

STUDIES ON FLOTATION TECHNIQUES FOR THE RECOVERY OF HELMINTH EGGS
FROM SOIL AND THE PREVALENCE OF EGGS OF TOXOCARA SPP.

IN SOME KANSAS PUBLIC PLACES

by

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Introduction

According to Ehrenford,²² Toxocara canis (Werner, 1782) is a common parasite of dogs in the midwestern area of the United States. In 1952, Beaver et al.⁸ identified Toxocara canis as the etiological agent of visceral larva migrans, and since then most active works on Toxocariasis were done in Great Britain.^{12,44,45,55}

However, the recent work of Borg and Woodruff¹² has stimulated our interest to examine soils for the presence of Toxocara spp. eggs in the public places in Manhattan, Kansas.

Review of Literature

Advances in Methodology

Isolation of nematode eggs and larvae from soil is an important matter in some aspects of the study of the epidemiology of nematodiasis. Most references to extraction of eggs from soil seem to start with a report by the Caldwells.¹⁶ The Caldwells' method consisted of mixing the soil to be examined with antiformin solution which was said to free the eggs from the soil. Sugar solution of high specific gravity was then added to the mixture to bring the eggs to the surface of the liquid where they could be removed by wire loop.

Although they claimed that their method gave a quantitatively accurate picture of the conditions, the efficiency of their method was not mentioned.

Other workers^{14,39,52} have used the modified form of Caldwell's method, although no mention was made about the quantitative values of their own techniques. Since then there has been considerable amount of work done (with variable success) by several other workers on the recovery techniques of nematode eggs from soil.^{11,17,27,30,31,35,36,37,48,53}

The World Health Organization^{56,57} (WHO) suggested some techniques of soil examination for recovery of helminth eggs. They brought into focus the problems associated with collection and examination of soil samples. They suggested 20-25 grams of crude soil as adequate sample size for examination and sodium hypochlorite (obtained as 6% commercial bleaching powder) solution diluted to 30% original strength as anti-formin. Egg concentration could be done by gravity or centrifugal-sedimentation using "brine" flotation. Instead of using loop to collect eggs in the meniscus, the surface film was poured off into a clean centrifuge tube, diluted, centrifuged (or allowed to settle) and the sediments examined.

Pegg⁴⁴ used a modified WHO method and examined 5,450 soil samples from both open areas and private gardens in the United Kingdom. She found that 5.2% of the samples in both groups contained T. canis eggs.

Borg and Woodruff¹² in their own technique collected 250-gram samples of soil and aliquots of 1 gram were processed as follows:

one part soil was triturated with 10 parts tap water and after a thorough shaking, it was centrifuged at 2,000 rpm for one minute. Zinc sulfate (s. gr. 1.18) was used for concentration and six bacteriological loopfuls from the solution at the surface were removed to a microscope slide and examined. Using this technique, they found a 24.4% pollution of British public places by Toxocara spp. eggs.

Problems Associated with Recoveries of Nematode Eggs From Soil.

Accuracy in taking a census of nematode egg population depends upon the percentage of nematode eggs recovered from sample residues. Several factors significantly reduce the flotation efficiencies of the different recovery methods. Variations in relative solution specific gravity, viscosity, rate of plasmolysis of eggs, temperature and pH have considerably lowered the efficiencies of the various levitation solutions.^{10,17,20,21,23,24,25,32,34,46,47,48,53}

Also, variations in percentages of eggs recovered from different soil samples using the same technique were said to be due to one or more of the following factors:^{30,56,57}

- (i) size of soil particles,
- (ii) organic matter content of soil,
- (ii) moisture content of soil,
- (iv) degree of contamination by eggs, and
- (v) inconsistent sampling procedures.

The biological status of the eggs under natural conditions has a lot of influence upon recovery ratio of eggs from soil. A large percentage of the eggs found in sediments of levitation solutions of

higher specific gravities were dead.⁴⁷ Also, egg disintegrations in soils have resulted from the combined effects of a series of interdependent factors, with resultant variations in the recovery ratio of nematode eggs in the soil during various seasons.^{42,43}

Human factors have been reported to be a major source of low recovery efficiency of techniques in use. Variations in light infestation due to missing eggs when egg-counting had been reported to affect the final results very adversely.¹⁵

Some workers reported the need for greater consistence in centrifugation procedures. These include the type and amount of diluent, the amount of material centrifuged, the speed of the centrifuge, the time allowed for each centrifugation, the number of times the material is centrifuged and the specific gravity of levitation solution.²⁴ Furthermore, consistent sampling procedures coupled with thorough mixing of samples before processing aliquots have been reported to produce higher nematode egg recoveries from soil samples.^{34,35,36}

Public Health Aspects of Soil-Transmitted Non-Patent Nematodiasis.

In considering the parasitic diseases of animals in relation to the public health, the most important type of accident that may befall the infective stages of parasites is to get into some unnatural host such as man. Of all the animals associated with man, the dog is probably the most important as a source of parasitic diseases.

In some regions, the most common infection derived from dogs is creeping eruption or cutaneous larva migrans. A clinical syndrome closely related to creeping eruption and one which likewise involves

dogs and cats is visceral larva migrans. The one common feature in these various infections is that none of them produce mature infections after a normal period of prepatency. Therefore, collectively they may be referred to as non-patent nematodiasis, although the more familiar term, larval migrans, had been used as having much the same meaning, and in addition to cutaneous larval migrans, incorporating chiefly visceral larval migrans, larval gnathostomiasis and larval filariasis.^{3,4}

According to Manson-Bahr,³⁸ larval migrans was first described by Lee as "myiasis linearis" as long ago as 1874. The erratic migrations of various nematodes and muscid larvae in the subcutaneous and dermal tissues give rise to slowly advancing linear and gyrating red eruptions on feet, legs, buttocks and sometimes on the back.

The term "larval migrans", as generally used, denotes prolonged migration of a larval parasite in the skin or internal organs of an abnormal host, usually man. The offending parasite may be an insect or helminth, but in either case its path through the tissues is marked by a progressive linear lesion produced by pathological reactions of the host's tissues in the vicinity of the wandering larva, or in its wake, depending upon the rate of migration. In most instances the offending larva is a nematode.^{4,9,51}

Cutaneous larval migrans (CLM), or creeping eruption is widely recognized to be caused by Ancylostoma braziliense, one of the several species of hookworm of dogs and cats. A. braziliense is regarded as the chief etiological agent of CLM because the disease is most prevalent within the known geographic range of the worm.³ In the United States CLM was reported to be endemic in southeastern coastal regions and was

considered purely a cutaneous disease.⁵⁸ However, recent studies have shown that other species, such as Uncinaria stenocephala, Ancylostoma caninum, Bunostomum phlebotomum and Ancylostoma ceylanicum were capable of producing this type of infection.³

Generalizing from these observations, CLM may be viewed as a type of parasitic infection resulting from various species of nematode larvae which in their normal hosts reach the intestine by a migration route from soil to skin, through skin to blood stream, to lungs, trachea, pharynx, esophagus and finally the intestine. When these larvae penetrate into the skin of man, their further migration to the blood stream and lungs is delayed and they move about for a more or less lengthy period in the other layers of the skin, setting up a reaction which marks the path of their seemingly aimless wandering.^{3,4,5,6,7,22,58}

Visceral larval migrans (VLM) was first formulated in 1952, and the term was applied to a clinical syndrome resulting from the invasion of human viscera by the larvae of nematodes normally parasitic in lower animals.^{8,18}

Visceral larval migrans as a disease of unknown etiology had been earlier reported under various designations such as familial eosinophilia, Weingarten's disease, Frimodt-Moller's syndrome, eosinophilic pseudoleukemia, eosinophilic leukemoides and other such terms generally focusing in extraordinary eosinophilia of undetermined etiology,^{3,4,8,18,50}

The problem with VLM is due to tissue damages caused by wandering nematode larvae incapable of undergoing a normal life cycle in man. The larval parasites remain in the invaded tissues and may be viable for a year or more.^{3,6,33,40} Such larval stages have been detected

in the liver,^{33,41} lungs,¹³ brains,¹ heart,^{18,22} eyes,⁵⁴ kidneys,^{9,51} and striated muscles^{9,51} depending on the severity of infection. Also, such larval localization in tissues and vital organs of the body have resulted in considerable loss of function in the affected organs.⁵⁵

Prevalence of *Toxocara* spp. Eggs in Soils.

The etiology of VLM seems now to be rather clearly established. Nearly all patients were dirt eaters and/or had contact with dogs.⁵⁰ *Toxocara canis* infections were known to have a wide geographic distribution, and studies conducted in New Orleans and Georgia demonstrated that *Toxocara* eggs occur frequently and abundantly in dooryard soils.^{2, 8,29} Forty-five percent of 74 dooryard soil samples from New Orleans, and 4 out of 17 dooryards in southern Georgia were positive for *Toxocara* eggs.^{8,31} A high incidence of contamination with *Toxocara* spp. eggs was reported in two city parks in Philadelphia.¹⁹ In different parts of the United Kingdom several workers,^{12,44,45} have reported different prevalence of *Toxocara* spp. eggs in soils in public places. In Milan public parks in Italy a prevalence of 21.0% was reported for *Toxocara* spp. eggs.²⁶

In the midwest area of the United States, although *Toxocara canis* infection in dogs was reported to be very common,²² no work has been done to determine the extent of soil pollution by *Toxocara* spp. eggs in different public places.

SECTION I

Standardization of Technical ProceduresIntroduction.

Because of persistent inconsistency reported by several workers^{11, 30, 31, 35, 36, 39} in their attempt to recover nematode eggs from soil, the need for a standard recovery method was obvious before any meaningful survey work could be done. This experiment was designed to investigate the possibility of standardizing our technique and improving the efficiency of recovery method.

Materials and Methods.*

One hundred grams of sterilized clay soil sample was seeded with 3.0 ml solution containing 21,000 Toxocara canis eggs (Figure 1, Appendix II). The infected clay soil was put into a blending machine (Waring blender) and blended for one minute. After blending, the soil particles were allowed to settle and the entire soil sample transferred into a small Dispo[®] container and covered up. A rubber spatula was used to clean the inside walls of the blending machine and residual infected soil sample added to the remainder in the container.

Two aliquots of 1 gram each, of seeded clay soil were processed together each time as follows: An applicator stick was used to mix the seeded soil sample thoroughly in the Dispo[®] cups and 1.0 gram of soil was weighed and transferred into each of the two 12.0 ml ground

* See Appendix I.

[®] Scientific Products Co. C8826.

top centrifuge tubes marked A and B. 9.0 ml of distilled water or decinormal sodium hydroxide were added into each of the two tubes containing 1 gram of seeded clay soil sample; the tubes were corked and their contents thoroughly mixed by shaking over Vortex mixer* for a minute.

The rubber corks were removed from the tubes and the two tubes were centrifuged at 1,000 rpm for 5 minutes. The supernatant was decanted, and levitation solution added to one inch from the top. The corks were replaced and the tubes shaken thoroughly over Vortex mixer for 2-4 minutes. Each cork was carefully removed and its tip washed into its tube using the same levitation solution. Each centrifuge tube was carefully filled to the brim with the same levitation solution such that a small air bubble was formed when an 18 X 18 mm coverslip was placed on the ground top tube. The two tubes (A and B) were centrifuged at 1,000.0 rpm for 5 minutes. After this centrifugation, the coverslip on tube A was carefully removed immediately onto a microscope slide and examined under low power (100 X). The number of eggs recovered was counted using a hand tally and the results recorded. Tube B was treated the same way as with tube A after 5-minute waiting period.

For a second coverslip recovery, a small, fine metal wire piece (4-1/2 X 0.016") was used to scratch the inside of each tube, thereby mechanically freeing more of the sticky eggs on the inside walls of tubes into levitation medium. Each tube was carefully trimmed up with the same levitation solution (as used previously) and the tubes centrifuged again at 1,000 rpm for 5 minutes. After centrifugation tube A

* Scientific Products Co., Vortex-Genie S8223.

was examined immediately and tube B after 5-minute waiting period, and the results recorded under the first coverslip recoveries for the two tubes.

Third, fourth and fifth coverslip recoveries were done for each tube by repeating the procedures in second coverslip recovery and examination and counting of recovered eggs done as in first coverslip recovery. Ten experiments (trials) were done for each interaction of levitation fluid, antiformin substitute, waiting period and coverslip recovery. Results were entered with each fractional recovery per trial and a percent total average recovery calculated.

Results.

The experimental results were summarized in the tables (I-II) below.

Discussion.

When our experimental results were statistically tested between each variable, the differences were not significant. However, when an analysis of variance was run on four variables (time, type of fluid, number of covers and type of wash), then ZnSO_4 s. gr. 1.20, decinormal sodium hydroxide, three covers and a 5-minute waiting period was significantly better than other four combinations at the 5% level. The efficiency of the method was fairly high and recovery ratio was more consistent than the results of many other workers (Table III).^{11,30,31,35,39,52}

The recovery efficiency of our new modified technique compared closely with the results of Lindquist (1966).³⁵ It should be mentioned,

TABLE I. Quantitative Recovery Results from 100.0 gram Clay Soil Samples Inoculated with Toxocara canis Eggs.

Experimental Conditions:

One gram soil mixed with 9.0 cc water.
 Centrifugation was for 5 minutes at 1000.0 rpm.
 Eggs inoculated per gram of soil = 210.

Examination Period	Amount of Sample Processed	Flotation Fluid	Specific Gravity	Total Numbers of Eggs Recovered		
				Range		Mean (%)
				Absolute figures	Percentage	
Immediate	10 - 1.0 gram	ZnSO ₄	1.18	138 - 140	65.71 to 66.67	66.19
	10 - 1.0 gram	Na ₂ Cr ₂ O ₇	1.20	117 - 118	55.71 to 56.19	55.95
	10 - 1.0 gram	ZnSO ₄	1.20	131 - 134	62.38 to 63.81	62.90
	10 - 1.0 gram	HgI ₂	1.63	115 - 117	54.76 to 55.71	55.24
Wait 5 minutes	10 - 1.0 gram	ZnSO ₄	1.18	134 - 137	63.81 to 65.24	64.67
	10 - 1.0 gram	Na ₂ Cr ₂ O ₇	1.20	112 - 115	53.33 to 54.76	54.10
	10 - 1.0 gram	ZnSO ₄	1.20	128 - 132	60.95 to 62.86	61.95
	10 - 1.0 gram	HgI ₂	1.63	117 - 121	55.71 to 56.62	56.67

TABLE II. Quantitative Recovery Results from 100.0 gram Clay Soil Samples Inoculated with Toxocara canis Eggs.Experimental Conditions:

One gram soil mixed with 9.0 cc decinormal NaOH.

Centrifugation was for 5 minutes at 1000.0 rpm.

Eggs inoculated per gram of soil = 210.

Examination Period	Amount of Sample Processed	Flotation Fluid	Specific Gravity	Total Numbers of Eggs Recovered		
				Range		Mean (%)
				Absolute figures	Percentage	
Immediate	10 - 1.0 gram	ZnSO ₄	1.18	128 - 130	60.95 to 61.90	61.43
	10 - 1.0 gram	Na ₂ Cr ₂ O ₇	1.20	109 - 111	51.90 to 52.86	52.24
	10 - 1.0 gram	ZnSO ₄	1.20	122 - 125	58.10 to 59.53	58.67
	10 - 1.0 gram	HgI ₂	1.63	102 - 105	48.57 to 50.00	49.05
Wait 5 minutes	10 - 1.0 gram	ZnSO ₄	1.18	110 - 114	52.38 to 54.29	53.24
	10 - 1.0 gram	Na ₂ Cr ₂ O ₇	1.20	118 - 120	56.19 to 57.14	56.71
	10 - 1.0 gram	ZnSO ₄	1.20	140 - 143	66.67 to 68.10	67.52
	10 - 1.0 gram	HgI ₂	1.63	96 - 99	45.71 to 47.14	46.29

Table III: Reported Efficiencies (%) of Worm Egg Recovery Techniques

Recovery Efficiency of Technique (%)	Reference(s)	Type of Worm Eggs
3.2	Spindler (1929)	<u>Ascaris</u>
15.1	Maplestone and Mukerji (1936)	<u>Ascaris</u>
26.6	Berlinguer (1962)	<u>Ascaris</u>
50.0 (Sandy Soil)	Ito and Natsume (1964)	<u>Ascaris</u>
10.0 (Clay Soil)	Ito and Natsume (1964)	<u>Ascaris</u>
32.0	Jacquemin and Vincent (1955)	<u>Ascaris</u>
68.0	Lindquist (1966)	<u>Ascaris</u> (Decorticated)
67.5	Dada (1976)	<u>Ascaris</u>

however, that Lindquist (loc. cit.) was working with decorticated Ascaris eggs, and with such preconditioned worm eggs the attractive force between such worm eggs and the particles of soil substance may have been highly reduced. The reduction of attractive forces between worm eggs and soil particles might probably account for his higher recoveries.

Also, as regards our technique, several factors might be responsible for such a fairly high recovery ratio, viz: The use of Waring blender enabled us to process all our samples without discarding any portion which might possibly contain some sticky eggs. Also, soil samples were processed into much finer particles than was achieved when mesh screens of graded sizes were used.^{35,36,56,57} In this way loss of eggs through "egg-screen" attachment was totally eliminated and the resulting soil particles from Waring blender were better processed.

Although none of the antiformin substitutes seemed to effect release of eggs too well from soil samples, extraneous matters were very much removed from the processed samples. The use of a mechanical stirrer to release eggs sticking to the inside walls of test tubes might have aided the recovery ratio. Because none of the flotation fluids crystallized within the experimental examination periods, there was no problem as to viewing recovered eggs. The eggs were clearly visible and mostly intact.

The use of mercuric iodide solution in the recovery trials was interesting since this solution was usually reserved for heavier fluke

eggs.⁴⁹ Although the yield (46.29 - 56.67%) with mercuric iodide solution was high, its cost and toxicity might not lend it for routine laboratory work.

Finally, although the chance of worm eggs recovered in this work has been increased by undergoing five coverslip recoveries on each sample processed, most of the eggs recovered were obtained with the first 3 coverslips.

Conclusion.

In this work a new modified technique for the extraction of Toxocara spp. eggs from soil has been devised. This consisted of comminuting soil samples into finer particle sizes by the use of Waring blender, followed by mixing soil samples with decinormal NaOH in a Vortex mixer, and floating with ZnSO_4 (s. gr. 1.20) solution. The eggs floated were recovered by coverslips and examined five minutes after centrifugation.

Though the experimental results showed that the modified new technique can be used quantitatively for replicated experiments, its costs and uses in (a) epidemiological surveys for handling larger soil samples, and (b) routine laboratory diagnosis of soil pollution with ascarid eggs would need further evaluations.

SECTION II

Prevalence of *Toxocara* spp. Eggs in Manhattan Public Grounds
and Some Kansas Highway Rest Areas

Introduction.

The city of Manhattan, Kansas covers an area of 8.5 square miles. There has been a steady increase of licensed dogs at the rate of about 100 per year since 1972. As of 1975 there were 1,297 licensed animals and an unknown number of unlicensed ones. The average weight of these dogs is about 20 lbs. Carnivores are known to deposit about 3% of their body weight in feces per 24 hours which is about 0.6 lb per day times the number of dogs equals 778.2 lbs per day or 284,043 lbs per year, or roughly 142 tons per year on this 8.5 square mile area. The data above gives us an insight into the amount of canine solid contamination in the Manhattan area and the basis of comparison for other workers.

Little or none of this goes through sanitary sewers, is burned or buried, but rather is disseminated by rainfall and much finds its way to untreated storm sewers and the river drainage system. With the increased interest in pets coupled with the recent revival of interest in visceral larval migrans, we undertook to examine worm egg contamination in Manhattan public places (school yards, city and state parks, university married housing play grounds) and some Kansas highway rest stops.

Materials and Methods.

Specimens of soil were collected from public places in widely separated areas throughout the entire city of Manhattan, Kansas and from well-traveled routes U.S. 54, 70 and 156 across the state of Kansas. These soil specimens were collected during late spring and throughout summer months.

Our selection of collecting areas was where the ground was bare and had little drainage, like around swings, picnic tables, merry-go-rounds, slides, teeters, refuse cans and ball diamonds. Our collection technique was to scrape with a garden trowel an area of soil 50 square inches at about 1/4 to 1/2 inch deep. This filled a 1/2-pint ice cream container and was approximately 250 grams of soil. The soil was allowed to dry in the laboratory and then entirely mixed in a Waring blender.

From the mixed soil we selected four 1-gram samples and processed according to our technique as follows: To each 1-gram soil sample in the centrifuge tube was added 9.0 cc decinormal sodium hydroxide and after thorough shaking over Vortex mixer, was centrifuged at 1,000 rpm for 5 minutes and the sodium hydroxide discarded. To the remaining sediment was added sufficient 1.20 s. gr. zinc sulfate to fill the tube one inch from the top. The tube was stoppered and mixed again with the Vortex mixer. The cork was removed and washed into the tube with more zinc sulfate solution. The tube was trimmed to a meniscus and a coverslip added. Again, we centrifuged at 1,000 rpm for 5 minutes. Then the coverslip was carefully lifted 5 minutes after centrifugation, placed on a slide and examined for Toxocara spp. eggs.

The tube sediment and sides were stirred with a fine wire and trimmed with more zinc sulfate and a second cover added, centrifuged, removed and examined. This procedure was done for 3 consecutive covers for each 1-gram sample to get the total eggs recoverable from the sample.

Results

The experimental results were summarized in the tables (IV-VII) below.

TABLE IV. Numbers of Soil Samples Collected in Manhattan Elementary
Schools Containing Toxocara spp. Eggs

Site of Soil Collection	Number of Samples Collected	Number of Samples Containing <u>Toxocara</u> spp. Eggs
Marlatt	5	4
Bluemont	5	0
Woodrow Wilson	5	0
Theodore Roosevelt	5	1
Eugene Field	5	1
Day Care Center (Federation of Handicapped Children)	5	1
Lee School	5	0
Seven Dolors	10	0
Northview	5	2
College Hill (Marlatt Annex)	5	3
Strong School	6	2
Total: 11 Elementary Schools	61	14

TABLE V. Numbers of Soil Samples Collected in City and State Parks
Containing Toxocara spp. Eggs

Site of Soil Collection	Number of Samples Collected	Number of Samples Containing <u>Toxocara</u> spp. Eggs
CiCo	5	2
Warner Memorial	5	1
Sunset	10	3
City	15	4
Goodnow	10	1
Girl Scout	7	0
Long's	5	1
Douglas	5	0
Tuttle Creek	51	9
Pottawatomie	12	2
Total: 10 parks	125	23

TABLE VI. Numbers of Soil Samples Collected in University Married Student Housing Playgrounds, Dykstra Veterinary Hospital, Hamilton's Dog Kennels and Kansas Highway Rest Areas Containing Toxocara spp. Eggs

Site of Soil Collection	Number of Samples Collected	Number of Samples Containing <u>Toxocara</u> spp. Eggs
KSU Married Students' Quarters		
a. Sandboxes	23	9
b. Swings	23	4
Dykstra Veterinary Hospital (front lawn)	5	5
Hamilton's Dog Kennels (vacated 9 months ago)	16	6
Kansas Highways (rest areas)	50	8
Total:	117	32

TABLE VII. Prevalence of Toxocara spp. Eggs in Soil in Public Places

Number	Place	Percent	Ratio of Positive Samples for <u>Toxocara</u> spp. Eggs
11	Schools	22.95	14/61
10	Parks	18.40	23/125
15	Highway Rest Stops	16.0	8/50
Married Students' Quarters			
23	Sandboxes	39.13	9/23
23	Swings	17.39	4/23

Discussion.

From the results presented in tables IV-VII, the following prevalences of Toxocara spp. eggs in soils were recorded for the several public areas sampled: elementary schools 22.95% (14/61); city and state parks 18.4% (23/125); Kansas State University married quarters - sandboxes 39.13% (9/23) and swings 17.39% (4/23); and highway rest areas 16.0% (8/50). The front lawn of Dykstra Veterinary Hospital which is used frequently for walking dogs was sampled and all our samples (5/5) were positive for Toxocara spp. eggs. Also, 16 samples were obtained from Hamilton's Dog Kennels vacated about 9 months ago and 6 samples contained Toxocara eggs.

There were some variations in prevalences between the several public places sampled and mention should be made that the highway rest stops were on well-traveled routes U.S. 54, 70 and 156 across Kansas in the summer time. Also, it was interesting that the sandboxes in the married students' quarters had a high percentage of positives. Although animals were not permitted in this housing unit, some illegal cats and dogs do reside there and perhaps their use of sandboxes has increased the worm egg distribution. Covered sandboxes might be a solution.

Out of a total of 282 soil samples obtained from these public areas (Table VII), 58 samples contained Toxocara spp. eggs. This gave an overall prevalence of 20.57%. This figure compared very well with reported prevalences in the United Kingdom,^{12,45} and Italy²⁶ (Table VIII). While the situations regarding leash laws in the United Kingdom and Italy were not known, the leash laws operating in the U.S. did not

TABLE VIII. Reported Prevalence of Toxocara spp. Eggs in Public Grounds
in Some Parts of the World.

Reported Prevalence (%)	Country	Location	Reference
21.0	Italy	Milan Public Parks	Genchi and Locatelli (1974)
24.40	United Kingdom	Multiple Areas	Borg and Woodruff (1973)
Abundant	U.S.A.	New Orleans and Georgia	Beaver (1954)
High	U.S.A.	Philadelphia City Parks	Dubin and Segall (1973)
20.57	U.S.A.	Manhattan, Kansas	Dada (1976)
5.2	England	N.W. London (open areas)	Pegg (1975)
5.2	United Kingdom	S.W. London/N.E. Scotland (private gardens)	Pegg (1975)
13.30	United Kingdom	Leeds (streets)	Read and Thompson (1976)
4.0 ^a	U.K. (Scotland)	Edinburgh (public areas)	Sewell (1976)
6.0 ^b	U.K. (Scotland)	Glasgow (public areas)	Sewell (1976)

a and b = Sewell, M. M. H. (1976) (personal communications).

seem to work well. We have observed that though these dogs were on leash, they were often walked about anywhere by their owners, and their (dogs') feces were usually not disposed of in refuse cans. Hence, it was apparent that contamination of public grounds resulted from feces deposited by dogs being walked about on streets, parks, school yards and other public grounds by their owners. Perhaps one way to solve this problem of soil contamination would be the provision of separate areas in public places designated for dog walks.

So far our work was purely qualitative and if positives occurred in any one of the four 1-gram samples on any of the 3 covers, it was recorded positive. Neither did we attempt to ascertain viability of eggs or record those embryonated or non-embryonated, although both were seen frequently. Also, in many cases since low numbers of eggs were recovered, it was doubtful if we could have checked viability by animal passage.

In this work, although our main interest was in soil pollution resulting from Toxocara spp. eggs, we were also watching for Trichuris and we found their eggs from time to time as shown in Table IX. While cats are not noted for having whipworms, the sandboxes still had the highest percentages of whipworm eggs. A probable reason for this situation might be a possible occasional visitation of these sandboxes by dogs as well, or Trichuris eggs may be freed from sand easier than clay soil.

Although T. vulpis has not been reported to be of any public health hazard, a case involving this parasite has been reported in a small boy.²⁸

TABLE IX. Prevalence of Trichuris vulpis Eggs in Soil in Public Places

Number	Place	Percent	Ratio of Positive Samples for <u>Trichuris vulpis</u> Eggs
11	Schools	3.30	2/61
10	Parks	5.60	7/125
15	Highway Rest Stops	6.0	3/50
Married Students' Quarters			
23	Sandboxes	13.04	3/23
23	Swings	0	0/23

As regards our new modified soil technique, it has been experienced in this work that it was often considerably easier to recover the eggs from artificially infected soils than from naturally infected soils.

Conclusion.

In conclusion, it was found that there was an average of about 20.57% positive Toxocara soil samples when collected from Manhattan public grounds. Also, highway rest areas were contaminated and children's sandboxes have the highest recoverable percentage of all.

Secondly, it would appear that a potential health hazard from Toxocara spp. could exist in Manhattan, Kansas.

Thirdly, because of relatively common T. vulpis infection in dogs in this area, further research is advocated for evaluation of its public health significance.

APPENDIX I

Details of Materials and Methods for Standardization of Technical
Procedure

I. Sterilization of Soil Samples

Crude clay soil was collected from the heap of soil by means of gardener's trowel outside Veterinary Medical Teaching Building, Kansas State University. Two hundred and fifty grams of crude clay soil was weighed out and heated in the oven at 180°F for 30 minutes to incinerate any possible egg content.

II. Preparation of Flotation Solution

All chemicals used were purchased from Kansas State University Chemistry Store.

- A. Zinc sulfate solution (s. gr. 1.18): 331 grams ZnSO_4 were dissolved in 1,000 ml distilled water. An hydrometer was used to determine the specific gravity at room temperature.
- B. Sodium bichromate solution (s. gr. 1.20): 180.0 grams of sodium bichromate was added to 1,000 ml of distilled water and specific gravity determined at room temperature by hydrometer.
- C. Mercuric iodide (s. gr. 1.630):
 - Potassium iodide - 32.0 gm
 - Mercuric iodide - 50.0 gm
 - Distilled water - 90 ml

Potassium iodide was dissolved in water and then mercuric iodide was added with gentle heating if necessary. The specific gravity was determined at room temperature by hydrometer.

D. Zinc sulfate (s. gr. 1.20): 385.0 grams of zinc sulfate was dissolved in 1,000 ml of distilled water and specific gravity tested at room temperature.

E. Sodium hydroxide (0.1 N): 4.0 grams of sodium hydroxide was added to 1,000.0 ml of distilled water to produce 0.1 N solution.

III. A. Isolation and Concentration of Toxocara canis Eggs From Fecal Materials.

Fecal samples were obtained from the Animal Shelter at Sunset Zoo in Manhattan, Kansas. The feces were qualitatively screened and the ones positive for T. canis infection were processed as follows:

Fecal material was mixed thoroughly with cold water, strained through cheese cloth into graduated cylinder and allowed to settle overnight in the refrigerator. The supernatant was decanted the following day and more water was added with stirring and allowed to settle overnight in the refrigerator. This process was repeated every day until the water on top of sediment became clear.

The clear tap water was decanted and sediments transferred from the graduated cylinder into ground top centrifuge tubes. Zinc sulfate (s. gr. 1.18) solution was added to the tubes, and 18 x 18 mm cover-slips added such that small air bubble would be formed. The tubes were centrifuged at 1,000 rpm for 5 minutes.

After centrifugation the coverslips were carefully lifted and placed on microscope slides and examined for presence of Toxocara canis eggs under low power (100 X). The coverslips with worm eggs were washed into a container with distilled water. The entire recovery process was repeated until most of the eggs in the feces were recovered.

Then the recovered eggs were washed clean of extraneous matter by adding water, centrifuging at 1,000 rpm for 5 minutes and decanting the supernatant. This washing process was repeated three times.

B. Determination of amount of eggs per milliliter of solution for seeding soil samples.

At the last egg washing stage, the supernatant was decanted by means of Pasteur pipette and the egg sediment was suspended in a known volume of water. The test tube containing this concentrate was labeled "original concentrated solution (A)".

The concentrated solution A was thoroughly shaken over Vortex mixer for 20 seconds and 1.0 ml was quickly pipetted into another test tube containing 9.0 mls of tap water. This test tube was labeled "1:10 dilution egg solution - (B)". The test tube B containing 1:10 dilution egg solution was corked, shaken over Vortex mixer for 20 seconds and solution was quickly pipetted using 1 cc pipette. A drop of solution B was put on the microscope slide, covered carefully with a coverslip and the slide was examined systematically to count the number of eggs per drop of solution B.

The above step was repeated 10 times and average count per drop was determined. In this work the average count of eggs per drop was 35.0, i.e.:

In solution B, 1.0 drop contained 35 eggs.

In solution B, 20.0 drops (= 1.0 ml) would contain 700.0 eggs.

Hence, in solution A (= 10 X concentration of solution B),

1.0 ml of solution would contain 7,000.0 eggs.

APPENDIX II

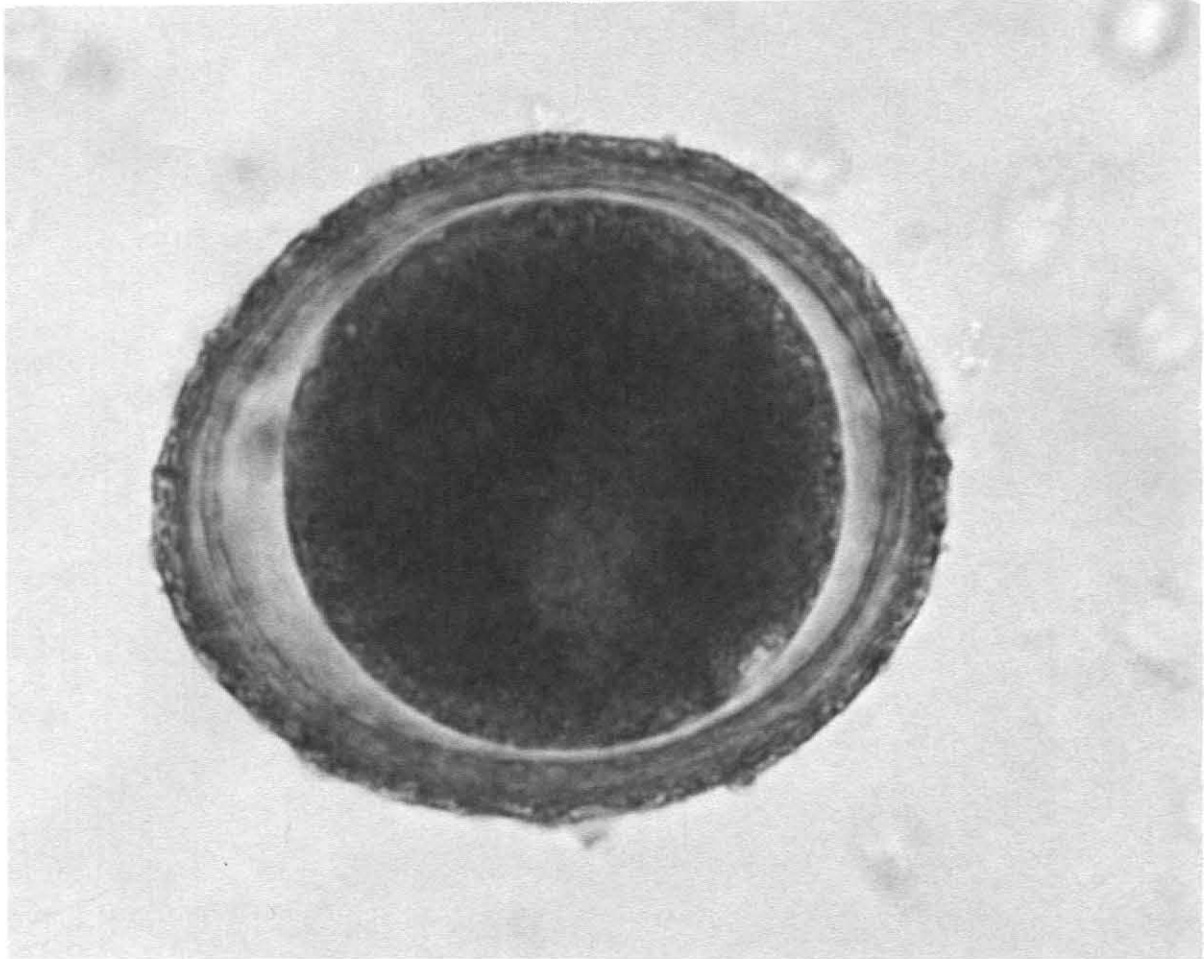


Figure 1: Unembryonated Toxocara canis egg.

APPENDIX III

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BIBLIOGRAPHY

1. Beautyman, W. and Woolf, A. L.: An Ascaris larva in the brain in association with acute anterior poliomyelitis. J. Path. Bact. 63, (1951): 635-647.
2. Beaver, P. C.: Observations on the epidemiology of ascariasis in a region of high hookworm endemicity. J. Parasit. 38, (1952): 445-453.
3. Beaver, P. C.: Parasitic diseases of animals and their relation to public health. Vet. Med. 49, (1954): 199-205.
4. Beaver, P. C.: Larval migrans. Expt. Parasit. 5, (1956): 586-621.
5. Beaver, P. C.: Wandering nematodes as a cause of disability and disease. Am. J. Trop. Med. and Hyg. 6, (1957): 433-440.
6. Beaver, P. C.: Animal parasites and human diseases. Pediatrics 22, (1958): 380-384.
7. Beaver, P. C.: Visceral and cutaneous larva migrans. U.S. Public Health Reports, 74, (1959): 328-332.
8. Beaver, P. C.; Snyder, C. H.; Carrera, G. M.; Dent, J. H., and Lafferty, J. W.: Chronic eosinophilia due to visceral larva migrans. Pediatrics 9, (1952): 7-19.
9. Belding, David L.: Textbook of Parasitology. Meredith Publishing Company, N.Y. 3rd Edition. (1965): 481-486.

10. Bello, T. R.: Comparison of the flotation of Metastrongylus and Ascaris eggs in different levitation solutions. Am. J. Vet. Res. 22, (1961): 597-600.
11. Berlinguer, G.: Ricerca e conteggio delle uova di elminti nel terreno con particolare riguardo agli ascaridi. (English Translation). Parasitologia. 4, (1962): 147-154.
12. Borg, O. A., and Woodruff, A. W.: Prevalence of infective ova of Toxocara species in public places. Brit. Med. J. 4, (1973): 470-472.
13. Brill, R.; Churg, J.; and Beaver, P. C.: Allergic granulomatosis associated with visceral larva migrans. Am. J. Clinical Path. 23, (1953): 1208-1215.
14. Brown, H. W.: Studies on the rate of development and viability of the eggs of Ascaris lumbricoides and Trichuris trichura under field conditions. J. Parasit. 14, (1927a): 1-15
15. Brown, H. W.: A study of the regularity of egg production of Ascaris lumbricoides, Necator americanus and Trichuris trichura. J. Parasit. 14, (1927b): 110-119.
16. Caldwell, Fred C. and Caldwell, Elfreda L.: Preliminary report on observations on the development of ova of pig and human Ascaris under natural conditions, and studies of factors influencing development. J. Parasit. 14, (1928): 254-260.

17. Caveness, F. E. and Jensen, H. J.: Modification of centrifugal-flotation technique for the isolation and concentration of nematodes and their eggs from soil and plant tissue. Proc Helminth. Soc. Wash. 22 (2), (1955): 87-90.
18. Dent, J. H.; Nichols, R. L.; Beaver, P. C.; Carrera, G. M. and Staggers, R. J.: Visceral larva migrans with a case report. Am. J. Path. 32, (1956): 777-803.
19. Dubin, S. and Segall, S.: Contamination of soil with canine nematode larvae in two city parks. J.A.V.M.A. 163 (10), (1973): 1193.
20. Dunn, D. R.: Studies on the pig lungworm (Metastrongylus spp.): II--Experimental infection of pigs with M. apri. Brit. Vet. J. 112, (1956): 327-337.
21. Dunn, D. R.; Gentles, M. A.; and White, E. G.: Studies on the pig lungworm (Metastrongylus spp.): I--Observations on natural infection in the pig in Great Britain. Brit. Vet. J. 111, (1955): 271-281.
22. Ehrenford, Frank A.: Canine ascariasis as a potential source of visceral larva migrans. Am. J. Trop. Med. & Hyg. 6, (1957): 166-170.
23. Farr, M. M. and Luttermoser, G. W.: Comparative efficiency of zinc sulfate and sugar solutions for simultaneous flotation of Coccidia oocysts and helminth eggs. J. Parasit. 27, (1941): 417-424.

24. Faust, E. C.; D'Antoni, J. S.; Odom, V.; Miller, M. J.; Peres, C.; Sawitz, W.; Thomen, L. F.; Tobie, J. and Walker, J. H.: A critical study of clinical laboratory technics for the diagnosis of protozoan cysts and helminth eggs in feces. *Am. J. Trop. Med.* 18, (1938): 169-183.
25. Faust, E. C.; Sawitz, W.; Tobie, J.; Odom, V.; Peres, C.; and Lincicome, D. R.: Comparative efficiency of various technics for the diagnosis of protozoa and helminths in feces. *J. Parasit.* 25, (1939): 241-262.
26. Genchi, C. and Locatelli, A.: Incidenza di uova di alcune specie di elminti intestinali del cane nei parchi pubblici della citta di Milano. *Atti dilla Societa Italiana delle scienze Veterinarie.* 28, (1974): 862-863. (English Translation)
27. Goodney, T.: Laboratory methods for work with plant and soil nematodes. Ministry of Agriculture and Fisheries, London. Technical Bulletin No. 2, (1949): 1-19.
28. Hall, J. E. and Sonnenberg, B.: An apparent case of human infection with the whipworm of dogs, Trichuris vulpis (Froelich, 1789). *J. Parasitol.* 42, (1956): 197-199.
29. Headlee, W. H.: The epidemiology of human ascariasis in metropolitan area of New Orleans, Louisiana. *Am. J. Hyg.* 24, (1936): 479-521.

30. Ito, J. and Natsume, T.: Studies on the effect of flotation technique for recovering of Ascaris ova from soil. Japanese J. of Parasit. 13, (1964): 238-242. (English Translation)
31. Jacquemin, P. and Vincent, G.: Nouvelle technique pour la detection des oeufs d'helminthes dans les poussières souillées. (English Translation). Bulletin de la Société D'Histoire Naturelle, 46, (1955): 295-300.
32. Jahnes, W. G. and Hodges, E. P.: An improved method of sedimenting Schistosoma japonicum and other helminth ova. J. Parasit. 33, (1947): 483-486.
33. Karpinski, Felix E., Jr.; Everts-Suarez, E. A.; and Sawitz, W. G.: Larval granulomatosis (visceral larval migrans). Am. J. Dis. Child. 92, (1956): 34-40.
34. Koutz, F. R.: A comparison of flotation solutions in the detection of parasite ova. Am. J. Vet. Res. 2, (1941): 95-100.
35. Lindquist, W. D.: An improved method for isolation of Ascaris eggs from soil. Paper presented at the 47th Annual Meeting of the Conference of Research Workers in Animal Diseases. Chicago, Illinois, U.S.A. (1966).
36. Lindquist, W. D.: Further studies on isolation of Ascaris eggs from soil. Paper presented at the 48th Annual Meeting of the Conference of Research Workers in Animal Diseases. Chicago, Illinois, U.S.A. (1967).

37. Lurain, S. T.: Extraction of parasitic nematode eggs from soil. Unpublished Report. Michigan State University, East Lansing, Michigan, U.S.A. (1966): 13 pp.
38. Manson-Bahr, Philip: The histological pattern of visceral larva migrans (parasitic granuloma) and its role in diagnosis. J. Trop. Med. & Hyg. 64, (1961): 129-136.
39. Maplestone, P. A. and Mukerji, P. K.: An improved technique for the isolation of Ascaris eggs from the soil. Ind. J. Med. Res. 23, (1936): 667-672.
40. Mercer, R. D.; Lund, H. Z.; Bloomfield, R. A. and Caldwell, F. E.: Larval ascariasis as a cause of chronic eosinophilia with visceral manifestations. Am. J. Dis. Child. 80, (1950): 46-58.
41. Milburn, C. L., Jr., and Ernst, K. F.: Eosinophilia-hepatomegaly syndrome of infants and young children: Report of a case due to invasion of liver by nematode larvae. Pediatrics. 11, (1953): 358-367.
42. Onorato, A. R.: The effects of temperature and humidity on the ova of Toxocara canis and Trichuris vulpis. Am. J. Hyg. 16, (1932): 266-287.
43. Owen, W. B.: Factors that influence the development and survival of the ova of an Ascarid roundworm Toxocara canis (Werner, 1782), (Stiles, 1905), under field conditions. University of Minnesota Agricultural Experiment Station, Technical Bulletin 71. (1930): 1-25.

44. Pegg, E. J.: Dog roundworms and public health. Vet. Rec. 97, (1975): 78.
45. Read, M. A. and Thompson, R. C. A.: Prevalence of Toxocara canis and Toxascaris leonina ova in dog faeces deposited on the streets of Leeds. J. Helminth. 50, (1976): 95-96.
46. Ritchie, L. S.; Lin, S.; Moon, A. P.; Frick, L. P.; Williams, J. E.; Asakura, S.; and Hishinuma, Y.: The possible effects of pH and specific gravity on the ether-sedimentation procedure in concentrating eggs and cysts. Am. J. Trop. Med. & Hyg. 9, (1960): 444-449.
47. Sawitz, W.; Tobie, J. E. and Katz, G.: The specific gravity of hookworm eggs. Am. J. Trop. Med. 19, (1939): 171-179.
48. Scott, J. A.: A simple substitute for antiformin for parasitological uses. J. Parasit. 23, (1937): 109.
49. Skerman, K. D. and Hillard, J. J.: A handbook for studies of helminth parasites of ruminants. Near East Animal Health Institutes, Iran Unit, UNDP/Special Fund. (1966): Sect. B: pg. 1-2 and Sect. C (CIV): pg. 1-3.
50. Snyder, C. H.: Visceral larva migrans: Ten years experience. Pediatrics. 28, (1961): 85-91.
51. Soulsby, E. J. L.: Helminths, Arthropods and Protozoa of Domesticated Animals. The Williams and Wilkins Co., Baltimore, MD, U.S.A., 6th Edition. (1975): pg. 159-165.

52. Spindler, L. A.: On the use of a method for the isolation of Ascaris eggs from soil. Am. J. Hyg. 10, (1929): 157-164.
53. Stevenson, R. T.: Use of calcium chloride to isolate helminth ova from soil. J. Parasit. 28, Suppl. 46 (1942): 24.
54. Wilder, H. C.: Nematode endophthalmitis. Trans. Am. Acad. Ophthal. 55, (1950-51): 99. Cited by Friedman, S. and Hervada, A. R.: Severe myocarditis with recovery in a child with visceral larva migrans. Jour. Pediatrics. 56, (1960): 91-96.
55. Woodruff, A. W.: Toxocariasis. Brit. Med. J. 3, (1970): 663-669.
56. World Health Organization. "Soil transmitted helminths". In: Technical Report Series No. 277, (1964): 1-69.
57. World Health Organization. "Control of Ascariasis". In: Technical Report Series No. 379, (1967): 1-47.
58. Wright, D. O. and Gold, E. M.: Löffler's syndrome associated with creeping eruption (cutaneous helminthiasis). Arch. Int. Med. 78, (1946): 303-312.

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STUDIES ON FLOTATION TECHNIQUES FOR THE RECOVERY OF HELMINTH EGGS
FROM SOIL AND THE PREVALENCE OF EGGS OF TOXOCARA SPP.

IN SOME KANSAS PUBLIC PLACES

by

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ABSTRACT

Beaver et al⁸ identified T. canis (Werner, 1782) as the etiologic agent of visceral larva migrans and infection was said to occur after eating soil contaminated by T. canis infective eggs.

In this work, a new modified technique (having about 68% recovery efficiency) for the extraction of Toxocara spp. eggs from the soil is described. The new modified technique can be used quantitatively for replicated experiments and its further usage for routine laboratory diagnosis and epidemiological surveys of worm egg pollution in soil is suggested.

Also, the new modified technique has been used to elucidate the degree of worm egg contamination of several public places in Manhattan, Kansas, and some Kansas highway rest areas. Based on these surveys the following prevalences were obtained: elementary schools 22.95% (14/61); city and state parks 18.40% (23/125); Kansas highway rest areas 16.0% (8/50) and Kansas State University married students' quarters 39.13% (9/23 sandboxes) and 17.39% (4/23 swing areas).