤虫感染率は8.1%を示して、予期以上にわが国に広く分布していることが判明した。またその種類には、*A. caninum* と *A. tubaeforme* の2種がある事実を確認し、従来の不明確な点を解明することを得た。

EXPLANATION OF PLATES

PLATE I

- Fig. 1. Teeth of *A. caninum*.
- Fig. 2. Teeth of *A. tubaeforme*.
- Fig. 3. Tail of female of *A. caninum*.
- Fig. 4. Tail of female of *A. tubaeforme*.
- Fig. 5. Cervical papillae and layer of cuticle of *A. caninum*.
- Fig. 6. Cervical papillae and layer of cuticle of *A. tubaeforme*.
- Fig. 7. Spicules of *A. caninum*.
- Fig. 8. Spicules of *A. tubaeforme*.

PLATE II

- Fig. 9. Gubernaculum of *A. caninum*.
- Fig. 10. Gubernaculum of *A. tubaeforme*.
- Fig. 11. Notch of spicule of *A. caninum*.
- Fig. 12. Notch of spicule of *A. tubaeforme*.
- Fig. 13. Pre-bursal papillae of *A. caninum*.
- Fig. 14. Pre-bursal papillae of *A. tubaeforme*.
- Fig. 15. Egg of *A. caninum* from a dog.
- Fig. 16. Egg of *A. caninum* from a cat.
- Fig. 17. Egg of *A. tubaeforme*.