EXPERIMENTAL STUDIES ON *TOXASCARIS LEONINA*

I. INCIDENCE OF *T. LEONINA* AMONG DOGS AND CATS IN JAPAN

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Three species of ascarids in Canidae and Felidae have been reported in the world. However, they resemble each other so closely in external shape that descriptions on them have been much confused until quite recently. At present, it has been recognized that the ascarids occurring in the Canidae are *Toxascaris leonina* (LINSTOW, 1902) and *Toxocara canis* (WERNER, 1782) and those occurring in the Felidae *T. leonina* and *Toxocara cati* (SCHRANK, 1788). As for *T. leonina*, its distribution in Canidae and Felidae has been reported from all over the world, including the regions of extreme climate, by such investigators as LEIPER⁸⁾, RAILLIET and HENRY¹⁵⁾, TAYLOR²¹⁾, and SPRENT¹⁷⁾.

In spite of the world-wide distribution of *T. leonina*, the occurrence of this parasite has been studied very seldom in Japan. SUGIMOTO¹⁹⁾ was the first to describe a feline case of *T. leonina* infection in Formosa. ITAGAKI⁴⁾ reported a few canine cases of this infection in Tokyo about 1930. The specimens of *T. leonina* obtained from a dog in 1930 and from a fox in 1937 have been preserved in the Faculty of Veterinary Medicine, Hokkaido University. After that, no reports on *T. leonina* infection had been presented nor any occurrence of this parasite had been detected in surveys conducted by many investigators for about twenty years. In 1954, NODA¹⁰⁾ reported *T. leonina* infection in imported dogs, tigers, and lions, the latter two of which were raised in a zoological garden in Osaka city. Continually, ISSHIKI et al.³⁾, NODA and ONO¹¹⁾, and UTIYAMA²³⁾ reported cases in tigers and lions in zoological gardens in Osaka and Hamamatsu cities. Nevertheless, there has been no evidence of *T. leonina* infection in dogs and cats born in Japan up to the present.

In 1961 and 1963, the senior author and his associates^{13,14)} reported a collective occurrence of *T. leonina* among dogs kept in the dog pound of the Faculty of Medicine, University of Tokyo. After that, *T. leonina* infection was often detected from among dogs or cats brought to the Veterinary Hospital, Faculty of Agriculture, University of Tokyo.

Recognizing the importance of this parasite in Japan, the authors carried out not only morphological and ecological experiments in comparison with *T. canis* and *T. cati*, but also investigation on host-specificity and larva migrans in abnormal hosts.

This paper presents the method of identification of *T. leonina* and the incidence of this worm in dogs and cats in the Tokyo district.

MORPHOLOGY OF ADULT *TOXASCARIS LEONINA*, AS COMPARED WITH *TOXOCARA CANIS* AND *TOXOCARA CATI*

Recently, the authors have revealed the existence of *T. leonina* in dogs and cats in Japan. In this report, the results of morphological observation on *T. leonina* are presented comparatively with those on *T. canis* and *T. cati*. These species resemble *T. leonina* in figure, though they belong to a different genus from this parasite. The detailed values of measurements will be presented in the succeeding paper, in which they are compared with those obtained from the parasites in other hosts than dogs and cats. *T. leonina* was identified morphologically, by referring to the precise studies of ascarids presented by LEIPER⁸⁾, TAYLOR²¹⁾, and SCHACHER¹⁶⁾.

1. Materials and Methods

Adult specimens of *T. leonina* were collected from naturally infected dogs and cats by anthelmintic treatment, and from the intestines of artificially infected dogs and cats by autopsy. The worms were fixed in 5% formalin and clarified with a lactophenol mixture after their living state was observed.

2. Results

a. Whole figure

The whole figures of each couple of *T. leonina*, *T. canis*, and *T. cati* are shown in Fig. 1. It has often been said that dead worms of *T. leonina* tend to flex dorsally at the anterior end, while those of the genus *Toxocara* flex ventrally. However, the authors observed many cases not showing such tendency. The measurements of length of *T. leonina* from dogs and cats are shown in Table 1, together with the data on three ascarids given by NEVEU-LEMAIRE⁹⁾. *T. canis* was the largest in size of the three species, but it was impossible to differentiate it from the others by comparison of the size alone. *T. leonina* from cats was smaller than that from dogs. Uncommonly large worms of *T. leonina* were occasionally observed among those from dogs; the largest of them was 117 cm long.

Table 1. Measurements of Three Species of Canine and Feline Ascarids

Species	<i>T. canis</i>	<i>T. cati</i>	<i>T. leonina</i>	<i>T. leonina</i>	
				Dog strain	Cat strain
Authors (Year)	NEVEU-LEMAIRE (1936)			OKOSHI & USUI (1966)	
Length of male (in mm)	50~100	30~60	20~60	42~38	38~57
Length of female (in mm)	90~180	40~100	60~100	55~73	38~67
Distances between striations of cuticle (in μ)	16~22	12~16	6~12	5.8~12.4	7.6~12.0
Size of egg (in μ)	75×80	65×75	75×85	65~84×82~99	63~76×63~88

b. Cervical alae

Figs. 4 to 6 are the microphotographs of the slightly pressed anterior portion of the three ascarids. At a glance, it is clear that the cervical alae are broad and short in *T. cati* (Fig. 5), but narrow and long in *T. canis* (Fig. 4) and *T. leonina* (Fig. 6). Therefore, *T. cati* can be differentiated from the other two species by macroscopic observation. There is no difference in cervical alae between *T. leonina* and *T. canis*.

c. Distance between transverse striations

The three ascarids have transverse striations in the cuticle. Figs. 7 to 9 are microphotographs at the same magnification showing ascarids at the esophagus-intestine junction.

The distance between striations is distinctly shorter in *T. leonina* (Fig. 9) than in *T. canis* (Fig. 7) and *T. cati* (Fig. 8). It is less than $12.4\ \mu$ in *T. leonina*, 15.5 to $22.5\ \mu$ in *T. canis*, and 14.0 to $18.0\ \mu$ in *T. cati*, as shown in Table 1.

d. Esophageal ventriculus

Figs. 10 to 12 are the microphotographs of clarified specimens showing esophagus-intestine junction. The esophageal ventriculus is distinctly observed in *T. canis* (Fig. 10) and *T. cati* (Fig. 11). The esophagus, however, is connected directly to the intestine in *T. leonina* (Fig. 12).

e. Posterior end of male worms

Figs. 13 to 15 show the posterior end of clarified male worms. There is a digitiform appendage in *T. canis* (Fig. 13) and *T. cati* (Fig. 14), but not in *T. leonina* (Fig. 15). Although not clearly shown in the figures, the spicules are winged in the *Toxocara* species, but not in *T. leonina*. They are long in *T. cati*, but short in *T. canis* and *T. leonina*.

f. Genital organs of female worms

The vulvae are located in the first quarter of the body in both *Toxocara* species and in the middle third in *T. leonina*. Only in *T. canis*, the oviduct extends to the anterior portion of the vulva.

g. Eggs

The sizes and figures of the eggs of the three species are shown in Table 1 and Figs 2, 3 and 16 to 18. There is a clear distinction between the eggs of *T. leonina* and the *Toxocara* species. The egg of *T. leonina* (Fig. 18) has a smooth egg-shell, much space between egg-ball and shell, and a transparent appearance. The eggs of both *Toxocara* species have a finely pitted egg-shell, an egg-ball almost entirely filling the inside of the shell, and a dark appearance. There is no difference, however, between the eggs of *T. canis* (Fig. 16) and *T. cati* (Fig. 17), except that the former is a little larger in size only when observed microscopically. Fig. 2 shows a canine case of concurrent infection with eggs of *T. leonina* and *T. canis* and Fig. 3 a feline case of infection with eggs of *T. leonina* and *T. cati*.

In view of the above-mentioned morphological differences among the three ascarids of dogs and cats, the authors performed those parasitological surveys presented in the following parts.

The eggs of *T. leonina* are so characteristically different from those of the *Toxocara* species that *T. leonina* infection can be identified by observing only the eggs detected from the feces or in the uteri of female worms. The two ascarid species infecting cats, *T. leonina* and *T. cati*, are identified by the morphological difference of the cervical alae, which can be recognized macroscopically. No such point of apparent difference, however, exists between the two ascarid species infecting dogs. Therefore, *T. leonina* and *T. canis* can be identified in dogs only by microscopic examination of all the above-mentioned points of difference. When detected in the intestine of dogs at a parasitological survey by autopsy, ascarid worms were apt to be identified as *T. canis* in the past in Japan. Ascarid worms less than 12 cm long should be examined microscopically in order to differentiate *T. leonina* from *T. canis*. As this procedure was neglected in the past survey there were possibilities for *T. leonina* to be erroneously described as *T. canis*.

INCIDENCE OF *T. LEONINA* AMONG DOGS IN JAPAN

Till quite recently, had it been wondered whether *T. leonina* infection in dogs existed in Japan or not. In 1961 and 1963, the senior author and his associates^{13,14)} reported the

occurrence of this worm among native dogs in Tokyo. Their report is summarized as follows: In 1958, *T. leonina* infection was found by autopsy in three of the six dogs kept in the Faculty of Medicine, University of Tokyo. In the same compound, in 1959, *T. leonina* was detected by fecal examination from 16 of the 106 dogs (15.09 %), and from six of the 64 dogs (9.38 %) in 1960. The infection route, however, could not be pursued, because all these dogs had been captured while straying and kept afterward for medical experiments.

By this collective-occurrence of infection, the existence of *T. leonina* was proved in Japanese dogs. Nevertheless, no description of *T. leonina* has appeared in the clinical records preserved in the Veterinary Hospital, University of Tokyo, until 1959. After the first case was observed in 1960, it was followed by several cases every year, as indicated in Table 2, together with the incidence of *T. canis*. In this table only those dogs which were subjected to fecal examination are presented after selected from among the patient dogs. Most of the infected dogs were reared in Tokyo and a few in the neighboring prefectures.

Table 2. Incidence of *T. leonina* and *T. canis* in Dogs at Veterinary Hospital, University of Tokyo

Year	Total No. examined	<i>T. leonina</i>		<i>T. canis</i>	
		No.	%	No.	%
1958	350 (6)	0 (3)	0 (50.00)	28	8.00
1959	424 (106)	0 (16)	0 (15.00)	50	11.79
1960	368 (64)	1 (6)	0.27 (9.38)	42	11.41
1961	357	3	0.84	35	9.80
1962	315	1	0.32	27	8.57
1963	285	1	0.35	28	9.82
1964	235	1	0.43	24	10.21
1965	313	1	0.32	33	10.54
1966	317	7	2.21	18	5.68
Total	2964 (176)	15 (25)	0.51 (14.21)	285	9.62
	3140	40	1.27		

Remarks: In parentheses is shown the number of dogs involved in collective infection in the dog-pound at the Faculty of Medicine, University of Tokyo.

The total incidence of *T. leonina* in dogs was 1.27 % (40/3,140) for the last nine years. It will decreased, however, to 0.51 % (15/2,964) if the collective occurrence in the dog pound at the Faculty of Medicine from 1958 to 1960 is excluded. It is also noticeable that the incidence rose suddenly to 2.21 % (7/317) in 1966.

Six of the 15 cases, the details of which are given in Table 3, happened to be in a single ward, Bunkyo-ku, of Tokyo. Such high concentration of cases in Bunkyo-ku may be partly due to the presence of an originally large number of patient dogs in this ward, and partly due to the primary existence of infected dogs in the dog pound at the Faculty of Medicine, University of Tokyo, located in the same ward. In this connection, KANEKO and MIYAMOTO⁵⁾ described the occurrence of *T. leonina* infection in the dog pound at another medical school in Bunkyo-ku.

All the 15 dogs, except two mongrel and three of the native Japanese breed, were of foreign breed and initially reared by breeders who had been keeping imported dogs. Therefore, these imported dogs were supposed to be the source of *T. leonina* infection in Japan. This was ascertain in Case No. 5 (Boxer) and Case No. 11 (White Terrior). Of the 15 dogs, five were involved in infection with *T. leonina* alone and the others in double

Table 3. Clinical Canine Cases of Infection with *T. leonina*

Dog No.	Date	Breed	Sex	Age ※	Other parasites	Locality
1	Jan. 1960	Scottish Terrier	Male	4 M	<i>T. canis</i>	Shibuya-ku, Tokyo
2	May 1961	Scottish Terrier	Female	10 M	<i>A. caninum</i>	Nerima-ku, Tokyo
3	Aug. 1961	Mongrel	Female	4 M	<i>A. caninum</i> <i>T. vulpis</i>	Bunkyo-ku, Tokyo
4	Nov. 1961	Dobermann	Male	5 M	<i>T. vulpis</i>	Bunkyo-ku, Tokyo
5	May 1962	Boxer	Female	1 Y		Kita-ku, Tokyo
6	Nov. 1963	Japanese native	Female	1 Y & 8 M		Sumida-ku, Tokyo
7	Apr. 1964	Mongrel	Male	1 Y & 8 M		Bunkyo-ku, Tokyo
8	Nov. 1965	Dachshund	Male	4 M	<i>A. caninum</i> <i>T. vulpis</i>	Minato-ku, Tokyo
9	Jan. 1966	Pekingese	Male	1 Y	<i>T. canis</i> <i>T. vulpis</i>	Bunkyo-ku, Tokyo
10	Jan. 1966	Japanese native	Male	1 Y	<i>T. canis</i>	Bunkyo-ku, Tokyo
11	Jan. 1966	White Terrier	Female	2 Y	<i>T. vulpis</i>	Daito-ku, Tokyo
12	Feb. 1966	Japanese native	Male	5 M	<i>T. vulpis</i>	Edogawa-ku, Tokyo
13	Mar. 1966	English Setter	Male	7 M		Tokorozawa City, Saitama Prefecture
14	May 1966	Pointer	Female	7 M	<i>T. vulpis</i>	Gotemba City, Shizuoka Prefecture
15	Dec. 1966	Collie	Male	5 M		Bunkyo-ku, Tokyo

※ M: Month Y: Year

or triple infection with *T. canis*, *Ancylostoma caninum*, and *Trichuris vulpis*.

When the 15 infected dogs were analyzed by age, the youngest was four months old and the oldest two years old. Seven dogs were adult (older than 8 months). Therefore, it was recognized that *T. leonina* infected young and adult alike. It was also perceived that no prenatal infection would occur with *T. leonina*, because there were no infected dogs less than four months old. This finding was confirmed in another experiment, which will be reported in the succeeding paper.

As also shown in Table 2, *T. canis* was found in 285 of the 2,964 dogs examined (9.62%). Of the 285 dogs, 237 (83.2%) were less than four months old, and only 20 dogs (7.0%) more than eight months old. This difference in age incidence between *T. leonina* and *T. canis* was reasoned out, taking the mode of infection into consideration. Prenatal infection is the main route of infection with *T. canis*.

Clinical symptoms manifested by the dogs infected with *T. leonina* were similar to those by the dogs with *T. canis*. Digestive disturbance, allotriophagia, and unthriftiness were observed in young dogs, but such symptoms were not observed in most cases of adult dogs.

INCIDENCE OF *T. LEONINA* IN CATS IN JAPAN

In Japan, *T. leonina* infection in cats has not been found in the authors' clinical records nor in the papers previously presented. *T. leonina* infection in dogs, however, began to be seen in 1958. Since then, the authors have been performing a careful fecal examination on patient cats with a conception that cats might also be infected with this worm.

Table 4, shows, the incidence of *T. leonina* and *T. cati* in cats as determined by the clinical records kept at the authors' hospital. One feline case of *T. leonina* infection was

Table 4. Incidence of *T. leonina* and *T. cati* in Cats at Veterinary Hospital, University of Tokyo

Year	Total No. examined	<i>T. leonina</i>		<i>T. cati</i>	
		No.	%	No.	%
1960	46	0	0	14	30.43
1961	38	0	0	14	36.84
1962	54	0	0	8	14.81
1963	48	0	0	5	10.42
1964	29	0	0	6	20.69
1965	40	1	2.50	9	22.50
1966	60	4	6.62	8	13.33
Total after 1965	100	5	5.00	17	17.00

Table 5. Clinical Feline Cases of Infection with *T. leonina*

Cat No.	Date	Breed	Sex	Age	Locality	Remarks
1	Mar. 1965	Persian	Female	8 Y	Shinjuku-ku, Tokyo	Imported from Hawaii 4 months before. Concurrently infected with <i>T. cati</i> .
2	Jan. 1966	Persian	Male	5 M	Minato-ku, Tokyo	Kept by the same breeder with cat No. 1.
3	Feb. 1966	Persian	Female	10 M	Shinjuku-ku, Tokyo	Kept by the same breeder with cat No. 1.
4	Nov. 1966	Siamese	Female	5 M	Koto-ku, Tokyo	Reared together with imported cats. Concurrently infected with <i>Isospora</i> sp.
5	Dec. 1966	Persian	Male	8 M	Kita-ku, Tokyo	Imported from California, U. S. A. 2 months before.

observed in 1965 for the first time, and followed by four cases in the next year. These cases are given in detail in Table 5. They could be diagnosed as *T. leonina* infection first by eggs found in the feces, and then by worms removed by anthelmintic treatment.

The two cases, Nos. 1 and 5, are imported cats which were assumed to have already been infected in other countries, judging from a short interval of time after their arrival in Japan and from the lack of report on the infection in the country. The cats of Cases 2 and 3 were born at the house rearing the cat of Case 1. Therefore, it is obvious that the infection was transmitted from Case 1 to the others. The cat of Case 4 was reared by an other breeder who also kept imported cats. Thus, the hitherto reported feline cases of infection were all related to purebred cats which had been imported or had come in contact with imported cats. No cases have been observed among native Japanese domestic cats.

Case 1 was one of concurrent infection with *T. leonina* and *T. cati* and Case 4 one of that with *T. leonina* and *Isospora* species. The others were cases of infection with *T. leonina* alone.

The incidence of *T. leonina* and *T. cati* in cats was 5% (5/100) and 17% (17/100), respectively, for the last two years.

No apparent deviation was observed in the age incidence of infection with both *T. leonina* and *T. cati* in cats.

DISCUSSION

SPRENT and BARRET¹⁸⁾ indicated the world-wide distribution of *T. leonina* in dogs and

cats, while BEAVER²⁾ pointed out regional deviation of its prevalence. Japan had been considered to be among poorly distributed countries. In spite of such initial rarity, *T. leonina* was readily spread in a group of animals, transmitted from one already infected animal kept in the group. In spite of many parasitological survey on dogs conducted in Japan by TOBARI (1938)²²⁾, NOSE (1939)¹²⁾, and ADACHI (1954)¹⁾, *T. leonina* had not been detected for 28 years from the first description by ITAGAKI⁴⁾ in 1930 to the authors' observation¹³⁾ in 1958. It is not clear, however, whether *T. leonina* extinguished during this period or could not be detected in spite of its real existence. Anyway, the collective occurrence observed in the dog-pound at the Faculty of Medicine was the first case of rediscovery in Japan. The infection route in this case cannot be pursued, because all the dogs examined were those captured while straying. However, the captive carnivores kept in the Ueno Zoo, which is located about two kilometers from the dog-pound, may be assumed as the source of infection. Such carnivores as these are known to be constantly infected with *T. leonina*. In fact, it was demonstrated later that dogs could be artificially infected with the eggs of *T. leonina* obtained from those carnivores. (This will be reported in the succeeding paper.)

Feline infection with *T. leonina* was reported in Formosa by SUGIMOTO¹⁹⁾ and in Sakhalin by KROTOV⁷⁾. Nevertheless, it had not been reported in Japan, which is in the neighborhood of these regions. This worm was not observed either in the parasitological surveys on cats conducted in various regions in Japan by KOGA (1951)⁶⁾, Yuta and TASHIRO (1958)²³⁾, and TAKAYAMA et al. (1963)²⁰⁾. Feline infection with *T. leonina* was first confirmed by the authors seven years after the first rediscovery of canine infection in Tokyo. All the cats involved in it were imported ones which had already been infected in other countries, or which had come in contact with imported cats. Therefore, it is assumed that no *T. leonina* had existed among cats in Japan till it was first recognized by the authors. This assumption is supported by the results of the authors' experiments, in which cats resisted to infection with eggs of *T. leonina* collected from dogs, but was successfully infected with those of the same species collected from imported cats. (These results will be reported in detail in the succeeding paper.)

From the clinical observation mentioned above, it is suggested that *T. leonina* infection may spread more widely among pet animals in future, in proportion to an increase in number of imported pet animals. Therefore, it is necessary to encourage breeders and owners of animals so that their animals may undergo anthelmintic treatment when found to have been infected with this parasite.

SUMMARY

The differences in morphology among three species of ascarids, *Toxascaris leonina*, *Toxocara canis*, and *T. cati* were discussed. Then the incidence of these parasites in the Veterinary Hospital, University of Tokyo, was mentioned. Results are summarized as follows.

1. *T. leonina* began to be observed in dogs in 1958. Its incidence among the dogs examined at the hospital during the past nine years was as low as 1.27% (40/3,140), while that of *T. canis* was 9.62% (284/2,963).

2. It was assumed that imported dogs might be the main source of *T. leonina* infection in dogs.

3. *T. leonina* was infective equally to young and adult dogs.

4. Five cats were found infected with *T. leonina* in 1965 and 1966. They were imported cats or cats which had got in touch with imported cats. This is the first

description of feline infection with *T. leonina* in Japan.

5. The incidence of *T. leonina* among cats for the past two years was 5.0% (5/100), and that of *T. cati* 17.0%.

6. It was suggested that the prevalence of *T. leonina* in dogs and cats in future might increase in accordance with an increase in number of imported pet animals.

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TOXASCARIS LEONINA に関する実験的研究

I. 犬および猫における感染状況

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Toxascaris leonina LINSTOW, 1902 は肉食獣の腸内に寄生する回虫の一種で、諸外国では犬猫に広く分布している。わが国では、動物園のライオン、虎および輸入犬の感染が報告されたのみで、一般には余り知られていなかった。内国産犬への寄生は、1930 年ごろに東京でまれに認められたと記載されている。その後最近まで、その姿は全く認められなかった。また猫への寄生は、従来わが国で、全然報告がなかった。

著者らは、かねてから東京における犬猫について、*T. leonina* 感染状況の詳細な調査を行なっていた。その結果、内国産犬では、1958 年以降にその寄生を認め、また猫については、1965 年に初めて本虫の寄生を発見した。その後、次第に犬猫へ蔓延の徴があることを知り得た。

わが国の犬猫に寄生する回虫には、*Toxocara canis*, *Toxocara cati* および *T. leonina* の 3 種がある。それらの外形は、たがいに酷似していて、区別が容易でない。そこで、3 種回虫の虫体および虫卵の形態について、まず *T. leonina* との相違点を明らかにした。

次いで東京大学付属家畜病院に来診した犬猫を材料として、前記の各種回虫に関する感染実態を、9 年間にわたって追究した。

1. 内国産犬においては、1958 年に *T. leonina* の寄生を認めるに至り、今日までの 9 年間の感染率は 1.27% (総検査数 3140 頭、陽性 40 頭) であった。なお、この期間中の *T. canis* の感染率は 9.62% であった。

犬への *T. leonina* 感染源は、主として輸入犬であると推定された。年令別には、幼犬にも、成犬にも、同様に感染を認めた。

2. 猫については、1965 年に初めて *T. leonina* の感染を認めた。その後今日までの 2 年間における感染率は 5.0% (総検査数 100 頭、陽性 5 頭) であった。

猫への感染源は、いずれも輸入猫か、または明らかに、それからの感染が確認されたものであって、*T. leonina* 感染犬との関係は、全く認められなかった。なお、同じ調査期間中における猫の *T. cati* 感染率は 17.0% であった。

EXPLANATION OF PLATES

PLATE I

- Fig. 1. Whole figures of a female and a male of three species of ascarids.
Fig. 2. Eggs of *Toxocara canis* and *Toxascaris leonina* (concurrent infection).
Fig. 3. Eggs of *Toxocara cati* and *T. leonina* (concurrent infection).
Fig. 4. *T. canis*.
Fig. 5. *T. cati*.
Fig. 6. *T. leonina*.
Fig. 7. *T. canis*.
Fig. 8. *T. cati*.
Fig. 9. *T. leonina*.
- } Cervical alae (same magnification).
} Striation of cuticle (same magnification).

PLATE II

- Fig. 10. *T. canis*.
Fig. 11. *T. cati*.
Fig. 12. *T. leonina*.
Fig. 13. *T. canis*.
Fig. 14. *T. cati*.
Fig. 15. *T. leonina*.
Fig. 16. *T. canis*.
Fig. 17. *T. cati*.
Fig. 18. *T. leonina*.
- } Muscular ventriculus (same magnification).
} Posterior end of male worms (same magnification).
} Eggs (same magnification).