THE EFFECTS OF PHYSICAL AND CHEMICAL FACTORS ON THE DEVELOPMENT OF EGGS OF *ASCARIS LUMBRICOIDES* AND *TRICHURIS TRICHIURA*

BY

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DECLARATION

I certify that this is my original work and has not been presented for a degree in any other University.

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This dissertation has been submitted for examination with our approval as University supervisors.

Signed...Richard Knight..... Date.....Jan 16th 1992.

Prof. Richard Knight

Signed..... Date.....

Dr. Benson B.A. Estambale

DEDICATION

TO

My husband and children.

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SUMMARY

The study was done in the Parasitology Laboratory at the Department of Medical Microbiology of the University of Nairobi. It was an *invitro* experimental study. Also the development of eggs (segmentation and embryonation) in the external soil environment and their occurrence in a natural peridomestic urban environment were studied. The objective was to study the effects of various physical and chemical factors on the development of eggs of *Ascaris lumbrocides* and *Trichuris trichiura*.

Positive stool specimens for eggs of *Ascaris lumbrocides* and/or *Trichuris trichiura* were identified from the collected and examined stool specimens of the patients at the Kenyatta National Hospital. Also soil specimens were collected and examined for similar eggs from two underprivileged areas in Nairobi. Stool specimens were re-examined and confirmed microscopically using the formal-ether concentration (FEC) method by the Principal investigator and a senior laboratory technologist. The positives were pooled together and analyzed in bulk. Control samples were also included.

The experiments done included the incubation of the stool samples in different physical and chemical conditions such as temperature, humidity, acidity and alkalinity. Samples were incubated for a duration of five weeks. They were examined weekly for egg development. These were then counted and recorded

as undeveloped, segmented or embryonated.

As described in the results, *Ascaris lumbricoides* eggs were the majority and *Trichuris trichiura* eggs were few. The results show that for the temperatures (22 to 28°c) studied, development of *Ascaris* eggs was best at 28°C, less well at 37°C and not at all at 4°C. The eggs developed at room temperature better than 37°C under similar conditions. The eggs developed better in acidic conditions compared to alkaline ones.

The eggs developed in both 4% and 10% formalin solutions but better in the former solution. The eggs developed in conditions of adequate moisture but not in those without moisture. In general, development of eggs was better aerobically than anaerobically except in 10% formalin solution.

In most of the experiments, the proportion of eggs developed was highest at week three and thereafter declined in weeks 4 and 5.This was more evident under alkaline conditions and at 37°C.

The results of egg development in the external soil environment showed that about one sixth of the eggs developed.The eggs seen and identified from examination of soil collected in the urban peridomestic environment were of *Ascaris lumbricoides* and few of them were segmented or embryonated.

INTRODUCTION

There are an estimated 500,000 species of nematodes, many of which are of considerable economic importance as plant parasites, others cause disease in animals and approximately twelve are commonly encountered in man (51,17,18,20). Intestinal nematode infections are of public health importance. This is due to their high prevalence, their effects on both nutritional and immune status of those affected, especially those living in the tropical and subtropical areas of the world (15,17,18,20 22,26,27,29,36,39,43). These effects are of special significance in undernourished populations because of poor usage of food, frequent short supply of food, and hence deficiencies in energy, proteins, vitamins and trace elements.

Studies have been done to assess the prevalence of intestinal nematodes world-wide (11,15,17,18,34). In addition, the numbers of those infected with each different type of gut helminthiasis in different areas of the world has been estimated (29). These studies indicate that globally, ascariasis is the dominant helminthic infection followed by hookworm (11,17). About one in four of the world population is infected with *Ascaris lumbricoides*.

In general, there are geographical variations in the prevalence and intensity of intestinal parasitic infections, and indeed most helminthiases. This is due to climatic variations in temperature, humidity, and also chemical differences in the type

of soil, and socio-economic status (1,3,5,6,11,37). These factors generally result in less intestinal parasitic infections in the temperate countries where temperatures are generally much lower than in the tropics and the subtropics (40,44).

Temperature plays a dominant role in the development of both *Ascaris* and *Trichuris* eggs (5,8,9,32,35). The other important physical factors include humidity, landscape, altitude, ultraviolet radiation from sunlight, and the type of soil (1,4,7,9,42). The presence or absence of chemical agents in the environment also plays an important role (13,16,35). In one such study in the Philippines, by Cabrera, it was apparent that ascariasis reinfection was highest when rainfall was minimal, and lowest when rainfall was at its peak (9).

The most important intestinal helminths in Kenya are *Necator americanus*, *Ascaris lumbricoides* and *Taenia saginata* (11). A recent review estimated that 25% of the total population of Kenya or over four million people at that time, were infected with *Ascaris*. A study by Pamba showed an overall prevalence of 42.2% infected by hookworm and 17.3% with *Ascaris lumbricoides* (28). In addition, in Kenya there are variations in climatic factors - such as temperature, humidity - and also type of soil. The coastal area of Kenya is hot and humid, the western part of the country also is hot but less humid, whereas the central part of the country especially Central Province generally has lower temperatures.

weeks at 25-28^{0}C (room temperature in tropical countries) and for two years at -5^{0}C to -10^{0}C (8,9). On the other hand, *Ascaris* eggs are destroyed in a few minutes in compost heaps of manure - which have an internal temperature of 50^{0}C. In general, eggs of *Trichuris trichiura* are less resistant to extremes of temperature than those of *Ascaris lumbricoides*.

Soil

The physical qualities of soil and the natural sorting action of physical forces (wind, landscape and rainfall) determine the survival of helminth eggs in the soil (3,25,42). These same factors also determine where the eggs can be collected in the environment. The eggs of *Ascaris lumbricoides* survive better in clay soil compared to sandy soil (42). The findings on soil examination for helminth eggs provide some information on the extent and intensity of environmental faecal pollution (3,4,5,42). One study done in a focus in Sumatra, Indonesia, found *Ascaris* eggs in 45% of samples of soil collected around nine farmer's houses (4). Several methods are available for detection of *Ascaris* and *Trichuris* eggs in soil and stool (10,19,21,23,33,42).

The intensity of infection with *Trichuris* depends on exposure to *Trichuris trichiura* eggs that are present in soil contaminated with human faecal material (6,7,14,32,35). It is apparent from the studies done in Columbia that the higher the intensity, the greater the chance of the symptomatic or severe

trichuriasis (44).

Chemical Agents

Ascaris eggs are resistant to chemical disinfectants and are able to withstand temporary immersion in strong chemical (42). They are viable and capable of developing after a period of eight months at 6^0 - 34^0C in 2.4% formaldehyde solution but not in a 7.4% formaldehyde solution (8). *Ascaris* and *Trichuris* eggs are killed in five minutes at 15 - 30^0C in solutions containing 100-250 ppm of free Iodine (42). Acidic soil is thought to enhance the development of geohelminths and an alkaline one to retard it (13,42).

More recent studies have been done, especially on dormancy and hatching of nematode eggs (8,30,31). A detailed study was done on the subject of the sequence of events during the hatching process of some parasitic nematodes showed that the trehalose content of perivitelline fluid and the permeability characteristics of the egg-shell influence hatching (30). In addition, reviews of the hatching mechanisms of nematodes and especially of the parasitic plant nematodes have demonstrated details of the hatching mechanisms (30). These findings may be relevant to the hatching of *A. lumbricoides* and *T. trichiura* eggs. The survival of nematode eggs in a dormant state, and the hatching of infective juveniles are interrelated; both require

physiological, behavioral and morphological adaptations by the parasite in order to exploit respective environments (30,35). It has been proposed that hatching is initiated when carbon dioxide has penetrated the lipid layer of the eggs, resulting in a structural change that makes it permeable to trehalose and enzymes (30). In addition, the eggshell and trehalose content of the perivitelline fluid protect the egg against desiccation (30).

Furthermore, enzymes are involved in the hatching of *Ascaris* eggs, knowledge of dormancy and hatching of nematodes is important in order to evaluate possible novel control strategies based on disruption of the normal life cycle process (2,7,42,43,44). There is need to link investigations of the hatching mechanisms, and behavioral responses of unhatched juveniles, to detailed examination of the physiological and biochemical adaptations for survival in a dormant state (30).

In addition, studies have been done on the prevalence of nematode eggs in public places (4,12,21,23,33). A study was done on the prevalence of *Toxocara* species in some public grounds and highway rest areas in Kansas (12). Also, a new technique for the recovery of *Toxocara* eggs from soil has been developed (12). This new technique may become useful in a country like Kenya in the near future.

OBJECTIVES

General Objective.

The objective of this study is to determine the effect of physical and chemical factors on the development of *Ascaris lumbricoides* and *Trichuris trichiura* eggs (most of the experiments were done in the laboratory and some in the environment).An additional objective is to promote more widespread similar studies in the community, especially in Kenya.

The specific objectives were:

1. To determine the effects of physical factors-temperature, soil humidity, aerobic and anaerobic atmospheres - on the development of eggs of *Ascaris lumbricoides* and *Trichuris trichiura*.

2. To determine the effects of chemical agents - acidity, alkalinity, formalin - on the development of eggs of *Ascaris lumbricoides* and *Trichuris trichiura*.

3. To study the development of eggs in the external soil environment.

4. To study the occurrence of eggs in a natural peridomestic *urban environment*

MATERIALS AND METHODS

DESIGN

Location of the study.

The laboratory work was carried out at Kenyatta National Hospital in the Department of Medical Microbiology, University of Nairobi (Parasitology section). This was an experimental study done to determine the effect of the following on the development of eggs of *Ascaris lumbricoides* and *Trichuris trichiura*:

1. Temperature; -4^{0}C, 28^{0}C room temperature and 37^{0}C
2. Humidity - no or very little moisture compared with more plentiful moisture.
3. Soil
4. Chemicals; 0.14% sodium hydroxide (ph=13) 0.14% sulphuric acid (ph=3); and formaldehyde solutions (4% and 10%).
5. The external environment: shaded soil outside the laboratory buildings.

Procedure

The specimens collected were stool and soil. The stool specimens were from the patients routinely examined at the Parasitology Section, Medical Microbiology laboratory at Kenyatta National Hospital. The soil specimens were from two underprivileged housing areas in Nairobi where sanitary standards were inadequate.

Stool.

The stool specimens were re-examined using the Formal Ether concentration method (FEC) by the author and a Senior laboratory Technologist. Those found positive for *Ascaris* and *Trichuris* eggs were kept at 4-8°C, in a refrigerator. Stool specimens were collected daily from Monday to Friday until enough material had been obtained.

The stool specimens with high egg counts for *Ascaris lumbricoides* and/or *Trichuris trichiura* were processed in bulk. First the specimens were emulsified in distilled water using a pestle and mortar. Applicator sticks were used to transfer specimens from the container to the mortar. These were then filtered (using surgical gauze and funnel) into two sedimentation jars which were then left to stand without disturbance for 30-60 minutes. After sedimentation, the supernatant was discarded. Sedimentation was repeated four times. The final sediment obtained was examined microscopically for eggs of *Ascaris lumbricoides and/or Trichuris trichiura* which were identified and recorded.

Experiments with eggs derived from stool.

To determine the effect of temperature and some chemicals on the development of eggs of *Ascaris lumbricoides* and *Trichuris trichiura*, equal aliquots of the stool sediment were put into Bijou bottles using a Pasteur pipette. The chemicals that were added to the bottles were as follows:- 0.14% sodium hydroxide, 0.14% sulphuric acid and 4% and 10% formalin solutions. The experiments were done in multiples of four. The bottles were then incubated at different temperatures: 4°C; 28°C room temperatures and 37°C. 28°C and 37°C were used because incubators set at these temperatures were available in the laboratory. The experiments were performed using these incubators in conjunction with other staff workers; hence their setting could not be changed. A refrigerator was used for 4°C.

To establish the effect of anaerobic conditions on egg development, a layer of liquid paraffin one centimeter in depth was put in half the bottles incubated at 28°C. All the other bottles were made aerobic and presumed to have the same amount of oxygen by loosening the caps of the Bijou bottles. control samples were included in the tests, for example sodium hydroxide solution was omitted in two bottles, which were put in similar conditions as those with 0.14% sodium hydroxide.

To determine the effect of humidity on the development of eggs of *Ascaris lumbricoides* and/or *Trichuris trichiura*, an aliquot from the processed stool was smeared on dry cotton wool and left to dry at room temperature (22-28°C). An equal amount

of aliquot was put in damp cotton wool to maintain humidity. The exact humidity in the different bottles was not measured due to lack of instrumentation.

Initially the specimens were examined weekly from the first to fifth week, but it was later decided to examine on the third, fourth and fifth week because the initial studies showed that egg development did not occur before the third week. It was decided to count and record the development of eggs up to the fifth week because some were expected to have broken up by then and also due to the availability of time.

A similar volume of measured(three drops) of each sample, from the various bottles were put on glass slides, using a Pasteur pipette, covered with a cover slip (22 x 22mm) and examined microscopically for the eggs and their development. Eggs were identified and counted as undeveloped (U), segmented (S) and embryonated (E). Egg viability was demonstrated by induced movements of embryo within the egg under bright light - by increasing the light intensity. All the eggs under each coverslip were counted and recorded.

Soil

The soil specimens were collected from two underprivileged housing areas in Nairobi where sanitary standards were inadequate. These areas were chosen because soil contamination with faecal material occurs in such areas. The soil was collected from the backyards of the houses and from under trees.

The soil was processed after collection using a method recommended by the World Health Organization (42). According to this method, solid soil particles are broken using equal amounts of distilled water and 1% sodium hypochlorite solution after which it is processed in a similar way to a stool sample.

The soil was processed using the zinc sulphate flotation method. Equal amounts of soil were put into Bijou bottles. These were then filled almost to the brim with 33% zinc sulphate solution (freshly prepared), they were then shaken for one minute after which they were completely filled and a coverslip placed on top of each bottle. These were left to stand for 30 minutes. the coverslips were briskly removed vertically and immediately placed on glass slides and examined for eggs of *Ascaris lumbricoides* and *Trichuris trichiura*. These were counted and recorded as undeveloped (U), segmented (S) and embryonated (E).

Development in the external soil environment:

In addition, stool specimens with high egg counts in which almost all the Ascaris and/or Trichuris eggs were undeveloped, were identified. Equal amounts of these were put in polythene bags. Small holes were made in the polythene bags to allow in adequate air. These were put under a tree on a shaded area. The specimens were examined weekly for egg development and were recorded as undeveloped, segmented and embryonated. This was done for a total duration of two months. The experiments were repeated three times.

RESULTS

At the start of experiments, no eggs of *Ascaris lumbricoides* and/or *Trichuris trichiura* were developed. Almost all eggs were of *Ascaris lumbricoides* and *Trichuris trichiura* eggs were few. In most of these experiments, the proportion of eggs developed was highest at week three and thereafter declined in weeks 4 and 5. This effect was noted particularly under alkaline conditions and at a temperature of 37°C.

The experiments done to show the effect of storage on egg viability and development at 4°C, showed no significant effect on later segmentation and embryonation except in one instance (Tables xii).

The results are presented and described under subheadings of temperature, chemicals, humidity and development in the external environment. They are tabulated in Tables I-XII and illustrated in figures IA - 6C. In tables 6 - 10, the figures for the replicates are combined and the proportion developed is given together with chi - square (X^2) analysis.The totals of the results at 37°C are higher because the experiments were repeated three times.

The Chi-square was used to test for statistical independence. Tests of independence may be used to indicate whether or not two random variables are statistically independent. The test of independence will show whether or not relationships exist between the attributes eg. time and egg development. It will not indicate the degree of association or

the direction of the dependency.

To conduct the test, a sample is drawn and the obtained frequencies are presented in a cross-classification contingency table. If the table has rows (R) and columns (C), it is an R x C contingency table. For example, in this case the tables had two rows and three columns. The frequencies in these tables are termed cell frequencies. The totals of the frequencies in each of the rows and columns are termed as marginal frequencies.

The general formula for obtaining the expected cell is:-

$$\text{Expected cell frequency} = \frac{R_i \, C_j}{T}$$

R_i - *Corresponding row frequency*

C_j - Corresponding column total frequency

T - The grand total of all frequencies

The test compares the expected and the observed cell frequencies as follows:-

$$X^2 = \sum_{i=1}^{k} \frac{(oi - ei)}{ei}, \quad k = rc$$

The distribution of the observed X^2 approximates the theoretical X^2 distribution with degrees of freedom = (r - 1) (c - 1). Most of the tables used had 2 degrees of freedom.

oi - Observed frequency

ei - Expected frequency

r - Number of rows

c - Number of columns

Example;

	W3	W4	W5
UN	a	b	c
D	d	e	f

key.

W3 - Week three

W4 - Week four

W5 - Week five

UN - Number of undeveloped eggs

D - Number of developed eggs

Note.

Degree of freedom = (number of column -1) (number of rows-1)

= (3-1) (2-1) = 2x1=2.

A. Experimental laboratory work results.

A.I Temperature.

At 4°C, under different chemical conditions and humidity, neither *Ascaris lumbricoides* nor *Trichuris trichiura* eggs developed. In general, the eggs of *Ascaris lumbricoides* developed better at 28°C. At 28°C plus 0.14% sulphuric acid (pH=3) under aerobic conditions, 78.8%, 88.9% and 89.3% (Tables IIA, IIB and

VIII and figure 2A and 2B) of eggs of *Ascaris lumbricoides* developed (segmented + embryonated) on the third, fourth and fifth weeks of examination respectively. The respective proportions under similar conditions but at 37°C in weeks three, four and five were: 92.4%, 17.0% and 36.0% (Tables VB and XI).

At room temperature, the proportions of eggs that developed (segmented + embryonated) in an alkaline medium in weeks three, four and five were:- 90.6%, 46.6% and 36.2% respectively. At a similar temperature but in an acidic medium the proportions of eggs that developed in weeks three, four and five were: 92.2%, 76.3%, and 60.8% respectively.

Analysis of results by the chi-square method, at a temperature of 28°C plus sulphuric acid, under aerobic conditions with time of examination, showed no significant statistical difference($p > 0.1$, $X^2 = 2.34$). Analysis of results under similar conditions but at 37°C also showed they were of no statistical difference ($p > 0.1$ X^2 3.82).

Chemicals

In general, under similar conditions of temperature and atmosphere (aerobic and anaerobic), the eggs of *Ascaris lumbricoides* developed better in acidic than in alkaline conditions.

At 28°C,under aerobic conditions plus 0.14% sodium hydroxide 77.7%, 76.8% and 84.8% of eggs developed on examination in the third, fourth and fifth weeks respectively (Tables IA and VII). The figures under similar conditions but plus 0.14% sulphuric acid were 78.8%, 88.9% and 89.3% of eggs respectively during the same examination period (Tables IIA, and VIII). The eggs in 0.14% sodium hydroxide solution were noted to have degenerated egg - shells as development proceeded with time.

The eggs of *Ascaris lumbricoides* developed in both 4% and 10% formalin solutions. In 4% formalin solution under aerobic conditions (Table IIIA and IX and figure 3A) 85.2%, 90.2% and 85.3% of eggs developed in the third, fourth and fifth weeks of examination respectively. The results in 10% formalin solution under similar conditions (Table IVA and figure 4A) were 44.6%, 35.1% and 59.0% in the third, fourth and fifth weeks of examination respectively. On analysis of the last mentioned results by the chi-square method ($p < 0.05$ $X^2 = 6.90$), they were of statistical significance.

Aerobic and anaerobic conditions.

In general, under similar conditions, the eggs of *Ascaris lumbricoides* developed better in aerobic than in anaerobic conditions (Tables I-IV and figures 1-4). The results show that in all the conditions tested, development was better aerobically than anaerobically, except in 10% formalin solution.

At 28°C, plus 0.14% sulphuric acid under aerobic conditions 78.8%, 88.9% and 89.3% of eggs developed in weeks three, four and five respectively (Table VIII). The respective figures under similar conditions but in anaerobic atmosphere were, 50.0%, 46.4% and 69.4% (Table VIII). Analysis of the last mentioned results by the chi-square method showed that they were of statistical significance ($p < 0.01$ $X^2 = 7.89$).

Humidity

Four independent experiments on the effect of humidity were done under adequate conditions of moisture, 37(20%) of the total number of 187 eggs of *Ascaris lumbricoides* were noted to have developed (segmentation or embryonation) after five weeks of incubation. There was no egg development noted among several hundred eggs incubated without moisture (dry cotton wool) for the same period.

B. STUDY OF SOIL COLLECTED IN URBAN ENVIRONMENT.

A total of thirty five (35) soil samples were examined for eggs of *Ascaris lumbricoides* . All eggs identified were of *Ascaris Lumbricoides*. No *Trichuris trichiura* eggs were identified. Of the total of 35 samples examined only 8, contained eggs of *Ascaris lumbricoides*. A total of 86 eggs were identified, but only 11 were either segmented or embryonated. One third (33%) of the embryonated eggs were viable and the rest were not viable.

C. Egg development in the external soil environment

A total of 16 eggs developed (segmented + embryonated) in the external soil environment under tree shades. Seven eggs were viable and the rest were not viable. This was out of a total of 95 eggs.

TABLE IA: Number of eggs of *Ascaris lumbricoides* and stage of development at a temperature of 28^0C plus 0.14% NaOH under aerobic conditions.

Week 3				Week 4				Week 5			
U	S	E	TOT	U	S	E	TOT	U	S	E	TOT
4	8	17	29	6	9	12	27	2	4	11	17
6	10	4	20	4	6	8	18	2	10	6	18
5	7	6	18	3	9	10	22	4	6	11	21
7	8	10	25	7	2	10	19	4	7	12	23
22	33	37	92	20	26	40	86	12	27	40	79

U - Number of undeveloped eggs

S - Number of segmented eggs

E - Number of embryonated eggs

TOT - Total number of eggs

TABLE IB: Number of eggs of *Ascaris lumbricoides* and stage of development at a temperature of 28^0C plus 0.14% NaOH under anaerobic conditions.

Week 3				Week 4				Week 5			
U	S	E	TOT	U	S	E	TOT	U	S	E	TOT
9	4	5	18	4	12	2	18	11	1	2	14
8	2	9	19	11	1	2	14	9	6	2	17
6	8	1	15	7	7	1	15	7	6	3	16
7	7	3	17	7	1	0	8	8	7	3	18
30	21	18	69	29	21	5	55	35	20	10	65

U - Number of undeveloped eggs

S - Number of segmented eggs

E - Number of embryonated eggs

TOT - Total number of eggs

TABLE IIA: Number of eggs of *Ascaris lumbricoides* and stage of development at 28^{0}C plus 0.14% sulphuric acid under aerobic conditions.

Week 3				Week 4				Week 5			
U	S	E	TOT	U	S	E	TOT	U	S	E	TOT
6	6	8	20	1	4	9	14	1	1	13	15
1	4	20	25	4	3	12	19	1	0	9	10
4	3	10	17	0	2	10	12	2	2	10	14
6	5	7	18	1	2	6	9	2	1	14	17
17	18	45	80	6	11	37	54	6	4	46	56

U - Number of undeveloped eggs

S - Number of segmented eggs

E - Number of embryonated eggs

TOT - Total number of eggs

TABLE IIB: Number of eggs of *Ascaris lumbricoides* and stage of development at 28^{0}C plus 0.14% sulphuric acid under anaerobic conditions.

Week 3				Week 4				Week 5			
U	S	E	TOT	U	S	E	TOT	U	S	E	TOT
7	6	4	17	7	5	6	18	2	6	5	13
9	3	3	15	14	3	2	19	2	7	5	14
7	6	3	16	9	2	6	17	11	1	6	18
10	4	4	18	7	7	1	15	4	9	4	17
33	19	14	63	37	17	15	69	19	23	20	62

U - Number of undeveloped eggs

S - Number of segmented eggs

E - Number of embryonated eggs

TOT - Total number of eggs

TABLE IIIA: Number of eggs of *Ascaris lumbricoides* and stage of development at a temperature of 28^{0}C plus 4% formalin solution under aerobic conditions.

Week 3				Week 4				Week 5			
U	S	E	TOT	U	S	E	TOT	U	S	E	TOT
2	4	10	16	1	2	10	13	2	1	14	17
4	2	8	14	2	3	11	16	3	5	11	19
1	5	10	16	1	1	17	19	2	2	14	18
2	3	10	15	2	1	10	13	3	0	11	14
9	14	38	61	6	7	48	61	10	8	50	68

U - Number of undeveloped eggs

S - Number of segmented eggs

E - Number of embryonated eggs

TOT - Total number of eggs

TABLE IIIB: Number of eggs of *Ascaris lumbricoides* and stage of development at a temperature of 28^0C plus 4% formalin solution under anaerobic conditions.

Week 3				Week 4				Week 5			
U	S	E	TOT	U	S	E	TOT	U	S	E	TOT
8	2	0	10	6	4	2	12	8	7	2	17
6	7	3	16	5	6	4	15	7	4	3	14
7	5	5	17	7	5	5	17	9	5	2	16
13	7	3	23	11	7	1	19	7	4	7	18
34	21	11	66	29	22	12	63	31	20	14	65

U - Number of undeveloped eggs

S - Number of segmented eggs

E - Number of embryonated eggs

TOT - Total number of eggs

TABLE IVA: Number of eggs of *Ascaris lumbricoides* and stage of development at a temperature of 28°C plus 10% formalin solution under aerobic conditions.

Week 3				Week 4				Week 5			
U	S	E	TOT	U	S	E	TOT	U	S	E	TOT
8	8	0	16	8	0	1	9	10	6	3	19
7	5	1	13	11	4	2	17	8	6	1	15
8	5	1	14	10	4	2	16	3	6	4	13
8	2	3	13	8	4	3	15	4	6	4	14
31	20	5	56	37	12	8	57	25	24	12	61

U - Number of undeveloped eggs

S - Number of segmented eggs

E - Number of embryonated eggs

TOT - Total number of eggs

TABLE IVB: Number of eggs of *Ascaris lumbricoides* and stage of development at a temperature of 28^{0}C plus 10% formalin solution under anaerobic conditions.

Week 3				Week 4				Week 5			
U	S	E	TOT	U	S	E	TOT	U	S	E	TOT
4	6	5	15	11	1	1	13	3	6	4	13
8	7	3	18	8	7	2	17	10	3	3	16
6	4	5	15	10	6	0	16	9	6	2	17
10	5	2	17	12	5	2	19	8	3	2	13
28	22	15	65	41	19	5	65	30	18	11	59

U - Number of undeveloped eggs

S - Number of segmented eggs

E - Number of embryonated eggs

TOT - Total number of eggs

TABLE VA: Number of eggs of *Ascaris lumbricoides* and stage of development at a temperature of 37^0C plus 0.14% sodium hydroxide under aerobic conditions.

Week 3				Week 4				Week 5			
U	S	E	TOT	U	S	E	TOT	U	S	E	TOT
21	30	1	52	20	17	7	44	27	3	1	31
18	19	4	41	35	1	1	37	42	5	4	51
8	29	5	42	38	3	7	48	24	6	3	33
15	18	6	39	33	14	7	54	48	6	5	59
62	96	16	174	126	35	22	185	141	20	13	174

U - Number of undeveloped eggs

S - Number of segmented eggs

E - Number of embryonated eggs

TOT - Total number of eggs

TABLE VB: Number of eggs of *Ascaris lumbricoides* and stage of development at a temperature of 37^0C plus 0.14% sulphuric acid.

Week 3				Week 4				Week 5			
U	S	E	TOT	U	S	E	TOT	U	S	E	TOT
1	42	1	44	60	10	2	72	60	6	0	66
3	39	12	54	36	6	0	42	38	10	3	51
6	33	0	39	42	12	0	54	42	5	1	48
3	30	0	33	28	3	1	32	20	9	2	31
13	144	14	171	166	31	3	200	160	30	6	196

U - Number of undeveloped eggs

S - Number of segmented eggs

E - Number of embryonated eggs

TOT - Total number of eggs

TABLE VC: Number of eggs of *Ascaris lumbricoides* and stage of development at a temperature of 37^0C plus 4% formalin solution.

Week 3				Week 4				Week 5			
U	S	E	TOT	U	S	E	TOT	U	S	E	TOT
9	35	1	45	45	5	1	50	48	8	1	57
28	1	0	29	48	11	1	50	45	4	2	51
38	8	2	46	35	1	0	36	33	4	2	39
21	5	1	27	31	2	2	35	20	1	2	23
94	49	4	147	158	19	4	181	146	17	7	170

U - Number of undeveloped eggs

S - Number of segmented eggs

E - Number of embryonated eggs

TOT - Total number of eggs

TABLE VIA: Number of eggs of *Ascaris lumbricoides* and stage of development at room temperature plus 0.14% sulphuric acid.

Week 3				Week 4				Week 5			
U	S	E	TOT	U	S	E	TOT	U	S	E	TOT
3	6	19	28	6	12	7	25	8	13	9	30
3	7	21	31	5	15	10	30	5	16	4	25
2	8	19	29	10	14	10	34	12	8	3	23
1	5	22	28	5	9	7	21	15	4	5	24
9	26	81	116	26	50	34	110	40	41	21	102

U - Number of undeveloped eggs

S - Number of segmented eggs

E - Number of embryonated eggs

TOT - Total number of eggs

TABLE VIB: Number of eggs of *Ascaris lumbricoides* and stage of development at room temperature plus 0.14% sodium hydroxide.

Week 3				Week 4				Week 5			
U	S	E	TOT	U	S	E	TOT	U	S	E	TOT
2	1	19	22	13	6	5	24	16	3	2	21
0	2	24	26	6	18	2	26	20	7	1	28
5	3	20	28	19	7	4	30	10	8	5	23
2	8	10	20	17	4	2	23	19	9	2	30
9	14	73	96	55	35	13	103	65	27	10	102

U - Number of undeveloped eggs

S - Number of segmented eggs

E - Number of embryonated eggs

TOT - Total number of eggs

TABLE VIC: Number of eggs of *Ascaris lumbricoides* and stage of development at room temperature plus 4% formalin solution.

Week 3				Week 4				Week 5			
U	S	E	TOT	U	S	E	TOT	U	S	E	TOT
3	1	21	25	3	7	11	21	16	9	1	26
0	0	19	19	2	16	5	23	14	7	2	23
5	4	20	29	11	4	7	22	23	5	0	28
0	1	23	24	5	13	5	23	19	4	1	24
8	6	83	97	21	40	28	89	72	25	4	101

U - Number of undeveloped eggs

S - Number of segmented eggs

E - Number of embryonated eggs

TOT - Total number of eggs

TABLE VID: Number of eggs of *Ascaris lumbricoides* and stage of development at room temperature plus 10% formalin solution.

Week 3				Week 4				Week 5			
U	S	E	TOT	U	S	E	TOT	U	S	E	TOT
3	1	21	25	3	7	11	21	16	9	1	26
0	0	19	19	2	16	5	23	14	7	2	23
5	4	20	29	11	4	7	22	23	5	0	28
0	1	23	24	5	13	5	23	19	4	1	24
8	6	83	97	21	40	28	89	72	25	4	101

U - Number of undeveloped eggs

S - Number of segmented eggs

E - Number of embryonated eggs

TOT - Total number of eggs

VIII: Number of eggs (totals) and their stage of development with time at 28°C plus 0.14% sulphuric acid (pH=3) under aerobic and anaerobic conditions.The proportions and chi-squares are also tabulated.

tion PROP	Week 3 D	TOT	Prop.	Week 4 D	TOT	Prop.	Week 5 D	TOT	Prop.	X^2
28 C under bic	63	80	78.8%	48	54	88.9%	50	56	89.3%	3.82
as e but r robic	33	66	50.0%	22	69	46.4%	43	62	69.4%	7.89

- Total number of eggs
- Number of developed eggs
- Chi-square with 2 degrees of freedom

IX: Number of eggs (totals) and their stage of development with time at 28°C plus 4% formalin solution under aerobic and anaerobic conditions. The proportions and chi-squares are also tabulated.

tion PROP	Week 3 D	TOT	Prop.	Week 4 D	TOT	Prop.	Week 5 D	TOT	Prop.	X^2
28°C ormalin bic	32	61	85.2%	55	61	90.2%	58	68	85.3%	0.87
as e but obic	32	66	49.2%	34	63	54.0%	34	65	52.3%	0.30

- Total number of eggs
- Number of developed eggs

Chi square with 2 degrees of freedom

Number of eggs (totals) and their stage of development with time at 28°C plus 10% formalin solution under aerobic and anaerobic conditions. The proportions and chi-squares are also tabulated.

tion PROP	Week 3 D	TOT	Prop.	Week 4 D	TOT	Prop.	Week 5 D	TOT	Prop.	X^2
28 C alin ic as e but r robic	25	56	44.6%	28	57	35.1%	36	61	59.0%	6.90
	37	65	56.9%	24	65	36.9%	29	59	49.2%	5.30

- Total number of eggs
- Number of developed eggs
- chi square with 2 degrees of freedom

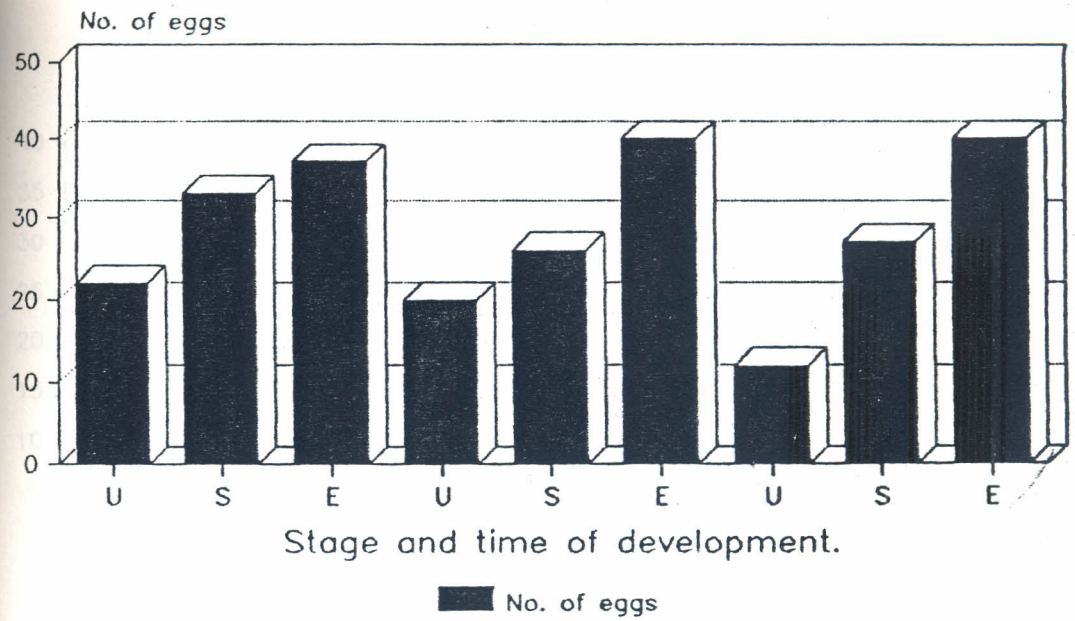
XI: Number of eggs (totals) and their stage of development with time at 37°C plus 0.14% sodium hydroxide (pH=13), 0.14% sulphuric acid (pH=3) and 4% formalin solution under aerobic conditions.The proportions and chi-squares are also tabulated.

	Week 3			Week 4			Week 5			
	D	TOT	Prop.	D	TOT	Prop.	D	TOT	Prop.	X^2
bic	112	174	64.4%	57	183	31.1%	33	174	18.9%	8.17
lar	158	171	92.4%	34	200	17.0%	36	196	36.0%	27.7
plus huric										
lar bove plus alin	53	147	36.0%	23	181	12.7%	24	170	14.1%	3.3

- Total number of eggs
- Number of developed eggs
- chi square with 2 degrees of freedom

Histogram showing egg development with time at 28°C plus 0.14% sodium hydroxide under aerobic conditions.

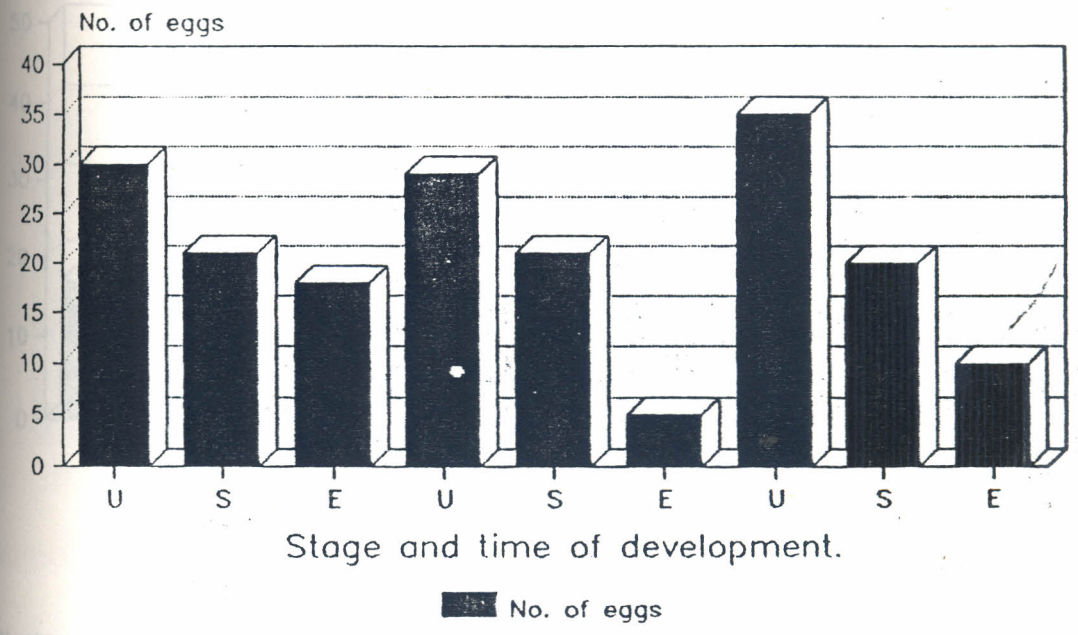
FIGURE 1A



U=Number of undeveloped eggs.
S=Number of segmented eggs.
E=Number of embryonated eggs.

1B: Histogram showing egg development with time at 28°C plus 0.14% sodium hydroxide under anaerobic conditions.

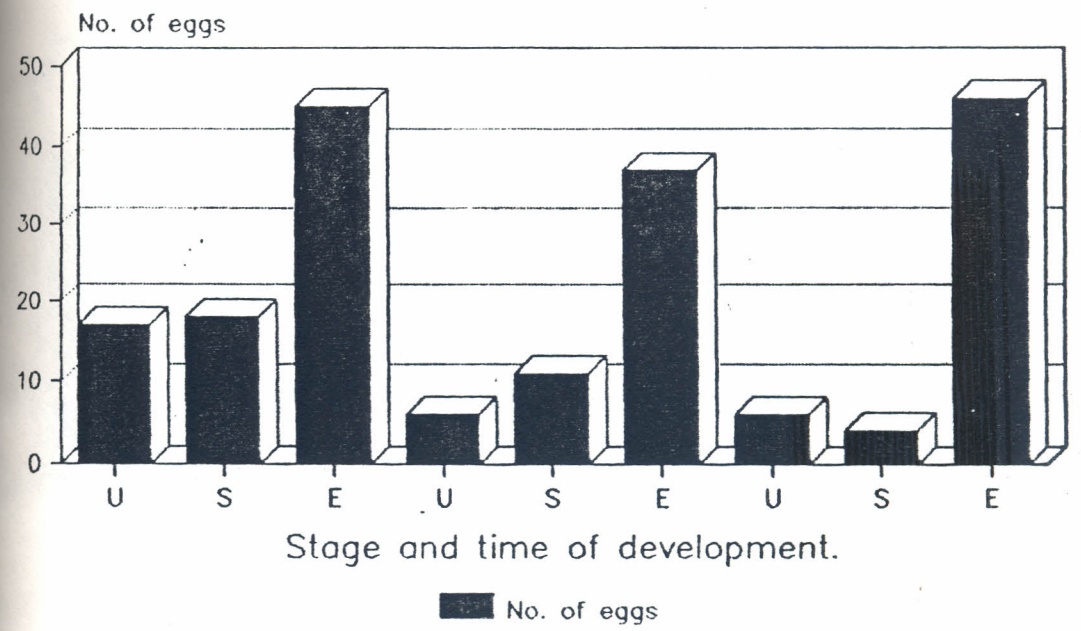
FIGURE 1B



U=Number of undeveloped eggs.
S=Number of segmented eggs.
E=Number of embryonated eggs.

E 2A Histogram showing egg development with time at 28°C plus 0.14% sulphuric acid under aerobic conditions.

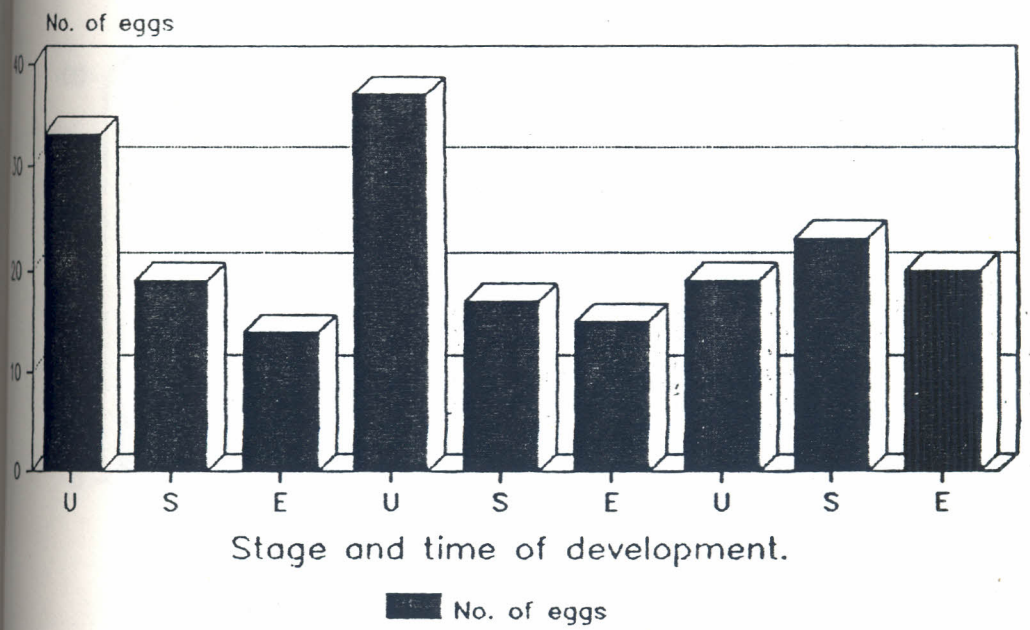
FIGURE 2A



U=Number of undeveloped eggs.
S=Number of segmented eggs.
E=Number of embryonated eggs.

B: Histogram showing egg development with time at 28°C plus 0.14% sulphuric acid under anaerobic conditions.

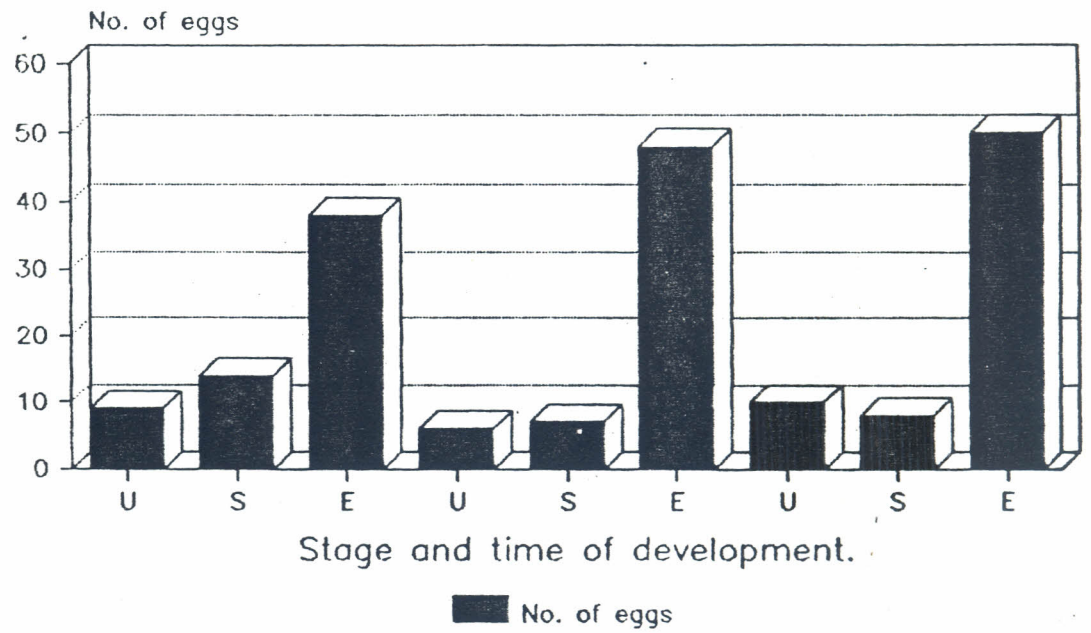
FIGURE 2B



Number of undeveloped eggs.
Number of segmented eggs.
Number of embryonated eggs.

E 3A: Histogram showing egg development with time at 28°C plus 4% formalin solution under aerobic conditions.

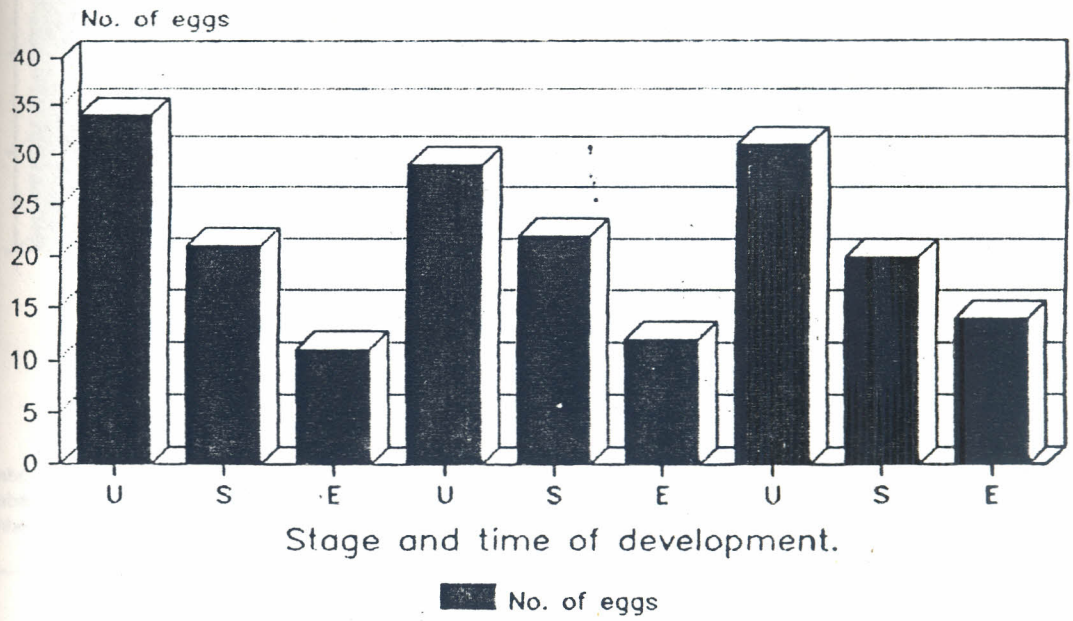
FIGURE 3A



U=Number of undeveloped eggs.
S=Number of segmented eggs.
E=Number of embryonated eggs.

E 3B: Histogram showing egg development at 28°C plus 4% formalin solution under anaerobic conditions.

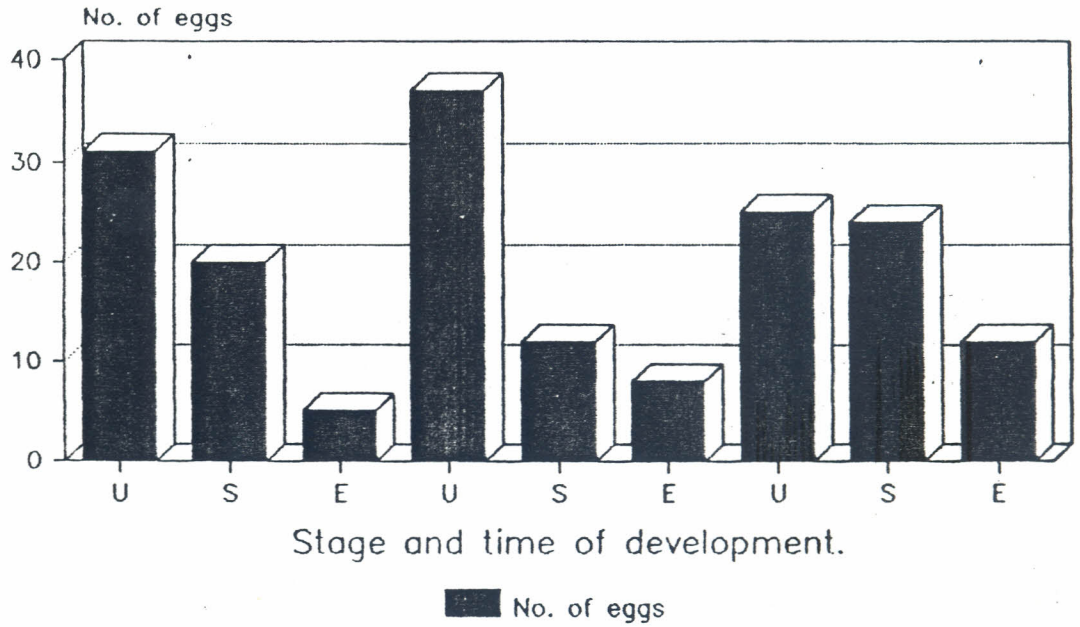
FIGURE 3B



U=Number of undeveloped eggs.
S=Number of segmented eggs.
E=Number of embryonated eggs.

4A: Histogram showing egg development with time at 28°C plus 10% formalin solution under aerobic conditions.

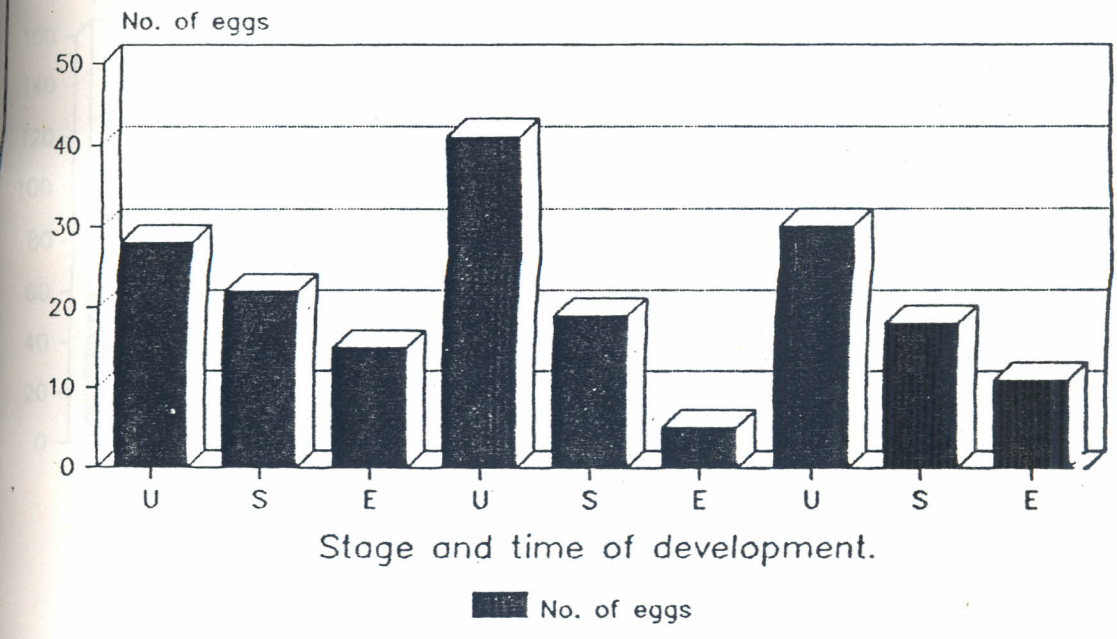
FIGURE 4A



U=Number of undeveloped eggs.
S=Number of segmented eggs.
E=Number of embryonated eggs.

4B: Histogram showing egg development with time at 28°C plus 10% formalin solution under anaerobic conditions.

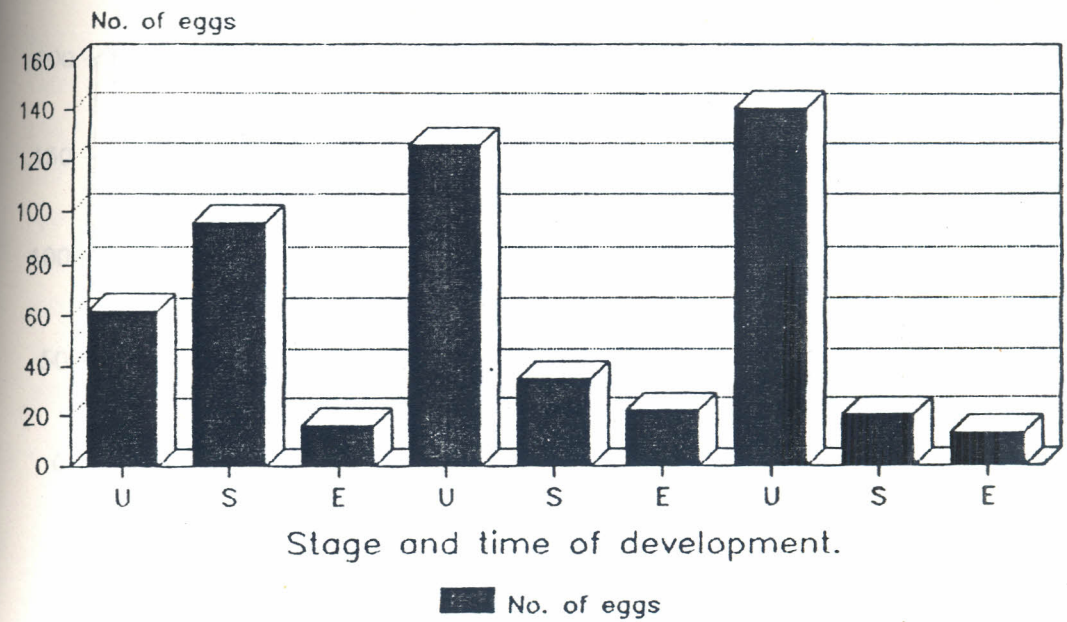
FIGURE 4B



U=Number of undeveloped eggs.
S=Number of segmented eggs.
E=Number of embryonated eggs.

5A: Histogram showing egg development with time at 37°C plus 0.14% sodium hydroxide solution under aerobic conditions.

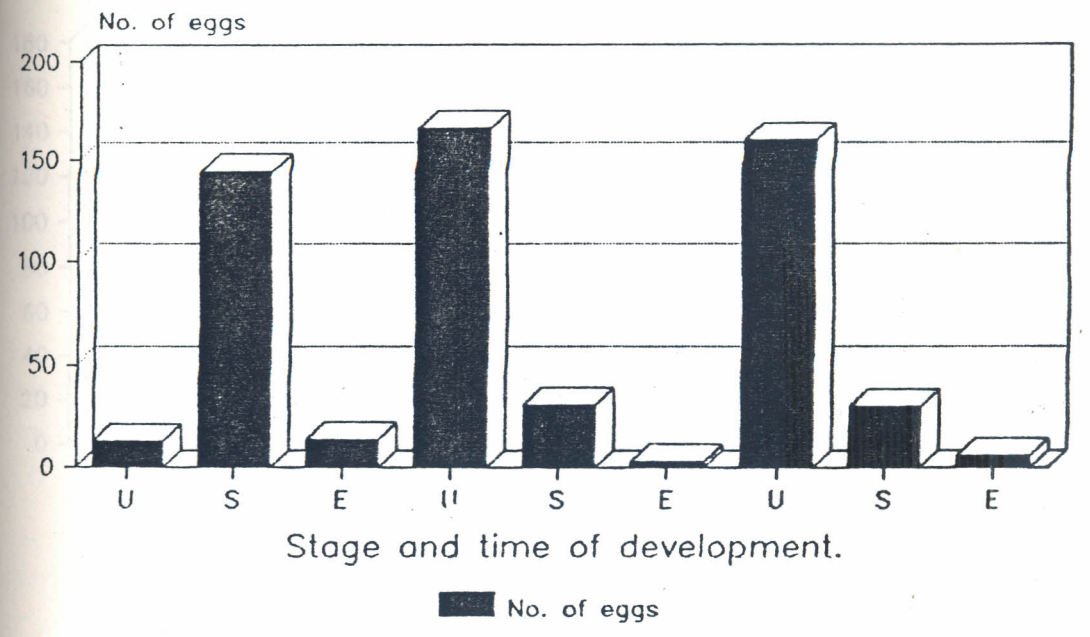
FIGURE 5A



U=Number of undeveloped eggs.
S=Number of segmented eggs.
E=Number of embryonated eggs.

5B: Histogram showing egg development with time at 37°C plus 0.14% sulphuric acid under aerobic conditions.

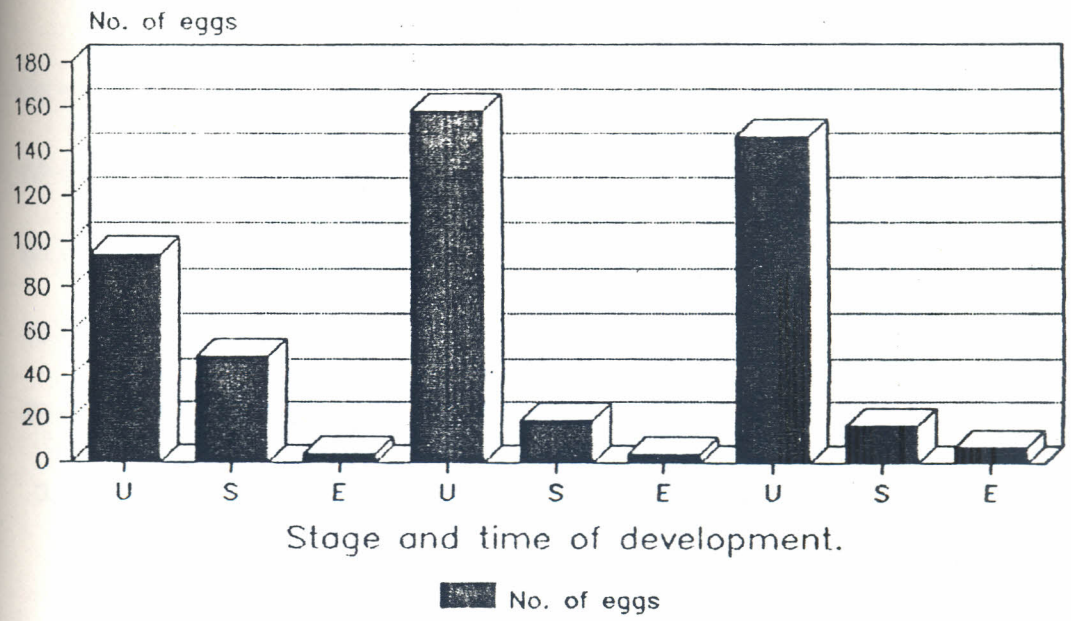
FIGURE 5B



U=Number of undeveloped eggs.
S=Number of segmented eggs.
E=Number of embryonated eggs.

5C: Histogram showing egg development with time at 37°C plus 4% formalin solution under aerobic conditions.

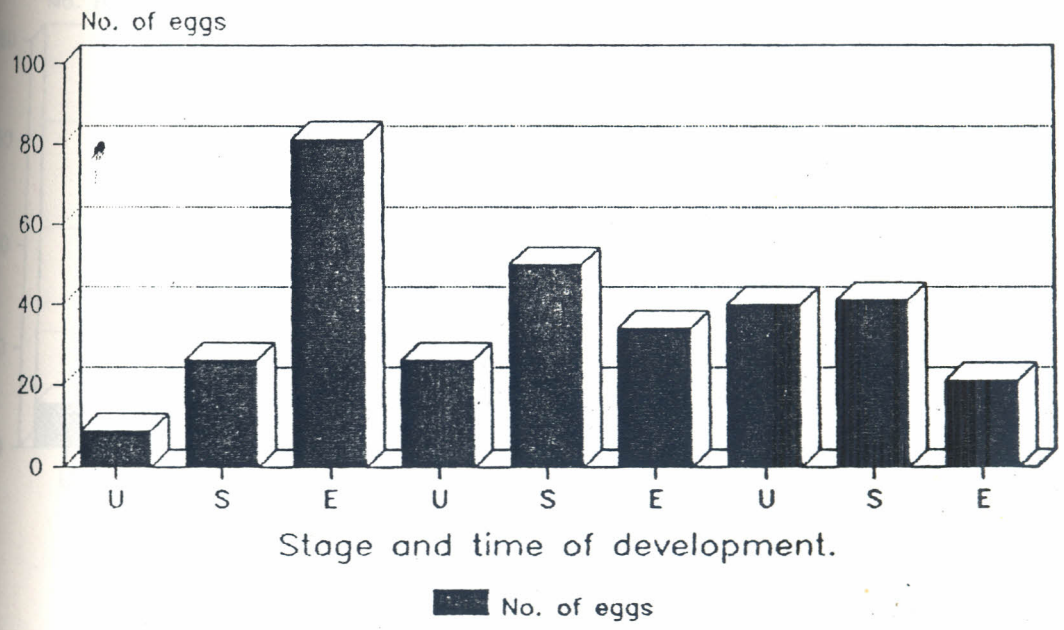
FIGURE 5C



U=Number of undeveloped eggs.
S=Number of segmented eggs.
E=Number of embryonated eggs.

6A: Histogram showing egg development at room temperature plus 0.14% sulphuric acid.

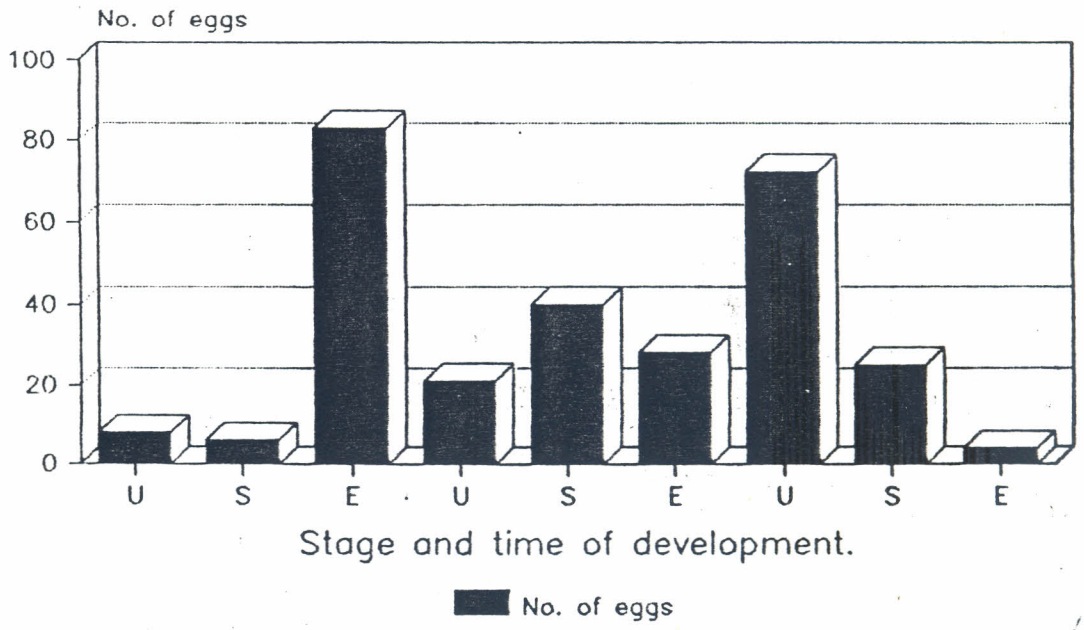
FIGURE 6A



U=Number of undeveloped eggs.
S=Number of segmented eggs.
E=Number of embryonated eggs.

RE 6C: Histogram showing egg development at room temperature plus 4% formalin solution.

FIGURE 6C



U=Number of undeveloped eggs.
S=Number of segmented eggs.
E=Number of embryonated eggs.

DISCUSSION

A number of studies have been done on the effects of environmental factors on the development of eggs of *Ascaris lumbricoides* and *Trichuris trichiura*. Brown in 1927 and Cabrera in 1981 in studies on the rate of development and viability of *Ascaris lumbricoides* and *Trichuris trichiura* under field conditions found that the respective eggs can survive desiccation for two to three weeks in temperatures of 25 - 28^{0}C (8,9). It is also evident from studies done that the physical and chemical properties of soil determine the survival of helminthic eggs (2,25,).

The effects of temperatures of 4^{0}c, 28^{0}C, room temperature and 37^{0}C were studied but not the other temperature ranges. This is a major limitation because little is known about the other temperature ranges. As it has been stated, it was not possible to study the effects of these ranges because the incubators were used in conjunction with the other workers. Results of the temperatures tested show that eggs of *Ascaris lumbricoides* developed best at a temperature of 28^{0}C, less well at 37^{0}C and did not develop at 4^{0}C under similar conditions. Studies done elsewhere showed that *Ascaris lumbricoides* eggs development was inhibited by a low temperature (8). It is also apparent from the results that *Ascaris lumbricoides* egg development (segmentation and embryonation) was better at room temperature

than at 37^{0}C under similar conditions.

The results on the effects of chemicals on the development *Ascaris lumbricoides* eggs show that under similar conditions they develop better in acidic than in an alkaline conditions. These concentrations of chemicals were used due to the fact that a similar concentration of sulphuric acid is used in the laboratory as an embryonation fluid and also 4% formalin solution is commonly used in the Parasitology laboratory where the study was done. It is an omission not to have included other concentrations of similar chemicals. A study by Brown in 1927, showed that *Ascaris* eggs were viable and capable of developing after a period of 8 months in a 2.4% formalin solution but not in a 7.4% formalin solution (8). The results show that *Ascaris lumbricoides* eggs developed in 10% formalin solution. This finding is in disagreement with that of a study done elsewhere (8).

Experiments to test the viability of the eggs was not done because it was not possible to culture the eggs which is the ideal method to demonstrate viability (24,42). It was evident from the findings of this study that storage of eggs at 4^{0}C, had no significant effect on later segmentation and embryonation. Egg viability was demonstrated by induced movements within the egg under bright light.

In general, under similar conditions, the eggs of *Ascaris lumbricoides* developed better in aerobic than in anaerobic conditions (31). *Ascaris lumbricoides* eggs developed

egmentation or embryonation) after five weeks of incubation in equate moisture, but they did not develop without moisture. ese findings were of statistical significance. Unfortunately e exact humidity was not measured due to lack of a hydrometer.

The results of the study of the soil collected in an urban eridomestic environment showed only *Ascaris* eggs. No *Trichuris richiura* eggs were identified. The lack of *Trichuris trichiura* ggs may be due to their inability to survive in the external nvironment after a period of time or the population living in hese areas did not harbour this particular infection. Published tudies done show that eggs of *Trichuris trichiura* are less esistant to extremes of temperature than those of *Ascaris lumbricoides* (5). Most of the soil samples collected were loam soil. A study by Storey and Phillips in 1985, showed that eggs of *Ascaris lumbricoides* survive better in clay soil compared to sandy soil (35).

CONCLUSIONS

1. *Ascaris lumbricoides* eggs develop best at a temperature of 28^0C. They did not develop at 4^0C.

2. *Ascaris lumbricoides* eggs develop better in acidic conditions compared to alkaline conditions.

3. *Ascaris lumbricoides* eggs develop better in aerobic than in anaerobic conditions.

4. *Ascaris lumbricoides* eggs developed in both 4% and 10% formalin solutions.

5. Eggs of *Ascaris lumbricoides* did not develop without moisture.

6. Eggs of *Ascaris lumbricoides* developed in the external environment (outside the parasitology laboratory).

N/B Only a few Trichuris trichiura eggs were identified hence these are not included in the conclusions.

RECOMMENDATIONS AND STUDY IMPLICATIONS

This study has attempted to highlight the effects of physical and chemical factors on the development of eggs of *Ascaris* and *Trichuris*. These factors play a significant role in the environment where the eggs of the mentioned parasites develop. It is therefore recommended that:-

1. Studies should be done of egg development and survival in the environment (field conditions) in different parts of Kenya to determine the effects of soil, temperature, humidity, rainfall, sewage treatment and chemical factors such as acidity and alkalinity on the development of the eggs of *Ascaris lumbricoides* and/or *Trichuris trichiura*.

2. Future epidemiological studies of *Ascaris lumbricoides* and *Trichuris trichiura* in Kenya should include detailed measurement of chemical and physical conditions of the soil where eggs are developing.

3. More studies need to be done on the effect of formaline on the development of *Ascaris lumbricoides* and/or *Trichuris trichiura* eggs as it is evident in this study that they developed in both 4% and 10% formaline solutions. There may

be a need to use higher concentration (>10%) of formaline or an alternative chemical agent to kill eggs of Ascaris lumbricoides in preserved specimens.

CONSTRAINTS.

One of the major problems was the availability of incubators. Those in use are also used by workers in the laboratory for various experiments or routine tests. The incubators had to be used in conjunction with others. This made it impossible for the investigator to test the effect of other temperatures (except 4^0C, 28^0C and 37^0C) on the development of *Ascaris* and *Trichuris* eggs. Ideally, the effect of temperature on the development of the mentioned eggs should be tested in a series of incubators set at a temperature difference of 3^0C between one incubator and the next.

Another major problem was lack of a hydrometer hence the exact humidity in the experiments to determine its effect were not measured. A bacteriological anaerobic jar would have provided and maintained anaerobic conditions more reliably. Unfortunately this equipment was not available.

Problems were encountered during specimen collection. There are specific boxes labelled Monday-Friday in which examined stool specimens should be kept, but these were at times kept in the wrong box(es). Sometimes all the specimens were kept in the same box irrespective of the day of examination. This made specimen collection difficult.At times the specimens and results of the examination were not recorded in the book for this purpose. One had to look for the request forms to find out if there were any positive specimens for Ascaris and *Trichuris* on that particular day(s).

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APPENDIX I

1. Approximate stool-sample bean or pea size - 1gm
2. In a mortar using a pestle emulsify in 7mls. of 4% Formal - saline.
3. Filter through 4 layers of surgical gauze using a funnel.
4. Balance and add 3 mls of anaesthetic ether, shake vigourously for 1 minute.
5. Centrifuge for a period of 3 minutes at 2,000 r.p.m..
6. Loosen fatty debris at the junction of the liquids with an applicator stick.
7. Pour away the whole of the supernatant together with the debris.
8. Mix the small deposit with the remaining drop of 4% Formal - saline.
9. Extract drop with pipette - and place on a slide and coverslip with size 22 x 22mm or 18 x 18mm.
10. Examine for protozoan cysts and all helminthic eggs.

APPENDIX II.

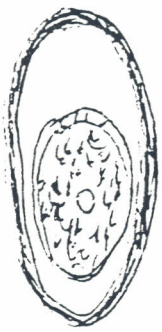
Zinc Sulphate Floatation technique.

1. Emulsify a portion of stool in a 33% zinc sulphate solution thoroughly.
2. Pour through a 60-mesh sieve into a Bijou bottle, fill to the brim. Put a 22 x 22mm coverslip on top of the bottle.
3. Leave to stand for 10 - 30 minutes.
4. Remove the coverslip and immediately put on a glass slide without turning it (coverslip).
5. Examine for all helminthic eggs.

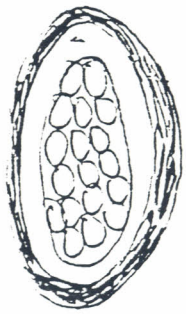
APPENDIX III

III: Developmental stages of eggs of *Ascaris lumbricoides* and *Trichuris trichiura*

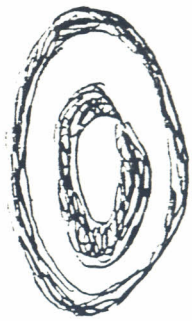
Eggs of Ascaris lumbricoides



Undeveloped eggs

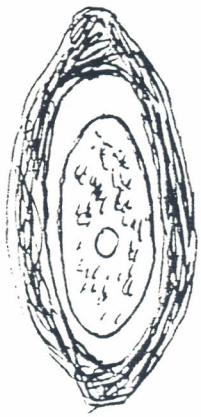


Segmented egg

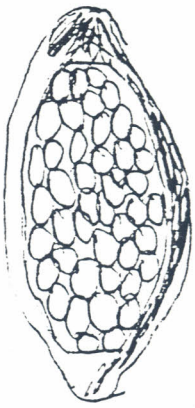


Embryonated egg

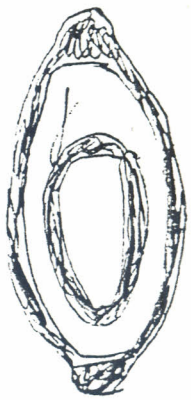
Eggs of Trichuris trichiura



Undeveloped egg



Segmented egg



Embryonated egg

APPENDIX IV

ams: "MEDSUP", Nairobi
hone: Nairobi 726300
replying please quote
No. .....
and date

KENYATTA NATIONAL HOSPITAL
P.O. Box 20723
NAIROBI
27th August, 1990

Dr. E. I. Odera,
Department of Human Pathology,
College of Health Sciences,
NAIROBI.

Dear Dr. Odera,

RE: THE EFFECTS OF VARIOUS PHYSICAL AND CHEMICAL FACTORS IN THE DEVELOPMENT OF EGGS OF ASCARIS LUMBRICOIDES AND TRICHUR TRICHURA (AN IN VITRO STUDY)

Your above research proposal was discussed by the Ethical and Research Committee and it was noted that this is a good proposa about a very important subject. The background information is well researched and the objectives and hypothesis are well thought out. I am pleased to inform you that clearance has been granted for you to embark on your research. However, you should let me have your response in the next fortnight, on a few disturbing things

(i) What do you mean by "the ethical consideration will be obtained"?

(ii) Give more information on how data will be analysed by the computer. What sort of data will be generated, how will it be analysed?

(iii) The budget is too brief. What kind of reagents will be used and at what rate?

Yours sincerely,

M. S. Riyat
Secretary - Ethical & Research Committee

C.C. Dr. F. E. Onyango - Chairman
Deputy Director - KNH
Mrs. S. Ikui - Senior Medical Records Officer
Prof. Knight - Department of Medical Microbiology (Supervisor)