STUDIES ON THE ASCARIS-BACTERIA RELATIONSHIP IN MAN AND PIGLETS

BY

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ABSTRACT

The paucity of knowledge of the inter-relationship of bacteria and *Ascaris* stimulated this study. Very little was known about the sources of *Ascaris* infection in man in Ibadan. Investigations conducted on the action of intestinal bacteria on the development of *Ascaris* eggs to the infective stages involved the growing of fertile eggs of *Ascaris lumbricoides* and *Ascaris suum* in diluted and undiluted overnight broth cultures of *Escherichia coli*, *Proteus mirabilis*, *Streptococcus faecalis* (Enterococci), *Bacillus subtilis*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Clostridium welchii* and also in sterile nutrient broth. The bacterial species used in the experiments inhibited the cleavage and development of both the human and porcine *Ascaris* eggs beyond 2-cell stage. The ovostatic action of the bacterial species on the eggs was found out to be related to respiratory processes of the actively growing and multiplying bacteria which consumed all the available

oxygen from the environment of the eggs. After the removal of bacteria from cultures, the eggs developed very well.

The bacterial flora of Ascaris suum and its relationship to the host flora was also investigated. The body surface of the adult worms was cultured on selective media. The different parts of the adult worms gut were cultured for isolation of micro-organisms. The faeces collected from pigs were also cultured. E. coli, Streptococcus faecalis, Staphylococcus aureus, Staphylococcus albus, Proteus vulgaris, Proteus mirabilis, Pseudomonas aeruginosa, Lactobacillus acidophilus, Bacillus subtilis and Candida albicans were isolated from the cultures but no anaerobic organism was isolated. The faeces cultures yielded the same genera of bacteria as in Ascaris suum adult worms, but Clostridium welchii, an anaerobic organism was isolated from the faeces. The results showed that adult Ascaris suum can act as a vehicle

of bacterial infections in ascariasis where the adult worm is active and migratory. In this case the pig bacteria which are limited in its ability to penetrate the intact epithelium of the animal can often be deposited on internal tissue by the migrating worms where untold problems could be set up.

The effect of intestinal flora on the establishment, development and pathogenicity of Ascaris suum larvae in piglets was also investigated.

The results have shown that development of Ascaris suum larvae to adult worms took place in the presence of a normal intestinal flora in piglets. Furthermore, the results have shown that the two agents (Ascaris suum larvae and bacterial species) worked together to produce a disease condition more severe than the sum total of effect produced by either the worm or the bacteria independently. Finally, investigations were conducted to find out sources of infection with human ascariasis in Ibadan. The results

have shown that common food items like fruits, vegetables, gari and palm-wine are contaminated by *Ascaris* eggs and therefore could serve as sources of *Ascaris* infection to those people who eat these food items raw or uncooked. It was observed that the *Ascaris* infection could occur through contaminated fingers of egg-passers, through dust and through the activity of flies. *Ascaris* eggs were found on edible vegetables and fruits and this observation emphasises the need for strict observation of simple hygiene methods aimed at eliminating the *Ascaris* eggs before consumption of the uncooked food items.

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CERTIFICATION BY SUPERVISORS

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D E D I C A T I O N

To my late Father and Mother

Pa AKANBI ADEDEJI

and

Madam ADEOTI ANIKE ADEDEJI

for their contributions to my education.

MAY THEIR SOULS REST IN PERFECT PEACE.

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TABLE OF CONTENTS

APPENDIX

APPENDIX

LIST OF FIGURES

3.4 FIGURE

3.4 FIGURE

3.4 FIGURE

3.4 FIGURE

LIST OF TABLES

3.2 TABLE

3.3 TABLE

3.3 TABLE

3.4 TABLE

3.4 TABLE

3.5 TABLE

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INTRODUCTION

1.1 General

Microbial inter-relationships are involved essentially in the aetiology, pathogenesis, and pathology of intestinal infection of protozoa and metazoa (Philips, *et al* 1958). The theory that bacteria may participate in the aetiology of amoebic dysentery was offered first by Losch in 1875, and has been adhered to by other investigators including Councilman & Lafleur (1891), Walker & Sellards (1913), Cleveland & Saunders (1930) and Westphal (1937). However, strong clinical evidence in support of the theory was not forthcoming until 1946 at which time Ellenberg presented data which suggested that symbiosis with bacteria may be essential to amoebic pathogenicity.

Studies on the interaction of host microflora and metazoan or protozoan parasites became practical with development of gnotobiotic techniques (Reynier, 1959). In gnotobiotic animals infected with monoaultures of bacteria the development was much worse (Philips et al 1955, Przyjalkowski, 1969, Chang & Wescott, 1972, Hungate, 1972, Johnson & Reid, 1973), while in germfree animals the parasite either showed no development or they developed to a very low degree (Philips et al 1955, Wescott, 1970, Przyjalkowski, 1973a).

However, the nature of the influence of the accompanying intestinal flora on the development and pathogenicity of parasites in animals is still not clear. Possibly nutritional factors come here into play, but according to Stefanski (1965), a host organism plays, with regard to its parasite, the same role as the external environment plays for each free-living organism. A permanent exchange takes place

between the milieu of the parasite and the external environment and from the parasitological viewpoint the exchange resulting from the question of food is most important. However, the alimentary tract mostly involved in this exchange, maintains some stable features such as temperature, pH, and Oxygen pressure. This refers mainly to the paramucosal environment in which among others, the oxygen pressure is greater. A definite bacteria flora is present in the alimentary tract and it only undergoes changes as a result of disease conditions, changes in diet and administration of some medicines, in particular antibiotics. Each segment of the alimentary tract has its own characteristic bacterial flora and the bacteria play an important role in meeting the demands for certain vitamins such as vitamins B, E and K groups which are usually produced by commensals of the intestinal tract and are subsequently absorbed into the blood and utilized by the host.

Both the aerobic, microaerophilic and anaerobic forms of microflora are seen in the alimentary canal of man and domesticated animal. The coliform organisms predominate amongst aerobic commensal bacteria, but Lactobacillus species excel amongst the microaerophilic. Clostridium perfringens (Clostridium welchii) are, however, in the lead amongst the anaerobic ones (Cruickshank, 1965).

Microorganisms alone do not have the exclusive ability to colonise human and animal body, but large organisms as well including members of the group Helminths and Arthropoda commonly invade and colonise various tissues of man and animal. The nematodes are especially an important group because many of their members have successfully invaded and established themselves in almost all the tissues of man. These nematodes are inside the human body and after a period of development, they settle down in various parts of the body including the alimentary tract to which they have adjusted physiologically and morphologically.

Although both the micro-organisms and the larger organisms are individual entities and they are capable of independent existence, they all inhabit a common environment of the gut and are subjected at times to similar circumstances. Some of them may have entered into relationships which are mutually beneficial, others may be antagonistic and still others may just have an accidental association and are just occuring as transport agent. Hutchinson (1965) established a relationship between Neoascaris egg and Toxoplasma transmission in which Toxoplasma organisms were incorporated into the egg of Neoascaris worm inhabiting the intestinal lumen of infected individuals. The excited Ascaris lumbricoides has often been accused of being a passive agent of contamination of different tissues of the body with pathogenic bacteria from intestinal tract. It will be also informative to ascertain to what extent the digestive system of Ascaris lumbricoides and Ascaris suum are invaded by

bacteria. For instance, Markell & Kuritsubo (1967) recorded that the trophozoites of Entamoeba coli do ingest Giardia intestinalis and thereby assist in keeping down on their population in the intestinal lumen.

1.2 Sources and consequences of Ascaris infections in man and piglets

Gastrointestinal infections of man and domesticated animals are still of paramount public health importance in all parts of the world in spite of the wide use of drugs. The infective agents of gastrointestinal tract include viruses, bacteria, fungi, protozoa and helminth (Banestiva, 1971). In the tropical and sub-tropical regions of the world where inadequate personal hygiene and environmental sanitation combine with the favourable weather conditions to enhance the growth and development of infecting agents, the incidence of these infections

is maximised. The parasite development is enhanced because of the favourable weather conditions all the year round. The sources of infection with Ascaris are generally warm, moist soil, vegetables especially leafy vegetables, root vegetables, fruit vegetables and water where the eggs flourish well. Ascariasis abound among the indegenous populations in many parts of the world where personal hygiene and environmental sanitation combine to favour embryonation of eggs on the polluted soil in the environments.

Intestinal nematodes abound in the rural and urban environment in this country because our tropical climate offers excellent opportunities for easy and rapid development of different stages of these parasites which are readily disseminated in our soil through our gross and indiscriminate defaecation habits. The Ibadan city presents an ideal environment for the study of the prevalence and problems of intestinal nematodes of man and

animal in Nigeria because of the low standard of public sanitation (Cowper & Woodward, 1961; Ogunba, 1974). Furthermore, because of lack of necessary health education resulting in our low standard of personal and environmental sanitation, the Ibadan population provides opportunities for constant contact between ourselves and infective stages of the worms. Some authors (Cowper & Woodward, 1961, and Ogunba, 1974) showed that infectivity with the trio Ascaris lumbricoides, Hookworm and Trichuris trichiura worms was always common amongst the Ibadan population. They also showed that in an environment which is highly polluted, opportunity for infection with one nematode opens the gate to several others at the same time. Furthermore, Ogunba, (1974) showed that the infectivity with intestinal nematodes varied in the ten zones of Ibadan that he studied which had varying degrees of sanitation. Infectivity was heaviest in zones with the lowest degree of environmental sanitation.

Ascaris infection has varied effects on man and animals. Therefore the consequences of *Ascaris* infection in man and piglets are great. Both man and animals living in areas of low degree of evironmental sanitation are staunted, anaemic and generally in poor health. It is a well known fact that adult *Ascaris* may wander up and down the intestine and into other organs outside their usual route. They may thus enter the stomach and may be vomitted, or they may enter the bile-ducts and occlude these, causing jaundice and other problems. Other symptoms may be caused by their migration elsewhere. There is in fact hardly any organ of the body of man and pig in which *Ascaris* has not been found. For instance, Beautyman & Woolf (1951) found *Ascaris* larva in the brain of a child. These wandering ascarids reaching abnormal foci provoke acute symptoms. Cerebral symptoms may occur in children infected with *Ascaris* in the absence of other helminthiasis or malaria. The most

common symptoms attendant on the presence of Ascaris in the intestine are vague abdominal discomfort and acute pains in the epigastric region, (Swartzwelder, 1946). Ascaris may possibly suck blood from the intestinal wall (Brown, 1934). In children, the presence and activity of worms are characteristically associated with fever. At other times, the symptoms may suggest abdominal tumour, gastric or duodenal ulcer. In the pigs, there is marked unthriftiness which may be so severe that the animals die; where they do not die, they have a very reduced market value.

In the early stages of infection with Ascaris, the migration of the larvae through the lungs may give rise to numerous minute haemorrhages, oedema and exudation; in severe infections, the worms may produce symptoms resembling lobar pneumonia and may cause death in both man and animal.

1.3 Objective of the Study

Very little is known about the role played by *Ascaris* of man and that of pig as a passive transport agent of contamination of different tissues of the body with pathogenic bacteria from intestinal tract. Furthermore, very little is known about the relationships which exist between the microflora and nematodes, particularly *Ascaris lumbricoides* and *Ascaris suum* in the intestinal tract of man and pigs. Apart from the work of Philips *et al*, (1958 and 1964), Wescott (1970) and Hungate (1972) little is known about the relationship which exist between metazoa, protozoa and bacteria inhabiting man and animal. The literature in this field is scanty and there has been no report on this subject in Nigeria. The first report (Adedeji, 1981) was based on the microflora and nematodes that occur simultaneously in human gut.

This investigation is therefore to

(1) determine the action of intestinal microorganisms on the developmental stages of Ascaris of man and pig;

(2) show what happens to embryonated eggs in an environment with different levels of bacterial contamination;

(3) show particularly that migrating larvae of Ascaris can carry with them micro-organisms and thus transmit and, or aggravate infection with micro-organisms;

(4) determine to what extent the digestive system of Ascaris suum of pig are invaded by bacteria and compare this with the bacteria flora of the host;

(5) show the sources of human infection with Ascaris lumbricoides in Ibadan.

It is believed that more information would be available to us at the end of this study about hitherto unknown aspects of Ascariasis in Nigeria.

LITERATURE REVIEW

2.10 INTRODUCTION

2.11 General Consideration

Ascaris lumbricoides, the largest of the human intestinal nematode which resembles the common earth-worm was first discovered by Linnaeus (1758). Apart from being the most common helminth of all intestinal nematode Ascaris lumbricoides has a cosmopolitan distribution throughout the world, and is most prevalent in the tropics and subtropics especially in rural populations. It thrives best in warm moist climates or in moist temperate regions where poor standards of personal hygiene and environmental conditions combine to favour embryonation of the eggs which are very resistant to adverse conditions.

Ascaris is the cause of Ascariasis among children. A wide variety of clinical symptoms is met with in such cases, often the disturbance is very severe and ends fatally. Williams (1938) observed that very little attention is paid in text-books of medicine to the condition, so that practitioners get little guidance in diagnosis and treatment of the subject. Perhaps, one reason for this obvious neglect of an important subject is the paucity of literature on clinical studies of Ascariasis. A search of the literature reveals a wealth of experimental and laboratory work on the subject and also a multitude of references dealing with isolated cases of complications including toxicity, but very few articles exist in which the subject is treated as a whole or which record the results of clinical studies of series of cases illustrating the various symptomatology, the relative frequency and incidence complication, their exciting causes and manifestations, and the mortality rates. Such studies are necessary if the importance of Ascariasis is to be more generally recognised.

As early as 1864, Cobbold noted that the human ascarid, Ascaris lumbricoides, is a different species from the equine ascarid Ascaris megalocephala, and swine ascarid Ascaris suilla; but it was not until the appearance of Neumann's paper "Sur l'' Ascaridae des Betes Bovine" (1883) that the bovine ascarid was generally accepted as a valid species. It was previously described as Ascaris lumbricoides or Ascaris megalocephala. Neumann's study of this worm showed, however, that the human Ascaris differs in many anatomical characters from the bovine one. Later investigators classified both the human ascarid and the bovine ascarid under the genus Ascaris. Consequently Stewart, 1916a, 1916b, 1916c, 1916d, 1917, 1918 referred to the pig ascarids as Ascaris suilla, (now known as Ascaris suum). Ransom & Foster (1917, 1919 and 1920, and Ransom (1919) referred to the human ascarid as Ascaris lumbricoides, while Martin (1926), Schwartz (1922) Griffiths (1922), Boulenger (1922) & Macfie (1922), referred to the bovine ascarid as Ascaris vitulorum. The present

systematic position of Ascaris according to Craig & Faust (1970) is as follows:

Superfamily:	-	Ascaridoidea (Railliet & Henry, (1915)
Family:	-	Ascarididae (Blanchard (1849) or Ascaridae (Cobbold, 1864)
Subfamily:	-	Toxocarinae (Hartwich, 1954)
Genus:	-	Ascaris (Linnaeus, 1758)
Species:	-	Ascaris lumbricoides (Linnaeus, 1758) Ascaris suum (Goeze, 1872).

2.12 Epidemiology and Prevalence

Although Ascaris lumbricoides affects all ages, it is more common in young children. A similar occurrence has been observed by Ransom & Foster (1920) in a closely related spp., . Ascaris suum of swine in which maximum infection is limited to between the third and fifth months of birth, although infection does extend beyond this period in some cases. However, in

the human Ascaris, the incidence of infection is higher in children than in adults (Brown, 1927, Cort (1929 and 1931), Cort & Otto (1933), Otto & Cort (1934), Headlee (1936), Winfield (1937), Scott (1939) Weir et al, (1952) and Chandler (1954). This is because human ascariasis is a household infection, primarilly propagatted by seeding of the soil immediately around the house with eggs present in the excreta of small children who in turn become reinfected from eggs which they pick up on their fingers and introduce into their mouths. The fact that workers like Grassi (1888), Lutz (1888) and Clandruccio (1886) cited by Stewart (1916a) could successfully infect themselves and adult volunteers, besides the incidence of adult ascariasis in tropical and subtropical countries (Brown (1927), Cort et al (1929 and 1930) Headlee (1936) Weir, (1952) and Chandler (1954)) shows that unlike the bovine and to some extent the swine infection, the acquisition of human ascariasis is not limited to

children. The essential difference between neo-ascariasis of calves and ascariasis of young pigs is that, while in the former infection is mainly prenatal, in the latter it is always oral as in human.

Martins (1926) who investigated the possibility of neonatal infection with Ascaris suum in pigs had this to say "from the data presented, it seems evident that intrauterine infection in swine is very uncommon, and if this phenomenon occurs at all in nature, it must be looked upon as being nothing, more than a biological curiosity". Later, Wall (1958) and Taff (1961) have also been unsuccessful in demonstrating prenatal infection in pigs with Ascaris suum.

In some countries in Europe and Orient where human excrements are utilized as fertilizers, field cultivators and vegetarians are exposed to the infection (Craig & Faust, 1970). The infection rates reported by the

World Health Organization (WHO), 1967 are as follows: Italy, 75% of 354 children in Rofrano, 40% of toddlers in Naples, 12% of 2126 children in San Marino, 9% of 207 School children in Matera, 2.5% of 550 University students in Pisa; 21% of 200 persons less than 10 years of age in Spain, 40-80% in Portugal, 7-9% and up to 20% in Yugoslavia and Czechoslovakia respectively. Albania; 18.3% of 283 pre-School children, Roumania; 18.3% of 124,420 persons surveyed. Significant infection levels were found in Poland and in many parts of USSR.

Although ascariasis is uncommon among urban population in Belgium, France and Germany (Craig & Faust, 1970) high prevalence, especially among children is occasionally reported from rural communities (Craig & Faust, 1970). In France overall prevalence among 3,797 School children was 17.8%; in one group of 162 School children it was 46%. In Japan in 1961, 2-5% of the population in large cities were found to be infected;

in rural areas the infection rate averaged 20%. It was observed by Craig and Faust, 1970 that ascariasis is highly prevalent in China. In most countries in central and South America, the average infection rate was approximately 45%. Small foci of high prevalence were found to persist in the South-eastern United States of America. Jeffrey et al (1963) reported an overall prevalence of 63.7% in rural population of South Carolina, the highest prevalence being in the six-eleven-year age group.

The Nigerian Experience

In Nigeria, Cowper and Woodward (1961) recorded 26.5% infection rate in 21,700 stool samples examined routinely at the University College Hospital, Ibadan over a three-year period. Okpala (1956) found an infection rate of 73% in 4,700 children in Lagos and later (1961) the same author recorded 71.5% infection rate in 515 Government Workers in Lagos. Nnochiri (1965)

found 52% infection rate in 42 patients seen at Lagos University Teaching Hospital outpatient department. Marsden (1960) recorded 53% of a series of 500 infected patients in Lagos, and Fisk (1939) found Ascaris in 90 out of a series of 120 post-mortems in Lagos. In Ibadan, Cowper & Woodward (1960) found Ascaris in 38 out of a hundred employees at the Moor Plantation Agricultural Centre. Gilles (1964) recorded infection rate of 70% of 600 villagers at Akùfo near Ibadan. Hinz, (1968) recorded infection rate of 52.3% of 230,197 people of nine Southern Nigerian States he examined. Ogunba (1970) found 29.8% infection rate of 12,384 hospital patients in Ibadan and Abioye and Ogunba (1972) found that 64.6% of 1,272 School children examined in Ibadan harboured Ascaris ova while Ogunba (1974) recorded 69.6% of the 1,130 School children in Ibadan positive for Ascaris infection.

Obiamiwe (1977) found 19.5% of 6,213 people of Benin City positive for Ascaris infection. Further north, the prevalence becomes lower. Ramsay (1934) found only 6% of over 7,000 persons examined in Jos, Plateau State infected and Collard (1962) in a survey in Katsina Province found ova of Ascaris on only 2.1% of 536 Habe and 0.4% of 236 Fulani examined. Ascaris infection is thus significantly lower in the drier north compared with the humid climate of Southern Nigeria.

Stoll (1947) estimated World incidence of Ascariasis as 644.4 million, consisting of 30 million in North America 42.0million in tropical America, 59.0 million in Africa, 320.0 million in Europe, 19.9 million in USSR, 488.0 million in Asia and 0.5 million in Pacific Islands. It has been estimated that one out of four people in the World's population was infected with Ascaris, and that this parasite has a very high prevalence in overcrowded towns of non-industrial centres and rural communities

where human faeces are utilized as fertilizers (WHO, 1967). It is observed that there are differences in the altitude of of various countries to the problems of ascariasis and it has been noted that the public health significance of ascariasis has not been assessed. Measures aimed at improving general sanitation and the self medication of infected individuals had little effect on the prevalence of the disease, yet in the USSR and Japan energetic measures have been introduced to control the infection.

In many parts of Europe, the parasite is still widespread, and the infection rate in children in some areas of the U.S.A. is still more than 20% (Otto & Cort, 1934). In communities when no effort has been made to control the disease, it was observed that intensity of infection was highest in the 1-4 age group (Otto & Cort, 1934).

2.13 Morphology and Biology of Ascaris

The superfamily Ascariodoidea (Railliet & Henry, 1915) AS fairly large or stout worms, having mouth provided with three conspicuous lips but lacking buccal capsule. The males are without bursa corpulatrix and usually without candal alae. Human representative is Ascaris lumbricoides, the largest of the human intestinal roundworms causing ascariasis. The pig representative is Ascaris suum. Like all other nematode species present in human gut, Ascaris is typically elongated, cylindrical in shape with a body cavity in which the organs lie. It is primarily bilaterally symetrical but with a secondary tri-radiate symmetry at the exterior end. The alimentary canal is characteristically a straight tube with a mouth and anus. The sexess are separate. The head portion is adorned with 3 large denticulated lips. The three lips lie, one dorsally and the other two sub-ventrally and they

are finely toothed. It is brownish-yellow in colour. The male is a little smaller than the female, having a length of about 25cm and maximum breadth of 4mm. Its posterior extremity is definitely pointed, and curved ventrally in the form of a hook. Two curved spicules can often be seen protruding from the orifice of the cloaca, around which there are large numbers of minute parpilla. The female measures up to 35cm in length and has a maximum diameter of about 5mm. The posterior extremity, though conical, is not pointed or ventrally curved as in the male, but straight. The vulva is very minute and situated ventrally at the junction of the anterior and middle-thirds of the body. It leads into a stout duct, the vagina which divides into two branches; these tubes lie anteroposteriorly within the worms. That portion of each duct close to the common vagina functions as the uterus. The middle part is the oviduct and the distal part the ovary

(Chitwood & Chitwood, 1974; Craig & Faust, 1970 and Davey & Crewe 1973). Ascaris lumbricoides egg was first described by Linnaeus (1758) as being round or oval in shape, brownish in colour and it measures about 60 /um in length by about 45 /um in breadth. The egg has a thick transparent shell. Wharton (1915) reported that the Ascaris lumbricoides egg is surrounded by a shell and an outer albuminous layer. Nelson (1851) reported that the number of layers surrounding the egg of ascarids is three and in the case of human ascarids Christenson (1940); Krenser (1953) and Frenzen (1954) disagreed with Nelson (1851) and they stated that Ascaris lumbricoides egg has four layers. This report was confirmed by Rogers (1956) using electron microscope in the study of the eggs of Ascaris lumbricoides var suum. The eggs are noted for their great resistance to extremes of temperature and chemicals. Davaine (1863) found the ova of Ascaris lumbricoides remained infective after storage for five years and Bailliet (1866)

observed that the eggs of A.suum remained viable after twelve months exposure to heat of summer and to the cold of winter. The effect of various temperatures on development of Ascaris eggs has been studied by Faure-Fremiet (1913) who showed that the ova of A.suum are capable of development at varying temperatures below 35°C, but that the optimum temperature is about 30°C. Wharton (1915), determined that at a temperature around 37°C, the ova of Ascaris lumbricoides developed to the eight-cell stage and then died. As reported by Nolf (1932), the ova of human Ascaris are more resistant to the effect of high temperature exposure than those of Trichuris, as shown by the fact that 68% of Ascaris ova eventually became embryonated following exposure for three minutes at 35°C whereas no Trichuris ova survived similar treatment. Ogata (1925) studied the effect of heat in the destruction of the ova of Ascaris lumbricoides and found that no eggs developed following exposure to

70°C for one minute, and that they were unaffected by exposure to temperatures up to 45°C for a period of one hour. The eggs remain infective for several months in the soil and on contaminated objects. For example, infective eggs have on many occasions been known to be transferred from contaminated fingers to paper money (Dolt & Theme, 1949). Reptiles and amphibians are known to be among the various vessels of transmission of infection. Marinkell & Williams(1964) showed that reptiles may act as potential mechanical vector of A.lumbricoides, as they found 206 Surinam toads heavily loaded with the eggs.

Although the eggs are resistant to extremes of climatic conditions, even with long freezing spells, dessication is however, lethal to them.

The fertilized egg undergoes the first pre-parasitic moult in 14 days to 18 days at 22° - 26°C opt. Lower temperatures prolong this period. The second moult

occurs about 12 - 14 days later, when it becomes infective. In all the egg requires about 30 days to become infective after being passed in the faeces under optimum conditions of temperature, oxygen and moisture. In effect, the bionomic requirements of the Ascaris egg are similar to those of the Strongyloides.

Favourable seed beds of Ascaris eggs may remain infective for many years from which infection can be obtained. The indistinguishability of the egg of the human species of Ascaris and the porcine variety was confirmed by Taffs & Voller (1963) using fluorescent antibody studies. They were also able to establish that both species share common antigens.

Unfertilized eggs also occur and are very common in human faeces. This may be due to the production of large number of eggs by female Ascaris lumbricoides and some of these eggs escape fertilization probably due

to absence of male worms in the host to effect fertilization. An unfertilized egg is more elongated than the normal egg. It has a small protein coat and atrophied ovum, marked by numerous highly refractile granules. In some cases, the mamillations commonly seen in fertilized eggs is lost and the egg is then at times difficult to recognise. Muira & Nishiuchi (1902) gave a comprehensive discussion of the appearance and occurence of the unfertilized eggs of Ascaris lumbricoides. Muira and Nishiuchi (1902) dealing with these unfertilized eggs and Foster (1914) considering the unusually large fertilized eggs sometimes seen, have pointed out that the diameters of all the different types of eggs of Ascaris lumbricoides are essentially the same, the variation in size being primarily in length. As already mentioned, solitary females or several females without males, are found when a person passing only unfertilized eggs is treated. Again, this may be due to the females never having been in copula or the supply of spermatozoa received by copula having

already been exhausted. Proof that copulation takes place more than once during life of the worm is equally difficult to obtain. However, Looss (1905) and Herrick (1928) both suggest from experimental evidence with hookworm that it does take place more than once. Thus a female probably does not receive and store in the seminal receptacle enough spermatozoa from one copulation for the fertilization of all the eggs to be produced. Hence if a solitary male were to be dislodged after its copulation, the female might easily use up its supply of spermatozoa after a time and produce unfertilized eggs thereafter.

2.14 Comparison of *Ascaris lumbricoides* and *Ascaris suum*

The human and pig adult *Ascaris* species are almost identical. Sprent (1952) established host specific differences within existing species through variations in size and shape of the dentigerous ridges.

He observed that the denticles of the pig Ascaris form a conspicuous row of more or less equilateral triangles, while the denticles of the human Ascaris are relatively much smaller and their edges are concave. Crewe& Smith (1971) corroborated Sprent's observation in making morphological distinction between the human and the porcine species by examining the teeth on the dentigerous ridges on a worm passed by a child. This worm was compared with random samples of specimens of Ascaris from the teaching collection at the Liverpool School of Tropical Medicine which had been obtained from pig and man respectively, and they were identical with Sprent's description, thus proving morphological difference between the human and the porcine strain based on the denticular arrangements. Dipeolu & Sellers (1977) confirmed that the Ascarids retrieved from the pigs were human ascarids (Ascaris lumbricoides) which is highly suggestive of the transmissibility of the

human strain to pigs, especially those roaming freely and foraging for food on dumps of household refuse and human excreta.

2.15 Infectivity of A. lumbricoides to Swine

Immunity of the pig to ascariasis has been regarded by helminthologists for some years as problematic. Ransom (1922) was of the opinion that the pig attains immunity at the age of 4 months, but this was subsequently refuted by Morgan (1931) who successfully infected pigs at 10 months of age, and Clapham (1934) at the age of one year. Sandground (1929) referred to the difficulty in establishing infections under normal conditions, however, the fact that almost every pig under field conditions pickSup an infection at an early age is well known. Clapham (1934) bottle-fed 8 pigs from birth and free from infection. These were experimentally fed with large

number of eggs of A.lumbricoides of pig origin. Only 4 infections resulted from the experiment.

A number of pigs of various ages and of known past history were used in an experiment. The Ascaris eggs were of pig origin. Two pigs aged 8 weeks were fed with a large number (unestimated) of infective eggs of A.lumbricoides in an emulsion of water. There were no signs of their migration through the liver and lungs, and no eggs were demonstrated in their faeces during the next four months. At the age of 6 months, these 2 pigs were again fed on enormous number of eggs in water as before. One pig died 6 days later, following signs of pneumonia. Larvae were recovered from both lungs and liver in large numbers when teased in physiological saline, and the tissues all showed lesions usually associated with Ascaris migration. The other pig survived and later survived a severe attack of diarrhoea lasting

about a week. With the restoration of the metabolism of the host, its general health improved. The pig became "clean" again in 23 weeks and a month later was successfully infected again with Ascaris eggs.

Subsequently 4 clean pigs 11 weeks old, and reared from a clean sow were used in an experiment, and these were fed with Ascaris eggs. On this occasion, the technique adopted was aimed at approximating the method to more natural conditions. Therefore, a number of small doses were given daily. The eggs were rather old and the culture not more than 50% infective. Each individual pig was given a feed of 200 infective eggs for 10 days giving a total of 4,000 eggs, with an experimental error of not more than 5%. Although empty shells were observed in the faeces following administration, there was no reaction until the 4th day when the pigs were less inclined than usual for their food. Between the 6th to

the 12th day after the first feeding, they were lethargic. One pig did not exhibit symptoms, but the other three developed a persistent cough and breathing was definitely laboured. At the end of a fortnight after the first dose and 4 days after the last, the symptoms began to moderate and the animals made a recovery. Ascaris eggs were later demonstrated in the stools showing that the infections had been successful.

Later another pig, aged 9 weeks, was fed with Ascaris eggs, 200 at a time, once a day in the food making a total of 2,000. This animal developed a slight cough with respiratory difficulties. The susceptibility of the animal regardless of the small dose was ascribable at least in part to the fact that it was the smallest one of the litter though it was perfectly normal and healthy.

This recalls Koino's experiment published in 1922, when a young man 21 years old showed pneumonia symptoms, following a dose of 500 eggs of Ascaris suum from the pig. Similar symptoms were observed by Koino himself after swallowing 2,000 Ascaris eggs of human origin. As early as 1888, Lutz fed a human patient with 100 eggs and later obtained 35 immature Ascaris worms, with a percentage closely approximating to that obtained by Koino. The experiments of Clapham (1934) would suggest that a very hopeful method of inducing an infestation with A.lumbricoides in the pig is by means of a series of comparatively small insignificant doses.

2.16 Developmental Cycle

The development of Ascaris lumbricoides is at present universally considered to be direct (Stewart, 1917). It is well known that the eggs passed in the faeces of man, undergo development in the outer world up to the formation

of a motile vermiform embryo. Infection is acquired through the ingestion of fully embryonated eggs which have been accidentally picked from polluted soil, from food or drink contaminated by viable embryonated eggs or by children who eat dirt. The second stage larva contained in the egg is released in the small intestine, the shell having been acted upon by enzymic action of the intestinal juices. The released second stage larvae reach the liver within 6 hours of infection where they moult to the third stage larvae. Four days post-infection, still in the third stage larvae, they leave the liver through the blood stream to the lungs, from whence tracheal migration commences. The two parasitic moults occur after they have reached the small intestine. Douvres (1967) states that the moult to the fourth larval stage occurs about the 10th day, in the intestine, this being contrary to the views of Roberts (1934) who stated that this moult occurred in the respiratory system and that only fourth stage

larvae were able to survive the acid environment of the stomach.

Douvres, Tomba and Matakatis (1969) confirmed the view that hatching of the eggs containing second stage larvae of A.suum in the swine occurred in the small intestine from whence migration proceeds to the liver within 6 hours from infection. The first parasitic moult occurs in the liver. The third stage larvae leaves the liver to the lungs via the blood stream four days post-infection. The remaining two parasitic moults occur in the small intestine after completion of tracheal migration. This hypothesis is based on the work of (Davaine, 1863, Grassi, 1887-88, Calandruccio 1886, Lutz, 1887-88, Epstein 1892, Jammös & Martin, 1906 & Martin & Wharton 1915)

Davaine (1863) administered ripe eggs to rats and found that after 12 hours, living larvae were found in the lower part of the small intestine. He also introduced ripe and unripe eggs in glass capsules closed with linen

into the alimentary canal of the dog and found that after the lapse of a certain period the ripe eggs had disappeared, whereas the unripe eggs remained. He concluded that hatching and development occurred in the alimentary canal of the definitive host. Grassi, (1888) administered ripe eggs to himself and two months later found eggs in his stool. Clandruccio (1886) cited by Stewart (1917) successfully infected a child of ten who had previously suffered from worms but had been relieved of these parasites by anthelminthics. Lutz, (1887-88) fed an adult on ripe eggs and found evidence of the subsequent appearance of adult worms. Epstein (1892) successfully infected a man. James & Martin (1906-8) allowed ripe eggs to hatch in artificial and natural solutions and found that hatching took place readily en masse in 0.8% salt solution at 37°C-40°C. Martin and Wharton (1915) found that the embryos of the ascarids from calf, pig, horse and dog, hatch in alkaline

solutions and that when developed eggs are introduced into the alimentary canal of an animal, they pass through the stomach unaffected and only hatch after they have been subjected to the action of the alkaline juices of the intestine. Wharton (1915) states that direct infection can take place, but that the embryo must be completely developed. He does not give the period necessary to secure this complete development.

In spite of the general acceptance of the hypothesis that infection takes place by the direct method, there is a considerable bulk of evidence against it. Davaine (1858) administered three to four hundred ripe eggs to an ox, an animal which is stated to harbour Ascaris lumbricoides and found that after four months, no worms were present in the intestine. Leuckart (1867) fed a rabbit on ripe eggs and found no worms after ten days; a dog was also treated and was equally unresponsive after

14 days. He also fed a pig for 3 weeks on several thousand of ripe eggs and did not find any worms on section.

Although Fulleborn (1922) suggested that certain Ascaris parasites of terrestrial mammals may utilise intermediate host, this possibility has been overlooked since the discovery by Stewart (1917) and (1918a) of the life history of Ascaris lumbricoides. Stewart, having discovered the lung migration of the larvae in rodent, at first concluded that rodents act as intermediate host in the spread of this parasite, but later in (1918b, 1919, and 1921) concluded that only one host was used.

The observation by Ransom and his co-workers (1917, 1919, 1920, 1921, 1922), Martin (1926), Roberts (1934) and de-Boer (1935) have amply confirmed that pigs may become infected with Ascaris lumbricoides directly by ingestion of embryonated eggs, the larvae migrating through

the liver and lungs before they reach maturity in the intestine. Koino (1922) demonstrated that human strain of A. lumbricoides makes a similar migration through the human body and confirmed earlier reports (See Martin, 1926) that human infection results from the ingestion of embryonated eggs.

From the above observation of earlier workers, it is apparent that it has been a rather difficult task explaining the early migratory course of Ascaris larvae on any basis other than its presentation of panoramic view of the evolutionary history of these worms. It appears that this migration results in the dispersal of the parasites throughout the body, inside as well as outside of the hepatic - pulmonary routes, and into organs and tissues from which they probably cannot extricate themselves rather than the circuitous journey of the larvae from the intestines to the lungs and back again to the intestine, and it does not appear to be an adaptation that has survival value for the species i.e.

A suum and A.lumbricoides. Fulleborn (1922) in accounting for the "superfluous" migratory route of these larvae suggested that Ascaris might have evolved from ancestral forms that penetrated the skin. Later, he advanced a more logical hypothesis suggesting that this larval migration probably points to the derivation of these worms from ancestral forms that migrated in an intermediate host.

Investigators who reported failures in their attempt to develop Ascaris in the intestine of pigs had no difficulty in raising the parasites in pigs to the stage that they attain in the lungs and, to some extent beyond that, in the intestines. There appears to be practically no known mammalian host specificity in the early development of Ascaris. A.lumbricoides as well as A.suum has repeatedly been shown to produce signs of pulmonary changes in pigs and other mammals. Host specificity becomes evident only when the worms return to the intestines. It is observed that in small mammals, e.g. mice, rats, guinea-pigs and rabbits, A. suum passes out of the

body shortly after returning to the intestine.

In goats, and more so in sheep and cattle, A. suum has been found to be capable of developing in the intestine and may attain about half its normal size. Half grown A.suum, the female containing eggs that could be cultured to the embryonated stage were found in a calf.

The fact that the behaviour of A.suum in pigs often parallels its behaviour in a strange host presents an anomally that is difficult to explain, except by postulating the development of a morked resistance to these worms by the host. This resistance could occur particularly during the wandering period of the larvae through the body.

Schwartz (1959) is of the opinion that the migration of newly hatched larvae appears to be of phylogenetic significance and probably points to the evolution of these worms from ancestral forms that had an indirect life cycle. On the basis of this assumption, he concluded that the life of A.suum and of related species apparently has become

compressed into one host, although there is still some evidence of an indirect development with one phase culminating in the lungs and the other in the intestine.

2.17 A.suum - Bionomics and Cross infectivity

A. suum has a common occurrence in domestic pigs, especially amongst free roaming ones foraging on garbage heaps in rural areas. 75% of pigs in the United States of America and Canada are infected before they are 6 months old. The principal effect is stunting of growths. S Spindler (1947) found that pigs infected with 20 or more worms at 8 weeks of age did not gain weight. One pig with 109 worms did not gain weight at all when compared with uninfected pigs gaining an average weight of 100lbs. (Chandler & Read, (1961)).

A.suum exhibits sexual dimorphism, the females often longer (25-40cm) and stouter than the males (15-25cm). The adults are provided with three denticulated

lips. These large worms inhabit the small intestines of the pigs. It is a common parasite of pigs in Nigeria. It is morphologically and serologically identical to A. lumbricoides of man. Dipeolu and Sellers (1977) observed in an investigation conducted in Eruwa, Western Nigeria, on the endoparasites of indigenous pigs that out of eleven (11) helminth species identified, A. suum was in the lead with highest incidence of 92%. Although for sometime, the specific identification of A. lumbricoides and A. suum has posed a problem to many scientists. Sprent (1952) claims on the morphological basis that both are identical, even though epidemiological evidence and experimental infections appear to indicate that thereis a difference in the two parasites. Sprent (1952) carrying out comparative studies on human and porcine ascarids showed that the pig ascarid form a conspicuous row of more or less equilateral triangles and that the edges of the denticles are straight. In contrast, the denticles of the human strain are considerably less conspicuous, being smaller and possessing concave edges. This observation was confirmed by Crew & Smith (1971) in

a morphological study of a worm passed by an infant.

As regards the cross infectivity, patent cross infection trials were carried out by several workers but they did not establish any proof. However, Clapham (1934) was able to infect young colostrum deprived piglets with human species.

2.18 Ascaris suum - Life Cycle

The ingested eggs hatch in the intestine and the larvae burrow into the wall of the gut. They pass through into the peritoneal cavity and thence to the liver; but majority reach this organ by way of the hepatoportal blood stream (Soulsby, 1973). The eggs of A.suum will hatch and the larvae migrate in many animals species, including man. Much work has been done with A.suum infections in guinea-pigs, rabbits, rats, and mice and in these, the migratory cycle is much the same as in the pig. There

are slight differences in size of larvae from various animals, thus larvae from mice are smaller than those from= swine. Normally, A.suum does not mature in such animals though Berger et al, (1961) have reported this in rabbit. The mode of migration of A.suum in laboratory animals, especially the white mice, has received attention by Sprent,(1956, 1958). Essentially this parasite follows the tracheal route of migration. On occasions in infections with A.suum in the pigs, earthworms and beetles have intervened as paratenic hosts. The eggs containing infective second stage larvae are ingested by earthworms and these beetles. The larvae hatch and remain alive in the tissues, fully infective for long periods.

2.19 Diagnosis of Ascaris infection

The diagnosis of the intestinal helminths which are found in man can be made readily by any one of the numerous methods of faecal examination. The recovery of the adult

worm, the mature stages or the eggs in an individual is confirmation of infection. Most of the eggs which are found in the faeces are characteristic for each worm. In the case of Ascaris lumbricoides, however, both fertilized and unfertilized eggs may be found. This fact introduces a possible source of error in the diagnosis of this particular parasite. Fertilized eggs are very characteristic, having a thick, clear, inner shell, covered by an irregular, warty, albuminous coat which is stained yellow or brown in the intestine. They are usually spherical or slightly oval in shape and measure from 40 to 75 micra in length by 35-50 micra in width. Unfertilized eggs are much more longer, narrower, more elliptical in shape. They measure from 70 to 88 micra in length by 38-50 micra in width. The inner structure is unorganised and frequently contains amorphous granular material or it may be vacuolated. The albuminous coat may be absent from both fertilized and unfertilised eggs.

The unfertilized eggs are usually bile-stained but are more likely to be colourless, grey, or unstained.

There is not much information available concerning the frequency with which fertilized and unfertilized Ascaris eggs occur in cases of ascariasis. However, Keller et al, (1933) has shown that the percentage of fertilized eggs increases with the intensity of infestation. It is interesting to note that in the 289 cases, he examined in which unfertilized eggs occurred alone, 265 (91.7%) had light infections. This is the group in which diagnosis is most likely to be missed if unfertilized Ascaris eggs are not recognised.

During lung migration of Ascaris larvae, there may be exhibitioin of scattered moulting in the lungs, accompanied by dyspnoea of an asthmatic type, fever up to 40°C and spasm of coughing. Occasionally, blood-tinged sputum and eosinophilia in the absence of tuberculosis is highly

suggestive of Ascaris pneumonia. Later, when the worms have matured in the small intestine, diagnosis is usually made on the recovery of the characteristic eggs (fertile or infertile) from the faecal samples. A single female worm produces a sufficient brood of eggs each day to guarantee their recovery in one or two direct faecal films. In patients with suspected Ascaris pneumonitis, a history of present or previous intestinal infection in the individual or in other members of the family, or of soil pollution, is suggestive. Keller et al (1932) stated that in children with negative tuberculin tests there may be evidence of a widening in the hilar areas of the lungs with increase in the broncho-vascular markings, presumably due to repeated migration of Ascaris larvae through the lungs.

The main materials that are examined for Ascaris infections are faeces, sputum, and bile samples. However, because of activities of houseflies, and other insects

which assist in disseminating Ascaris eggs as well as in contaminating fruits, root and leaf vegetables, these food items are also examined for ova that are stuck to their surfaces. In many areas of the world, human faeces is still actively used as fertilizer without proper treatment to kill the parasites in them The farmland is therefore grossly polluted with eggs which are later passively and widely disseminated by rain, floods and strong winds., It is therefore customary to examine soil and air samples as well to determine the level of contamination of the environment with Ascaris eggs.

Amongst the various diagnostic methods, the direct smear method is the oldest and simplest method. It is however inefficient in light infections except one uses a large sample material. Kato's cellophane thick smear method is very efficient and is recommended for mass examination of many faecal samples. It is simple, rapid and cheap (Kato & Miura 1954). It is however unreliable when the faecal material is

fibrous and contains fermentation gas. It detects all the common helminth ova except the minute ones. When the Kato's cellophane thick smear method is used, further examination of faecal sample by a concentration method is unnecessary.

The concentration methods are of two types. The floatation methods which make use of the differences in the specific gravity between the helminth ova and faecal materials in the medium. The objective is to float the ova on the medium surface. Commonly used salt solutions are those of sodium chloride and Magnesium sulphate. The sedimentation methods are better favoured and more widely used than floatation methods because they eliminate most of the faecal debris as well as increase yield of ova. The morphology of the ova is often distorted in Acid-ether sedimentation but the yield in the Formalin-ether test is good and can be stored in formalin (Ridley & Hawgood, 1956).

The quantitative methods that have been widely used include the following:

(i) Stoll dilution egg count method (Stoll, 1923,);

(ii) Beaver egg count method (Beaver, 1949, 1950);

(iii) McMaster egg count method.

The value of quantitative methods have been fully appreciated in helminthic infections where egg production is fairly constant, and the approximate daily output per female worm is known as in Ascaris, Trichuris and Hookworm infections. They, however, provide only estimates of the worm burden because of the many factors that are known to effect the total daily egg output. Some of these factors include the fluctuations that occur in the individual worm's egg output and the varying amounts of faeces passed. Nonetheless, the recorded egg counts enable us to classify infections as light, moderate and heavy and they are useful in determining the success or failure of control programmes and mass

chemotherapy. Since some intestinal helminths shed their ova ireegularly into faeces, modifications have been introduced to the commonly used quantitative methods to adjust for this peculiarity (Martin & Beaver, 1968).

The Stoll's dilution egg count method (1923,

The stoll egg count method was first introduced in 1923, but the small drop modification described by Stoll and Housheer in 1926 has been the most widely used quantitative method. This is a dilution method and the eggs are counted in 0.075ml sample from 5mg of faeces which is an estimated equivalent of 1 in 200 of the original faecal sample. It is a reliable method which has been widely accepted.

Beaver's Standard Smear Method (1949, 1950)

Beaver's egg count method uses a small sample of faeces when compared with Stoll's method. It is simpler to perform and less time consuming than the Stoll's method. Beaver's method is however not suitable for routine work in hospitals and clinics especially in developing countries because of the use of the photometer and the precise measurements required for the faecal slide density. The photometer which is essential, is easily damaged and needs to be constantly recaliberated. Although this method is known to give reliable counts with high reproducibility, Maldonado (1956) and Melvin et al, (1956) have suggested that Stoll's method shows no advantage over Beaver's despite the relatively small size used in Beaver's method.

McMaster's Egg Count Method

This method, like the Stoll's is a dilution method and it uses saturated salt solution and sieve to free helminth ova from the faecal debris. The amount of faecal

sample used is smaller than in Stoll's method and the helminth ova float on the saturated solution, being higher than the debris. These are counted in the counting chambers which have been calibrated. This method is unsuitable for trematode eggs which invariably collapse and refuse to float, nonetheless, it is a fairly reliable method. Other methods for quantitative estimation of eggs are Kato's Cellophane thick, smear method (Kato & Miura, 1954).

Ascaris infection can also be diagnosed by finding the adult worms in the stool, or vomit. When freshly passed out from the intestine the adult worm is light brown or pink in colour but it gradually changes to white. In shape, it is rounded and tapers at both ends, the anterior end being thinner than posterior. The mouth opens at the anterior end and possesses three finely toothed lips, one dorsal and two ventral. The posterior extremity is neither curved nor pointed but conical and straight.

X-Ray Diagnosis

The presence of Ascaris lumbricoides has been demonstrated by radiography with barium emulsion, which being ingested by the worm within 4 to 6 hours, casts an opaque string-like shadow.

Dermal Reaction (Allergic)

Scratch test with powdered Ascaris antigen has often been found to be positive but the results are variable. This reaction has some value in the diagnosis of Ascaris infection.

2.20 Control of Human Ascariasis

The control of ascariasis is very difficult especially in tropical countries where ascariasis is most common amongst young children, who habitually pollute the compounds with faeces and become infected while playing on the ground, as a result of infective eggs adhering to the fingers and

being later transferred to the mouth. Whenever possible, all infected patients should be given specific treatment, but this in itself, does not control infection, since reinfection from contaminated sites may occur repeatedly. The benefits of single treatments for Ascaris lumbricoides have been shown to be of short duration in areas where infections are perpetuated by gross pollution of dooryard and environs (Cort, Schapiro & Stoll, 1929; Otto, 1930; Otto & Cort, 1934 and Headlee, 1936).

Fayard (1949) treated 2,000 patients harbouring Ascaris lumbricoides using piperazine citrate and recorded 70 to 95% success. Mouriguand, Roman & Coisnard (1951) also recorded good results when he used piperazine to treat some patients. Other investigators like Oliver-Gonzalez, Santiago-Stevenson & Hewitt (1949); Etteldorf & Crawford (1950); Ghanem (1954), White & Standen (1953) and Bumbalo et al (1954) recorded some degree of success

and they condluded that if used over a period of time piperazine, a relatively non-toxic agent is useful in controlling Ascaris infection. Wykoff and Altmann (1956) had shown that some molluscicides that are often applied to soil have ovicidal activity on the embryonated eggs of Ascaris lumbricoides. It is of interest that all the 4-thicyano-compounds they tested were toxic at high dilution to Ascaris eggs. Muazzam et al (1960) using pyrantel pamoate, another potent, yet safe drug with minimum toxicity reported that in two different reports, the therapeutic success of these drugs has been from 76% to 88% (WHO., 1964) and the effect of other drugs such as thiobendazole has been even less (WHO., 1967). Ogata (1925) in a series of experiments on the destruction of Ascaris eggs concluded that Ascaris eggs were easily destroyed by heat at 100°C. At this temperature, the eggs lost their power of development and that Ascaris eggs containing embryos, after hot water had been poured on

them lost the ability to infect. Hoshi & Morizaki (1952) and Yamada (1952), dealing with some groups of people with large populations gradually lowered prevalence and density of Ascaris infection by repeated treatments, but complete elimination was not attained. While deliberate large-scale efforts to control ascariasis have been attempted in comparatively few countries, it can be assumed that its prevalence has gradually fallen in large part of Europe and America (WHO., 1967). This has occurred as an unplanned by-product of a generally improved standard of living and of the popular acceptance of relatively high standards of personal hygiene. The experience of a few countries, notably Japan and the USSR, indicates clearly, however, the results that may be expected from deliberate, planned efforts on a national scale. Adequate epidemiological data are available to assess the efficacy of measures carried out, and this experience in ascariasis control is particularly instructive in planning control efforts in other countries. In general, the approaches to ascariasis control have proved valuable:

(i) the treatment of infected populations;

(ii) measures to prevent environmental contamination with human faeces and

(iii) education of the public in personal hygiene.

In the most successful programmes, all the three measures have been undertaken simultaneously. Comparatively few attempts have been made to demonstrate the effect of any one of these approaches as the sole method of control, although in Singapore, a sharp decline in ascariasis was noted within a year of reallocating families in modern houses (WHO., 1967). Environmental measures, especially the provision of latrines and of safe sources of water, have not, in themselves eliminated ascariasis from certain parts of Western Europe and North America (WHO., 1967). In fact, high prevalences of infection still persist locally in both these areas. In addition, various studies have shown that the provision of latrines in villages as the sole preventive measure may have little or no effect upon prevalence. For example, in certain Egyptian villages

without latrines, the prevalence was 73% while in similar villages in which latrines were present, it was 70% (WHO., 1968). In such villages, less than one third of the latrines and the wells were in use after six to ten years. In area where Ascaris prevalence in the highest infected age group was 77% in 1926, in 1950-1951, it was 72%. In the same period, the prevalence of hookworm fell from 87% to 47%, thus showing that the methods usually employed to control hookworm infection have no effect in ascariasis control (Craig & Faust, 1970). In areas where human faeces is used as fertilizer for vegetables which are eaten raw, fermentation at 50°C or higher is needed to kill the eggs (Davey & Crewe, 1973). Furthermore, if a small plot of ground is known to be seeded with Ascaris eggs, thus exposing children, it may be desirable to spade under the top two inches of soil and bury the eggs or to sterilise the soil with live steam (Craig & Faust, 1970).

Control of Pig Ascariasis

The chief problem in the control of ascariasis in pigs is the great survival capacity of the egg. In houses, the use of caustic soda, applied concentrated to walls and floors and left for two or three days, to be hosed away before the pigs are re-introduced, is effective and, where it is available, live steam is also highly efficient. When the problem is a serious one in range pigs, the animals must be moved to fresh ground, and the old ground cultivated or grazed by other stock. The application of the new piperazine compounds or other effective anti-helminthics represents a possible means of controlling Ascaris suum by planned periodic treatment (Kelley, Olson & Garwood, 1956). In order to prevent the spreading of the eggs, A. suum should not be permitted to mature within the host. About 70 days are required for A. suum to reach maturity. Thus if the market pigs are treated at 2 and 4 months of age and the brood stock at bi-monthly intervals, the worms would not mature and no new eggs would be deposited on the soil.

This programme of planned treatment would lower the number of infective Ascaris eggs available and eventually eliminate Ascaris from the herd.

Vaccination with Whole Worm

Attempts to vaccinate against parasitic nematodes with dead whole organisms, homogenates or extracts began in the 1930s. Kerr (1938) could not detect any increased resistance to the dog hookworm Ancylostoma caninum in mice or Ascaris suum in guinea-pigs following repeated vaccination with heat-killed larvae or extracts prepared in various ways, Sprent & Chen (1949) did not detect any resistance against Ascaris lumbricoides in mice following vaccination with homogenates of worm tissues. The failure, or at most, marginal success, of the majority of these early attempts to vaccinate against parasitic nematodes gave rise to the view that the antigens needed to stimulate an effective immune response, the functional antigens, were not present in sufficient quantity in homogenates of dead worms, but

might be actively secreted by the living parasites.

Taffs (1961) reported that adult worms in infections of pigs or laboratory hosts with Ascaris suum may be expelled by a self-cure mechanism; and that the larvae of a challenge infection in an immune host are affected when they approach the moulting period. In one of the first investigations of immune response to gastro-intestinal nematodes in cattle and sheep, Stoll (1929) and Soulsby(1961), made some observations which strongly suggested that an immune reaction capable of blocking egg production and causing expulsion of many adult worms was triggered by an early stage in the development of a challenge infection. This so-called "self-cure" reaction has been most studied in the response of sheep to the stomach-worm, Haemonchus contortus. After sensitisation of sheep by several infections, a further challenge can result in a local hypersensitivity reaction in the abomasum which causes rejection of most of the adult worms from this site

(Stewart, 1953). This reaction occurs at a time when the larvae of the challenge exposure are moulting from the third to the fourth larval stage (Soulsby &Stewart, 1960).

2.21 Identification of Bacterial Microflora in Human alimentary tract

Bacteria are microscopic in size and relatively simple, often unicellular in structure. The morphological features of importance are the size, shape, and grouping of the cells, and their possession of distinctive structures such as endospores, flagella, capsules, and intracellular granules. Staining reactions are observed after treatment by special procedures such as Gram's stain (Gram, 1884). The Gram's stain divides bacteria into two categories in separate colours due to their permeability to certain decolourising agents. Limanna & Mallette (1959) also discussed the principle of different staining procedures and concluded that

staining helps in the grouping of bacteria. Furthermore, systematic position of microflora of the gut can be verified by other methods (Baltimore, 1957). Gram (1884) divided bacteria into Gram-negative bacteria and Gram-positive bacteria. These are many but those ones that are present in the alimentary tract of man include the following:

(a) Staphylococci:

These are Gram-positive cocci, usually arranged in clusters. Smears from liquid culture may show Gram-positive cocci in pairs, short chains or small clumps. Staphylococci are catalase positive, non-motile, non-spore forming. They grow on most laboratory media at 37°C in the presence of oxygen. Manitol-salt-agar is one of the selective media for the isolation of Staphylococci from contaminated specimens. Some strains show pigmentation which occurs better at room temperatures and some species are haemolytic. Staphylococci must be differentiated from Micrococci which are also Gram-positive cocci. This can be done by their

attack on glucose. Staphylococci ferment glucose whereas Micrococci attack glucose oxidatively or do not attack it at all. Coagulase testing separates the pathogenic strains of Staphylococci from the non-pathogenic strains, Cowan (1939). Thus coagulase positive Staphylococci are referred to as Staphylococcus aureus (Staphylococcus pyogenes) and coagulase negative ones as Staphylococcus albus. Staphylococcus aureus produce a substance called coagulase which, in-vivo deposits fibrin on the surface of Staphylococci rendering them less susceptible to phagocytosis.

(b) Streptococci:

They are Gram-positive cocci arranged in pairs, short or long chains. They are non-sporing, generally non-motile and catalase negative. Most species are aerobic, or facultative anaerobes, and a small group are obligatory anaerobes. Many species of Streptococci elaborate a substance called C-carbohydrate, which, when extracted by hydrochloric acid or formamide or lysed by enzymes, forms an antigen used

for their serological grouping (Lancefield's groups, Lancefield (1933). The common Lancefield's groups are A - G and Enterococci which are present in human alimentary tract belong to Lancefield's group D. Enterococci may be haemolytic showing alpha (α) or beta (β) haemolysis on Blood agar medium. On MacConkey's agar medium, they form small pink colonies. They are heat-resistant - 60°C for 30 minutes - and will grow at 45°C. They will also hydrolise aesculin agar slope.

(c) Lactobacilli:

The organism in this group are aciduric, Gram-positive, non-motile, non-sporing rods. They are catalase negative, often long and slender and sometimes pleomorphic. They grow best on a glucose-containing medium or on Tomato juice medium having an acid PH. The group is commonly found in faeces of both adults and infants.

(d) Clostridium perfringens (Clostridium welchii):

The Clostridia are anaerobic or microaerophilic Gram-positive rods, forming spores. The position of the spore is an aid in identification. Some species decompose protein (proteolytic); others ferment sugars (saccharolytic), while some species are both proteolytic and saccharolytic. The majority are saprophytic organisms, their natural habitat being the soil and intestine of man and higher animals. Many are pathogenic to man. Among the pathogens are the Clostridium tetani, the causative organism of tetanus, the Clostridium welchii (Clostridium perfringens) and the allied ones are organisms associated with gas gangrene. The isolation and identification of the Clostridia is not a simple task, as materials for cultures will invariably contain other organisms including Proteus. Isolation media should therefore include blood agar media, incubated aerobically and anaerobically; MacConkey's agar incubated aerobically and anaerobically and cooked

meat medium and Thioglycollate medium, which after an overnight incubation, is later subcultured onto solid blood agar plates for both aerobic and anaerobic incubation.

2. Gram-negative Bacteria:

These are also many in genera and species but those found in human alimentary canal of man include:

(a) Escherichia coli - which is the commonest type of faecal coliform and a frequent cause of urinary tract infections and gastro-enteritis in infants. It will grow readily on most laboratory media and ferments lactose and most sugars. It is nearly always indole positive and produces gas in MacConkey broth medium at 44°C (Eijkman's test). It does not utilise citrate medium. On account of its fermentation of lactose, it is pink in colour on MacConkey-Lactose-Bile-Salt medium (MCC).

(b) Proteus group of organisms are Gram-negative motile aerobic bacilli found in normal faeces, urinary tract infections, and in suppurating wounds. Most species tend to swarm over the surface of solid media such as Blood agar medium. There are four species of Proteus according to some carbohydrates and biochemical reactions. The species are P.vulgaris which ferments glucose, maltose and sucrose with acid and gas production. It is indole positive. It produces hydrogen sulphide; P.mirabilis which ferments glucose and sucrose with acid and gas production. It is the only species among the group that does not produce indole. P.morgani ferments glucose with acid and gas production. It is indole positive and it produces hydrogen sulphide. The fourth species is P.rettgeri which ferments glucose and mannitol with acid only. It does not produce gas in the sugars, It is indole positive, and hydrogen sulphide negative.

(c) Other Gram-negative organisms usually found in faeces of man include:

Klebsiella species - They are Gram-negative, non-motile, indole negative organisms. Some mucoid strains produce capsule and the virulent strains are highly pathogenic for mice, thus, mice are used for their diagnosis. Citrate is utilised whidch helps to separate these lactose fermenting organisms from Escherichia coli - another lactose fermenting organism. Also found in the alimentary canal of man is Pseudomonas aeruginosa, which is a motile Gram-negative rod-shaped organism. It may produce a yellow-green pigment and may also produce a characteristic smell. It is catalase positive and oxidase positive. Other Gram-negative organisms occasionally encountered in the alimentary canal of man apart from the above mentioned ones afe fully identified by the methods of Baltimore (1957), Cruickshank (1965) and Baker (1967).

2.22 Ascaris Lumbricoides and Bacteria:

In spite of the fact that Ascaris infection is one of the most cosmopolitan and most common helminth infections in man and domestic animals and although our knowledge of these worms dates far back to antiquity, a review of the literature shows that very little is known about how these parasites feed.

Leuchart (1866) observed in Enterobius vermicularis that the worm intestine usually contained yellow fluid which microscopic examination proved to be identical with the liquid masses normally found in the large intestine of the host between the solid part of the faeces. In Oxyuris equi, he was able to demonstrate that the intestinal contents contained small particles of vegetable material identical with the contents of the intestine of the horse.

Weinberg (1907) stated that he could not find red blood-corpuscles in the intestine of Ascaris but that they were usually very numerous in the intestine of Strongylus of the horse; he expressed, therefore, the opinion that Ascaris feeds on the contents of the intestine of the host. Hoeppli (1927) recorded an experiment carried out by Dr.Vogel in the Tropen-Institute at Hamburg. A patient, positive for Ascaris was fed with powdered animal charcoal three times a day for three days. On the fourth day, a female Ascaris was expelled with an ascaricide, but a thorough examination of the worm intestine showed no particles of charcoal; but the same author was later informed by Dr. Vogel that in a second experiment carried out in a similar way, numerous charcoal particles were found in the intestine of Ascaris lumbricoides.

Archer & Peterson (1929), in their experiment on "Roentgen Diagnosis of Ascariasis", made the interesting observation that soon after the ingestion of barium cereal

meal by the patients, a cylindrical filling defect showing the displacement by the Ascaris specimens could be seen in the jejunum. Later after the contrast meal had entirely passed out of the jejunum, stringlike shadows, representing the barium-filled enteric canal of the parasites remained still for sometime in the jejunum. Furthermore, they observed that the parasites would not ingest the barium, if the patient had eaten prior to drinking the contrast meal, and in that case, the enteric canals of the worms would not be outlined. Their explanation of this observation is that if the patient had eaten food prior to the administration of barium, the enteric canal of the parasites would be already filled so that no barium would be ingested. These observations indicate that Ascaris spp.(A.lumbricoides) swallowed the barium meal and that they normally feed on the intestinal contents of man.

2.23 INTERRELATIONSHIP OF BACTERIA AND INTESTINAL NEMATODES (ASCARIS SPECIES)

Although the literature reports of the interrelationship of bacteria and intestinal nematodes are scanty, Newton et al, (1959) and Wescott (1968) studied Nematospiroides dubius infection in germ-free mice and Przyjalkowski (1967) worked with Trichinella spiralis in germ-free and monocontaminated mice.

Microbe-microbe interactions are numerous and have been reviewed by Donaldson (1968), Broitman & Giannela (1971) and Bryant (1972). The antagonistic interactions include competition for nutrients, alteration of physical environment and elaboration of antagonistic substances or toxic metabolite, while the synergistic interactions involve production of growth factors, enzyme sharing and antibiotic resistance (Mettrick & Rodesta, 1974). Relationship between protozoa and bacteria of the gut and bacteria-nematode interactions have been reveiwed by Hungate (1972) and Wescott (1970).

Generally, bacteria-protozoa interactions are antagonistic, including competition for nutrient or, in some cases the protozoa ingesting the bacteria (Hungate, 1972). The relationship between nematodes and intestinal bacterial flora involve both synergistic and antagonistic component (Mettrick, 1973). The first is characterised by the microflora contributing to the survival and well-being of adult nematodes, increasing the percentage of larval nematodes developing to adults and improving the survival times and fecundity of the adult worms. The second is characterised by alterations in the host's response to the tissue stages of the parasites. Hosts with normal flora were more successful in preventing the larval nematodes from completing their migrations and were more successful in healing the lesions produced by migrating larvae (Wescott, 1970).

Studies of the interaction of host microflora and metazoan or protozoan parasites became practical with the development of gnotobiotic technique (Reynier, 1959). These techniques allowed the study of parasitism in host devoid of bacteria or with a flora of his choice. Philips & Wolff (1959) working with Entamoeba histolytica in germ-free guinea-pigs were the first to show experimental example of parasite-microflora interaction using bacteria-free hosts and revealed the importance of the application of gnotobiotics to the study of parasitism. Since that time, experimental infection of gnotobiotic hosts with metazoan and protozoan parasites has been reported by many investigators (Bradley & Reid, 1966; Doll & Franker, 1963, and 1964; Houser & Burns, 1968; Newtons, Weinstein & Jones, 1959; Newtons, Beardon & Deleva, 1960; Przyjalkowski, 1961, 1966, 1969 and 1973; Weinstein, Newton, Sawyer & Sommerville, 1969), thus increasing our knowledge about the bacteria-nematode interaction.

CHAPTER 1

3.1 DEVELOPMENT OF HUMAN AND PORCINE ASCARIS EGGS TO INFECTIVE LARVAE IN DISTILLED WATER AND IN 2% FORMALIN AT 25°C AND 27°C AND UNDER ACTUAL FIELD CONDITIONS

INTRODUCTION

Wharton (1915) reported that *Ascaris* eggs developed completely in 15 days to larval stage at 27°C. Schwartz (1922) observed that fresh unsegmented ascarid eggs cultured in a thin layer of 2% formalin solution in petri dishes developed to the first stage larvae in ten to twelve days at 25°C, Refuerzo, Alis Jimenez (1954), found that in distilled water culture at 27°C the eggs developed into the first stage larvae in six days. Tiner (1953) observed that in distilled water, ascarid eggs developed to the first stage larvae in 17 days at room temperature of 27°C. Further experiments are necessary to ascertain whether the difference in temperatures is responsible for the differences in incubation periods and also to find out the action of formalin

on the developing eggs. The study is also to find out the time it takes Ascaris eggs to develop to larval stages under actual field conditions in our tropical climate.

MATERIALS AND METHODS

Ascaris eggs used in this study were taken from the uteri of adult gravid female worms. Some of the eggs were harvested from faecal suspensions by sedimentation and floatation on saturated sodium chloride solution. The Ascaris eggs were obtained from living worms collected from freshly passed faeces from dewormed children. Ascaris suum eggs were collected from living worms collected from the abattoir. After the uteri were removed from the female worms and washed in several changes of sterile distilled water, they were placed in a petri-dish and the eggs were teased out of each uterus. Only the eggs in the portion of the uterus proximal to the vagina were

used because Ackert (1931) has shown that this part of the uterus contains a high percentage of fertile eggs. The eggs were cultured using a modified method of Hansen et al (1954). This modification involved the preparation of an artificial digestive juice which would break down the uterine wall surrounding the eggs. This solution contains 0.5% hydrochloric acid and 1% pepsin. In 2-3 minutes, contact with the solution, the uterine walls were completely digested leaving only the eggs. The eggs were washed 4-6 times in distilled water and were cultured in different media thus:

(1) Some eggs were cultured in ordinary distilled water and incubated at 25°C and 27°C;

(2) Some eggs were cultured in 2% formalin and were incubated at 25°C and 27°C.

Two incubators one of which was maintained at 25°C and the other 27°C were used. The temperatures of 25°C incubator and those of 27°C incubator were recorded by means of maximum and minimum thermometers kept close to the petridishes

The depth of the culture medium was about 2cm and the actual temperature of the cultures was measured by inserting a thermometer in the dishes. About half a dozen petri-dishes were used for each medium and for a particular temperature. The contents of the petri-dishes were agitated twice a day to aerate them. The cultures were observed under the light microscope everyday to see the rate of development. At each observation, 3 separate total differential egg counts were made from three of the culture dishes selceted at random. Both the low and high power objectives of the microscope were used in the process. For each count, the culture was mixed well, and then taken with a 50-dropper Pasteur pipette and delivered onto a clean microscope slide and covered with a coverslip. The eggs on the slide were counted under the microscope with a moving stage. Fresh pipette was used for each count and three such counts were made and the average taken. The

total counts per ml. was obtained by multiplying the number counted per slide by 50. Each stage of development was then counted and the percentage was calculated.

When the eggs appeared to have reached the first stage larvae at the end of the sixth day a quantity from each culture dish was fed to a white mouse. There was nothing that happened to the infected mice. Immediately the larvae moulted from the first stage larvae to the second stage larvae with their clearly discernible moulted cuticles on the thirteenth day of the experiment, a quantity from the culture dish was again fed to a white mouse. The infected animals did not show any sign of infection. When the cultures were seventeen days old, the feeding experiment of the white mice was repeated and all the infected animals died from Ascaris pneumonia. Ascaris larvae were recovered from the mice lungs thus showing that the eggs were then infective. The experiment was then terminated at the end of the eighteenth day. The whole experiment was repeated

using Ascaris suum eggs of pigs and the same results were obtained. The results recorded here are those of Ascaris lumbricoides of man. For the field conditions, Ascarid eggs were cultured in both distilled water and in 2% formalin and were incubated in pair thus:

(i) A pair consisting distilled water and 2% formalin cultures were incubated under direct sunlight;

(ii) Another pair under grasses and the last pair under big trees.

The cultures were examined daily and the temperatures measured with maximum and minimum thermometers. The average temperature under direct sunlight was 42°C, that of under grasses was 30°C and the temperature under the trees was 22°C. The aqueous media were replenished, as they dried up in cultures under direct sunlight, under grasses and under the tree.

RESULTS

Figs. 1 and 2 show Ascaris eggs with different stages of development. Fig. 2 particularly shows unfertilized egg showing vacuolated protoplasm with highly refractile granules; unsegmented fertile egg; advanced morula stage of development and early larval stage of development.

Fig. 3 shows fertile egg with advanced morula stage of development while Fig.4 shows tadpole-like larva inside the egg-shell and also first stage larva. Fig.5 shows second stage larva coming out of the egg-shell while Fig.6 shows second stage larva expressed out of the egg-shell with its clearly discernible sheath of the larva.

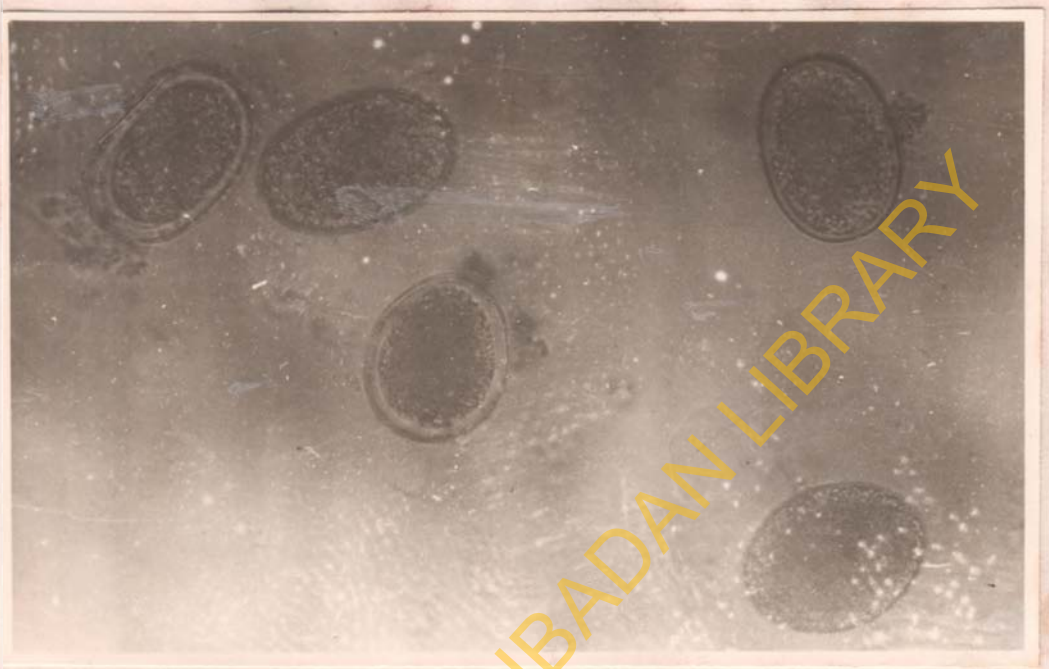

FIG.1 - Showing different stages of *Ascaris* development
2 days old x 200

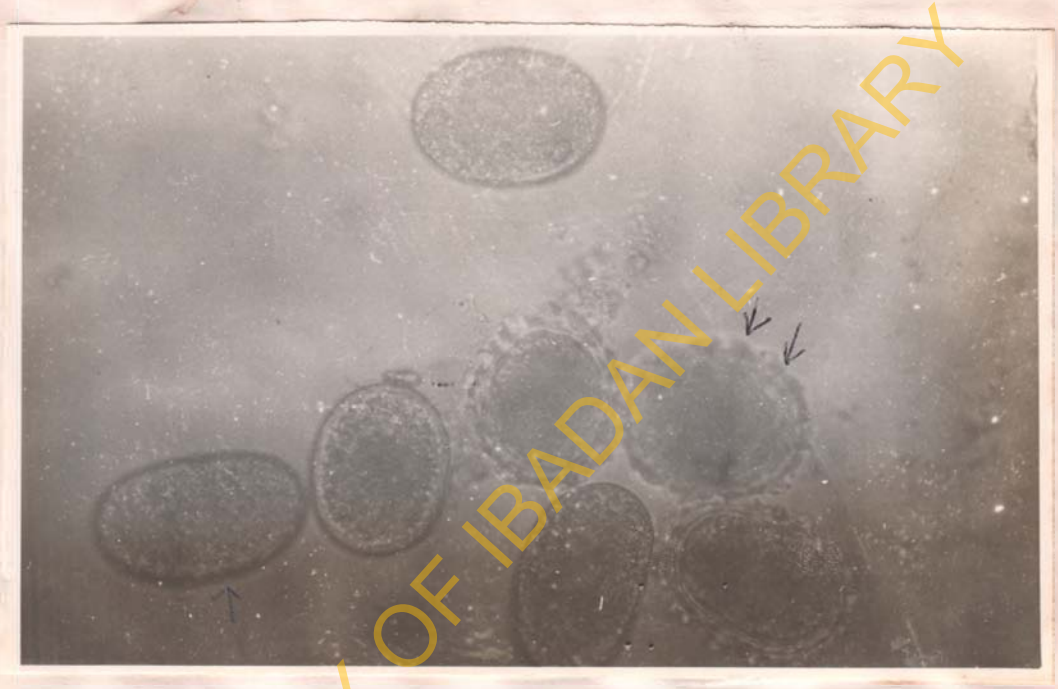

FIG.2 - Showing different stages of development. Notice both fertile and infertile eggs 2 days old x 200

FIG 3: - Showing fertile egg with advanced morula stage of development 3 days old x 200

FIG.4: - Showing Tadpole-like larva and first stage larva inside the egg-shells 4 days old x 200

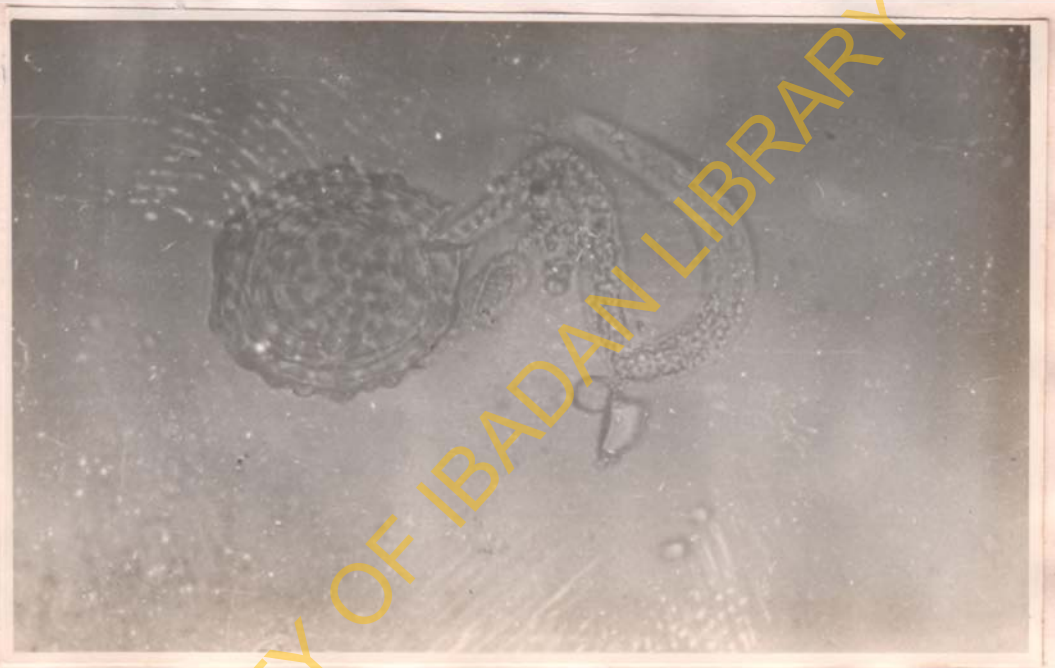

FIG.5:- Showing second stage larva coming out of egg-shell 13 days old x 200

FIG.6: - Showing 2nd stage larva which has been expressed out of the egg-shell. Notice the sheath of the infective larva and the egg-shell. 15 days old x 200

TABLE 1:

DEVELOPMENT OF ASCARIS LUMBRICOIDES EGGS IN DISTILLED WATER AND IN FORMALIN AT 27°C

DAY	TOTAL COUNTS Total Counts	ONE CELL No.	%	TWO CELLS No.	%	FOUR CELLS No.	%	EARLY MORULA No.	%	ADVANCED MORULA No.	%	PRE-LARVAL STAGE No.	%	TADPOLE LARVA No.	%	FIRST STAGE LARVA No.	%	2ND STAGE LARVA No.	%
1	1293	1293	100																
2	1033	14	1.4	303	29.3	498	48.2	209	20.2	9	0.9								
3	1129	10	0.9	58	5.1	101	8.9	49	4.3	911	80.7	14	1.2						
4	1040	11	1.1	9	0.9	26	2.5	14	1.3	895	86.1	76	7.3	12	1.2				
5	1072	10	0.9	1	0.09	2	0.19	0	0	762	71.1	241	22.5	56	5.2				
6	1013	9	0.9	0	0	0	0	0	0	281	27.7	455	44.9	268	26.5				
7	1003	8	0.7	0	0	0	0	0	0	11	1.0	20	1.8	887	80.5	76	16.0		
8	1241	11	0.9	0	0	0	0	0	0	0	0	7	0.6	412	33.2	811	65.4		
9	1232	10	0.8	0	0	0	0	0	0	0	0	0	0	239	19.4	983	79.8		
10	1219	11	0.9	0	0	0	0	0	0	0	0	0	0	74	6.1	1134	93.0		
11	1083	10	0.9	0	0	0	0	0	0	0	0	0	0	19	1.6	1054	97.3		
12	1070	11	1.0	0	0	0	0	0	0	0	0	0	0	0	0	1059	98.9		
13	1088	13	1.2	0	0	0	0	0	0	0	0	0	0	0	0	1041	96.0	34	3.1
14	1172	11	0.9	0	0	0	0	0	0	0	0	0	0	0	0	1001	86.0	154	13.1
15	1008	11	1.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	997	99.0
16	1015	11	1.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1004	99.0
17	1086	10	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1076	99.0
18	1061	11	1.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1050	99.0

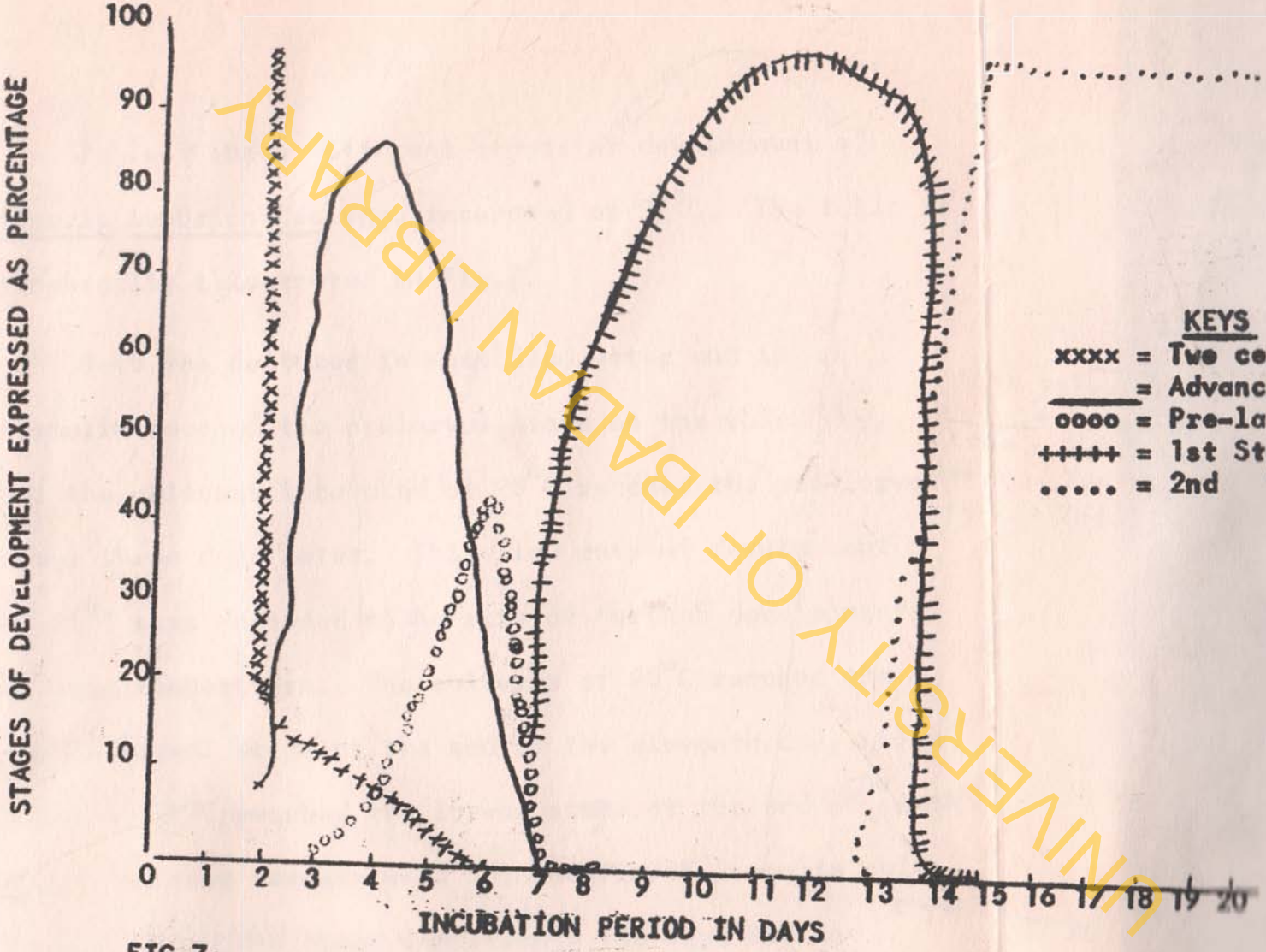

FIG.7:

DEVELOPMENT OF ASCARIS LUMBRICOIDES EGGS IN DISTILLED WATER AND IN 2% FORMALIN AT 27°C.

Table 1 shows different stages of development of Ascaris lumbricoides eggs incubated at 27°C. The table is graphically illustrated in Fig.7.

Both the cultures in distilled water and in 2% formalin reached the prelarval stage on the third day, but the cultures incubated at 25°C reached the pre-larval stage three days later. This slow rate of development at 25°C also happened to the rate of further development at this temperature. The cultures at 25°C reached the motile larval stage at the end of the eleventh day, while those at 27°C reached the larval stage at the end of the sixth day. The same results were obtained in 2% formalin cultures. Under the field conditions, the eggs did not develop beyond the 4-8 cell stages at 42°C. Under the grasses, the Ascaris lumbricoides eggs developed to the first stage larvae in 15 days and under big tree, the eggs developed to the first stage larvae in 21 days.

DISCUSSION

Fertilised eggs of Ascaris species require a period of incubation during which development and maturation takes place before they can be infective to the next host. This fact shows that immediate transfer of Ascaris eggs from one host to another does not happen in Ascaris infection. The eggs will have to undergo further development in the outer world before infection of the next host can take place. Ascaris eggs are complex organisms and before they can infect another animal must go through a period of development and adjustment. The percentage of fully embryonated eggs which develop and the rapidity of development depend on several environmental conditions. Commonest amongst these conditions are moisture, temperature, and oxygen.

Under laboratory conditions, the three pre-requisite requirements are provided for by cluturing the eggs in aqueous media and incubating them at favourable temperature. The dividing egg has a very high oxygen requirement and the development of the eggs incubated at 27°C (Table 1) reached the pre-larval stage on the third day. On the fifth day, the eggs developed into the tadpole-like larvae. Motile larvae clearly discernible inside the egg-shell were present at the end of the sixth day. This result does not agree with the findings of Tiner (1953) who reported that the development of Ascaris eggs reached the first stage larvae in 17 days, but the result of the 25°C cultures confirms the findings of Schwartz (1922) and Wharton (1915). The result of this study also confirms the findings of Refuerzo & Alis Jimenez (1954) and the result shows that the four-day difference between the two investigations were due to the 2°C difference in temperature.

This study shows that the use of 2% formalin had no effect whatsoever on the development of the eggs because both the eggs cultured in formalin solution and in ordinary distilled water developed to the first and second stage larvae at the same time.

There was an interval of about seven days between the development of the first stage larva and the second stage larva, giving a total period of about thirteen days for the unsegmented eggs to develop to the second stage larvae (Table 1). This interval is necessary because the first stage larvae (L_1) needs to feed vigorously and grows within the period. Furthermore, within the interval, there is always a stage of lethargus at which time the larva does not feed before it moults to the second stage larva.

In nature, the eggs of Ascaris lumbricoides and Ascaris suumtake ten days before the first stage larvae are formed and another eight days before the first stage larvae develop into the second stage larva (Dunn, 1978).

Therefore, the short incubation period of thirteen days from the unsegmented eggs to the formation of second stage larvae, in this study, is only possible under laboratory conditions. This observation is made plausible by the findings of Davey and Crewe (1973) who reported that the eggs of Ascaris developed to the infective second stage larvae in a suitable environment in about a month. This long incubation period under the field condition where the two most important bionomic factors are temperature and moisture is probably due to the prevention of proper aeration by the faecal pad and also to unstable temperature both in the day and the night.

The time interval between the first stage larvae and the second stage larvae is necessary because during this period the larvae must develop all necessary organs for their existence in the definitive host.

Alicata (1934) had shown that in eggs of certain members of the family Ascaridae (Ascaris lumbricoides, Ascaris suum, Parascaris equorum, Toxocara canis, Toxocara leonina), moulting is a pre-requisite for infectivity. The results of this study confirm his findings because the first stage larvae failed to infect the white mice. The results show that the first stage larvae were not infective to the mice because the larvae at this stage of development cannot survive in the animals and they died, for in the early stages of this nematode the larvae are adapted to free-living conditions (Dunn, 1978). Furthermore, the first stage larvae are incapable of secreting the hatching fluid which breaks down the egg shells and the sheaths. This observation is in agreement with Roger (1958) who showed that the larvae of ascarids secrete a moulting fluid which breaks down the egg shell to release itself. The results of this study also show that the second stage larvae failed to infect the mice

immediately the larvae reached the second stage. This shows that the second stage larvae still require few days post moulting period before they could become infective. The interval between the time the larvae moulted to the second stage larvae and the time they became infective seemed to be connected with the development of certain organs responsible for the secretion of the hatching fluid. The second stage larvae which are parasitic can infect the mice by ingestion. It is when the second stage larvae arrive in the stomach, hatch and cast off their sheaths that they can bore their way through the stomach wall. There are two factors responsible for the exsheathing, namely, the extrinsic and the intrinsic factors. The extrinsic factor is the preliminary stimulus and the factor which induces it is present in the gastric and duodenal contents. The action of the extrinsic factor results in the release by the larva itself of the intrinsic factor and it is this factor which accomplishes the process

of exsheathing. The secretory region for the intrinsic exsheathing fluid lies anteriorly in the larva between the excretory pore which is in itself about the mid oesophageal area and the base of the oesophagus. It is the infective stage larvae that possess this excretory pore thus the failure of newly formed second stage larvae to infect the mice. Finally, the results of this investigation show that the number of the infective larvae rose to a maximum by the close of the experiment, and the infective larvae (Figs 5 and 6) were typical sheathed filariform larvae (Fig.6). The study shows that the use of thermostatically controlled incubators for culturing the eggs of ascarids or other helminths will reduce the unnecessary lengthy period of incubation especially when there is not much time at the disposal of one carrying out the experiment. Under the field conditions, ascarid eggs failed to develop to the larval stage in six days as compared with the laboratory results. The eggs failed totally

to develop into larval stage under the direct sunlight. This probably shows that the sunlight had a direct lethal solar effect on the eggs. The average temperature recorded under direct sunlight was 42°C and this temperature was capable of destroying the eggs before larval formation. Wharton (1915) showed that at temperature around 37°C, ova of Ascaris lumbricoides developed to the eight-cell stage and then died. The results of this study agree with his findings. The results also agree with the work of Refuerzo & Albis-Jeminez (1954) who showed that Ascaris eggs were destroyed in five hours, thirty minutes when exposed to direct sunlight at temperature between 34°C and 42°C. The findings in this study show that in the tropical areas where people defaecate on big stones directly exposed to sunlight, Ascariasis may be relatively low as most of the eggs would have been destroyed by the direct sunlight. Again, the result here shows that direct solar effect could be used as a means of Ascaris control because once

the Ascaris eggs have failed to develop to larvae infection of people would be impracticable.

The results of the Ascaris eggs incubated under the grasses at an average temperature of 30°C shows that the eggs were capable of development to the larval stages in 15 days. This finding is not in agreement with the laboratory experiment of the egg cultures in which the eggs developed to the larval stage on the sixth day at 27°C; a temperature which is lower than 30°C. The explanation to this could probably be due to the drop in temperature during the nights and the early mornings of the period of experiment; the temperature was not constant at 30°C unlike the one in the laboratory with thermostatically controlled temperature. The finding, however, is in agreement with the result of the work of Brown (1927) who showed that eggs of Ascaris incubated under the field condition at 30°C, developed to the motile embryo in 15 days. He considered

this temperature to be the optimum. The result is also in accordance with the findings of the work of Fanret-Fremiet (1913) who showed that the ova of Ascaris suum are capable of development at varying temperatures below 35°C, but that the optimum temperature is about 30°C.

The results of the eggs culture incubated under the big tree at an average temperature of 22°C shows that the eggs developed into larval stage on the twentyfirst day of the experiment. This shows the slow rate of development at lower temperature. This slow rate of development was not unexpected as it is natural that under lower temperature, the metabolic rate of any living animal is greatly reduced. The slow rate of development showed the ability of the tree to cut off the lethal solar effect, to reduce the temperature and to prevent evaporation of aqueous media used. The tree also prevents the destruction of the eggs by the lethal solar effect. These findings

confirm Spindler's (1940) view that under natural conditions, ascarid eggs are protected by the faecal mass during early stages of development. It is likely that the embryonated eggs under this condition will survive for quite a long time. The observations here are made plausible by the findings of Refuerzo & Albis-Jeminez (1954) who showed that ascarid eggs under legume (Pueraria javanica) survived for 76 days while the eggs under grasses (Pollina fulva) survived for 66 days. This survival period is very important in the epidemiology of ascariasis and this could probably account for the long survival of the eggs in shaded moist environments in tropical areas.

CHAPTER 2

3.2 ACTION OF INTESTINAL BACTERIA ON THE DEVELOPMENT OF ASCARIS EGGS IN-VITRO

INTRODUCTION:

Although Grzyb & Szydlowska (1964) studied the effect of antibiotics on Ascaris suum eggs in cultures of Escherichia coli and Proteus and found that the bacteriostatic doses of antibiotics acted ovostatically, little or no work has been done on the effect of monocultures of intestinal bacteria on the development of ascarid eggs in-vitro. Therefore, in this study, the action of monocultures of intestinal bacteria on the cleavage and development of larval stages on Ascaris lumbricoides and Ascaris suum in-vitro was investigated.

MATERIALS AND METHODS

Experiment 1:

Fertile eggs of gravid female Ascaris lumbricoides were collected from uteri of gravid worms and were washed several times in sterile physiological saline (0.85% Nacl). The eggs were deshelled and sterilised with equal volumes of 3% sodium hydroxide and 3% sodium hypochlorite overnight using Hansen et al (1954 & 1956) method. The eggs were subsequently washed 4-6 times with sterile saline to remove the deshelling solution. They were then checked bacteriologically for their sterility by culturing them onto enriched and differential media and into Brewer's thioglycollate medium for both aerobic and anaerobic organisms.

The washed sterile eggs were suspended in small volume of sterile normal saline and were counted using improved Neubauer counting chamber. Ten thousand of the prepared eggs were put in each of ten 6½x⅝inch long test tubes

containing an overnight broth cultures of Excherichia coli; Proteus mirabilis, Staphylococcus aureus, Streptococcus faecalis (Enterococci), Bacillus subtilis, Bacillus cereus, Pseudomonas aeruginosa and Clostridium welchii (in Brewer's medium). A control tube containing eggs in sterile nutrient broth without bacteria was put up. The tubes were covered back aseptically with their sterile cotton wool covers. They were incubated at 30°-31°C for 14 days. One-day old cultures of bacteria were pipetted off using sterile Pasteur pipettes and a fresh sterile nutrient broth was added to a level of about one inch above the bottom of the tube. Brewer's medium was used for Clostridium welchii culture and the culture incubated anaerobically using Anaerobic jar. Each tube was examined everyday under the light microscope for its development. During this incubation and re-incubation periods, control egg cultures were tested for their sterility and the bacterial-egg-cultures for their monoculture state and their development.

This process requires strict aseptic precaution under the microflow cabinet.

After the incubation period of 14 days all the bacterial-egg-cultures were disinfected using clorox solution. After an overnight incubation in this solution - which is the same solution as that used to sterilise the surface of the eggs at the beginning of this exercise, the eggs were washed several times with sterile saline. This treatment removed both the bacteria and their metabolic by-products. After the last washing, each tube was tested for sterility as previously described Fresh sterile nutrient broth was added to each tube and fresh Brewer's medium added to Cl.welchii tube. All the tubes were incubated again at 30-31°C for a further period of 14 days.

Experiment 2:

Quantitative Determination of the used Bacteria Species

Dilutions of the overnight broth cultures of the test bacteria were carried out. The surface viable count of Miles & Mizra (1938) was used. This method of utilizing dropping-pipette is probably the most accurate of the viable count techniques (Baker, 1967).

Required:

50-drop pipettes
Extra dried nutrient agar plates
Sterile 3 x ½ inch tubes.

Method:

The nutrient agar plates were dried in drying oven on the tilt for two hours, to prevent a drop of fluid from running over the surface of the plates. With a grease pencil, the bottom of the plates was divided into 8 segments labelling them from 10^{-1}, 10^{-2} upwards. Using a dropping pipette 45 drops of sterile nutrient broth were added to each of 10 sterile 3x ½ inch tubes. The pipette was discarded

with a fresh dropping pipette, 5 drops of culture were added to tube 1. This pipette was also discarded. With a fresh sterile pipette the content of tube 1 was mixed ten times and 1 drop was added to segment labelled 10^{-1} and 5 drops were transferred to tube 2. The used pipette was discarded. With a fresh pipette, the operation with tube 2 was repeated upwards. The drops on the plates were allowed to be absorbed into the medium before the plates were incubated at 37°C for 24 hours. For accurate viable count, tubes 8,9 and 10 were used. The segment containing discrete colonies was counted. Two of such counts were made and the average found. The calculation of the number of colonies of organisms per ml was done.

The organisms used were:

Escherichia coli	-	2.0×10^{10} orgs/ml.
Staphylococcus aureus	-	7.5×10^{12} " "
Streptococcus faecalis	-	5.0×10^{12} " "
Pseudomonas aeruginosa	-	1.0×10^{11} orgs/ml.
Proteus mirabilis	-	8.9×10^{10} " "
Bacillus cereus	-	7.5×10^{11} " "

Doubling dilutions of each of the used organism were made in 10-15 sterile cotton-wool-plugged 6 x $\frac{5}{8}$ inch tubes, using fresh sterile pipette for each dilution and starting from the highest concentration to the lowest concentration of organism per ml. Four control tubes were set up for each organism - one control tube contained only nutrient broth plus eggs, the other contained distilled water plus eggs. One control tube contained nutrient broth only and the fourth control tube contained distilled water only.

Ten thousand eggs treated as in experiment 1 above was added to each tube including the controls except one tube containing nutrient broth only and one tube containing distilled water only. These two tubes were put up to test for the sterility of the broth, the distilled water and the egg. The tubes were incubated at 30-31°C for 14 days. The procedures used with the overnight broth cultures were also used in this series for checking for the development or otherwise of the eggs.

Experiments 1 and 2 were repeated using the eggs of Ascaris suum. The same results as in Ascaris lumbricoides eggs were obtained, so the data recorded here are for Ascaris lumbricoides of man.

RESULTS

The result of the bacteriological test on deshelled eggs after an overnight incubation period on different media was negative. The control tubes containing eggs plus nutrient broth/distilled water remained sterile throughout the period of the investigation; and the eggs in the control tubes developed into motile first and second stage larvae between the sixth and the fourteenth day of development (Table 1). Egg cultures in Gram-negative bacterial cultures with the exception of those in Pseudomonas aeruginosa developed into two-cell stages. Those eggs in culture of Bacillus species also did not develop at all. The use of Clorox (3% sodium hydroxide and 3% sodium hypochlorite) to sterilise the bacteria-egg-cultures did not

affect the protoplasm of the eggs as the eggs after washing in sterile saline and cultured in nutrient broth developed to larval stages.

Table 1, shows the effect of overnight broth cultures on the development of fertile Ascaris lumbricoides eggs. In this concentration, the eggs were found to have multiplied only to 2-cells stages in some cultures and there was no development at all in others. Table 2 shows the development of eggs into various developmental stages after killing off the bacteria with Clorox. Table 3 shows the development in various stages in dilutions of Escherichia coli, there was no development beyond 2-cell stage up to the concentration of 20×10^6 organism/ml, but the eggs started to develop as the number of bacteria decreased in the tubes.

Effect of Stapylococcus aureus on the development of the Ascaris eggs

In the cultures of this organism the eggs started to develop beyond 2-cell stage at the concentration of 75×10^3 organism/ml and the development reached the motile larval stage at a concentration of 750 organisms per ml and to a

second stage larva at a concentration of 0.75 orgs/ml (Table 6).

The Effect of Streptococcus faecalis on the development of the ascarid eggs

Table 5 shows this result. The higher concentrations of the bacteria inhibited the development beyond the 2-cell stage. However, as the bacteria decreased in number, the eggs started to develop. The development reached the motile larval stage at a concentration of 50.5 orgs/ml thus the critical concentration was 505 $\times 10^3$ organism per ml.

The Effect of different concentration of Pseudomonas aeruginosa on the development of Ascaris eggs

In the dilution of this organism, the eggs failed to develop until there were only 1000 organism per ml in the broth culture (Table 7). The eggs developed into larval stages where the tube contained almost no organism per ml of the broth, that is, when the concentration was 0.1 organisms per ml. The last dilution of this organism at which

the ascarid eggs will not develop was 10,000 organisms per ml.

The effect of different concentrations of Proteus mirabilis on the development of eggs is shown in Table 8. In this dilution of the organism, the eggs did not devekop at all until the bacterial concentration was reduced to 8900 organisms per ml. The development reached the first stage larva at a concentration of 8.9 organisms per ml. The critical concentration at which the eggs will not develop at all was the undiluted overnight broth culture.

TABLE 1: SHOWING DEVELOPMENT OF ASCARIS LUMBRICOIDES EGGS IN OVERNIGHT BROTH CULTURES OF DIFFERENT BACTERIAL SPECIES

DAY	E. coli	Pro-teus	Pseudo monas	Staph	Strep. faec.	B. sub tilis	B cereus	Cl. wel chii	CONTROLS Nut. bro.	CONTROLS Dis. water
1	-	-	-	-	-	-	-	-	-	-
2	2	2	-	2	2	-	-	-	2	2
3	2	2	-	2	2	-	-	-	4	4
4	2	2	-	2	2	-	-	-	M	M
5	2	2	-	2	2	-	-	-	TP	TP
6	2	2	-	2	2	-	-	-	L1	L1
7	2	2	-	2	2	-	-	-	L1	L1
8	2	2	-	2	2	-	-	-	L1	L1
9	2	2	-	2	2	-	-	-	L1	L1
10	2	2	-	2	2	-	-	-	L1	L1
11	2	2	-	2	2	-	-	-	L1	L1
12	2	2	-	2	2	-	-	-	L1	L1
13	2	2	-	2	2	-	-	-	L2	L2
14	2	2	-	2	2	-	-	-	L2	L2

KEYS:
- \- = No Development
- 2 = 2-cells stage
- 4 = 4-cells stage
- 8 = 8-cells stage
- M = Morula stage
- TP = Tadpole larva
- L1 = 1st stage larva
- L2 = 2nd stage larva

TABLE 2: SHOWING THE DEVELOPMENT OF ASCARIS LUMBRICOIDES EGGS AFTER KILLING THE BACTERIAL SPECIES WITH CLOROX

DAY	E. coli	Pro-teus	Staph.	Strep. faec.	B. subtilis	B. Cereus	Nut. Broth	Dis. water
1	-	-	-	-	-	-	-	-
2	2	2	2	2	2	2	2	2
3	4	4	4	4	4	4	4	4
4	M	M	M	M	M	M	M	M
5	M	M	M	M	M	M	M	M
6	TP	TP	TP	TP	TP	TP	TP	TP
7	L1	L1	L1	L1	L1	L1	L1	L1
8	L1	L1	L1	L1	L1	L1	L1	L1
9	L1	L1	L1	L1	L1	L1	L1	L1
10	L1	L1	L1	L1	L1	L1	L1	L1
11	L1	L1	L1	L1	L1	L1	L1	L1
12	L1	L1	L1	L1	L1	L1	L1	L1
13	L2	L2	L2	L2	L2	L2	L2	L2
14	L2	L2	L2	L2	L2	L2	L2	L2

KEYS:

- \- = No development
- 2 = 2-cells stage
- 4 = 4-cells stage
- 8 = 8-cells stage
- M = Morula stage
- TP = Tadpole larva
- L1 = 1st stage larva
- L2 = 2nd stage larva

TABLE 3: SHOWING THE EFFECT OF DIFFERENT CONCENTRATION OF ESCHERICHIA COLI ON THE DEVELOPMENT OF ASCARIS LUMBRICOIDES EGGS

TUBE NO.	E.coli Concentration In Broth	Incubation Period in Days	RESULTS
1	20×10^9	1	-
2	20×10^8	2	2
3	20×10^7	3	2
4	20×10^6	4	2-4
5	20×10^5	5	4-8-M
6	20×10^4	6	TP
7	20×10^3	7	L1
8	20×10^2	8	L1
9	20×10	9	L1
10	20	10	L1
11	2	11	L1
12	0.2	12	L1
13	0.02	13	L2
14	0.002	14	L2
15	0.0002		L2

KEYS:

-	=	No Development	M	=	Morula stage
2	=	2-cells stage	TP	=	Tadpole larva
4	=	4-cells stage	L1	=	1st stage larva
8	=	8-cells stage	L2	=	2nd " "

TABLE 4: SHOWING THE RESULTS OF THE FOUR CONTROLS SET UP FOR EACH SPECIES OF BACTERIA USED USING ASCARIS LUMBRICOIDES EGGS.

DAY	Nutrient broth only	Nutrient broth plus eggs	Distilled water only	Distilled water plus eggs
1	RS	-	RS	-
2	RS	2	RS	2
3	RS	4	RS	4
4	RS	8	RS	8
5	RS	M	RS	M
6	RS	TP	RS	TP
7	RS	L1	RS	L1
8	RS	L1	RS	L1
9	RS	L1	RS	L1
10	RS	L1	RS	L1
11	RS	L1	RS	L1
12	RS	L1	RS	L1
13	RS	L2	RS	L2
14	RS	L2	RS	L2

KEYS:

-	=	No Development
2	=	2-cells stage
4	=	4-cells stage
8	=	8-cells stage
M	=	Morula stage
TP	=	Tadpole larva
L1	=	1st stage larva
L2	=	2nd " "
RS	=	Remained Sterile.

TABLE 5: SHOWING DEVELOPMENT OF ASCARIS LUMBRICOIDES EGGS IN MONOCULTURE DILUTIONS OF STREPT. FAECALIS DURING 14 DAYS INCUBATION PERIOD

TUBE NO.	Strept. faecalis concentration in broth	Incubation period in Days	RESULTS
1	505×10^8	1	-
2	505×10^7	2	2
3	505×10^6	3	2
4	505×10^5	4	2
5	505×10^4	5	2
6	505×10^3	6	2
7	505×10^2	7	4
8	505×10	8	8-M
9	505	9	TP
10	50.5	10	L1
11	5.05	11	L1
12	0.505	12	L1
13	0.0505	13	L2
14	0.00505	14	L2
15	0.000505		L2

KEYS

-	=	No development	M	=	Morula stage
2	=	2-cells stage	TP	=	Tadpole larva
4	=	4-cells stage	L1	=	1st stage larva
8	=	8-cells stage	L2	=	2nd " "

TABLE 6: SHOWING DEVELOPMENT OF ASCARIS LUMBRICOIDES EGGS IN VARIOUS DILUTIONS OF AN OVERNIGHT BROTH CULTURE OF STAPHYLOCOCCUS AUREUS

TUBE NO.	Staph. aureus concentration in broth	Incubation period in Days	RESULTS
1	75×10^{11}	1	-
2	75×10^{10}	2	2
3	75×10^{9}	3	2
4	75×10^{8},	4	2
5	75×10^{7}	5	2
6	75×10^{6}	6	2
7	75×10^{5}	7	2
8	75×10^{4}	8	4
9	75×100^{3}	9	8 and M
10	75×10^{2}	10	TP
11	75×10	11	L1
12	75	12	L1
13	7.5	13	L2
14	0.75	14	L2
15	0.075		L2

KEYS:

-	=	No development	M	=	Morula stage
2	=	2-cells stage	TP	=	Tadpole larva
4	=	4-cells "	L1	=	1st stage larva
8	=	8-cells "	L2	=	2nd " "

TABLE 7: SHOWING DEVELOPING ASCARIS LUMBRICOIDES EGGS IN VARIOUS DILUTIONS OF PSEUDOMONAS AERUGINOSA

TUBE NO.	Pseudomonas concentration in broth	Incubation period in Days	RESULTS
1	10×10^9	1	-
2	10×10^8	2	-
3	10×10^7	3	-
4	10×10^6	4	-
5	10×10^5	5	-
6	10×10^4	6	-
7	10×10^3	7	-
8	10×10^2	8	2
9	10×10	9	2
10	10	10	4
11	1	11	8 and M
12	0.1	12	TP
13	0.01	13	L1
14	0.001	14	L2
15	0.0001		L2

KEYS:

-- = No development
2 = 2-cells stage
4 = 4-cells "
8 = 8-cells "
M = Morula stage
TP = Tadpole larva
L1 = 1st stage larva
L2 = 2nd " "

TABLE 8: SHOWING THE EFFECT OF DIFFERENT CONCENTRATIONS OF PROTEUS MIRABILIS ON THE DEVELOPMENT OF ASCARIS LUMBRICOIDES EGGS

TUBE NO.	Proteus concentration in Nutrient broth	Incubation period in Days	RESULTS
1	89×10^8	1	-
2	89×10^7	2	2
3	89×10^6	3	2
4	89×10^5	4	2
5	89×10^4	5	2
6	89×10^3	6	2
7	89×10^2	7	4
8	89×10	8	8 and M
9	89	9	TP
10	8.9	10	L1
11	0.89	11	L1
12	0.089	12	L1
13	0.0089	13	L2
14	0.00089	14	L2
15	0.000089		L2

KEYS:

-	=	No development	M	=	Morula stage
2	=	2-cells stage	TP	=	Tadpole larva
4	=	4-cells "	L1	=	1st stage larva
8	=	8-cells "	L2	=	2nd " "

TABLE 9: SHOWING THE EFFECT OF DIFFERENT CONCENTRATIONS OF BACILLUS CEREUS ON THE DEVELOPMENT OF ASCARIS LUMBRICOIDES EGGS

TUBE NO.	Bacillus concentration in Nutrient broth	Incubation period in Days	RESULTS
1	75×10^9	1	-
2	75×10^8	2	-
3	75×10^7	3	-
4	75×10^6	4	-
5	75×10^5	5	-
6	75×10^4	6	-
7	75×10^3	7	-
8	75×10^2	8	2 and 4
9	75×10	9	8 and M
10	75	10	TP
11	7.5	11	L1
12	0.75	12	L1
13	0.075	13	L2
14	0.0075	14	L2
15	0.00075		L2

KEYS:

-	=	No development	M	=	Morula stage
2	=	2-cells stage	TP	=	Tadpole larva
4	=	4-cells "	L1	=	1st stage larva
8	=	8-cells "	L2	=	2nd " "

Table 9 shows the result of the egg development in various concentrations of Bacillus cereus. Development did not begin until the number of organisms per ml dropped to 75,000 organisms per ml. Thereafter, the development got to the first larval stage at a concentration of 75 organisms per ml. thus showing increased ovostatic action of this bacterium on the egg.

DISCUSSION

Table 1 shows that almost all the species of bacteria used in this study were capable of unhibiting the cleavage and development of both the human and the porcine Ascaris eggs. According to Stefanski & Przyjalkowski (1965 & 1966), some helminth eggs need intestinal bacterial flora for their growth and maturation. On the other hand Przyjalkowski & Jaskowski (1968) and Przyjalkowski 1973 a and b) found that eggs of Ascaris suum, Ascaridia galli and Aspicularis tetraptera developed well without a bacterial flora in their

environment. The findings in this study are therefore in total agreement with the observation of the above-named workers. The inhibition of development of Ascaris lumbricoides eggs by the cultures of bacteria added to sterile broth was found to be ovostatic which is anatogous to bacteriostatic action exhibited by some antibiotics on certain organisms. In this case when cells which are inhibited by the presence of a bacteriostatic agent are removed by centrifugation, washed thoroughly in the centrifuge and resuspended in fresh growth medium, they will resume normal multiplication. In this study, the eggs of Ascaris lumbricoides that were freed from bacteria and were resuspended in sterile hutrient broth developed to motile larval stages after a period of re-incubation. This shows that the ovostatic action of bacteria occured only in the presence of living and actively multiplying bacterial cells. This finding agrees with the observation of Przyjalkowski (1973), who observed

that the ovostatic action of bacteria was not effected with the metabolic products of bacteria present in the filtrate of bacterial cultures or with unfiltered culture of bacteria killed after 48 hours of incubation. Furthermore, the ovostatic action of bacteria might be due to respiration processes. These processes are based on the oxidation-reduction reactions and, in the case of bacteria are, connected with= oxygen consumption. This assumption is made plausible by the observation in the study of egg development in cultures of strict aerobes and facultative anaerobes. The use of Clostridium welchii incubated anaerobically did not allow the development of Ascaris lumbricoides eggs because under anaerobic conditions, oxygen content in the anaerobic jar was replaced with hydrogen gas thereby leaving no oxygen for the growth and development of the ascarid eggs. Pseudomonas aeruginosa is a strict aerobe commonly used for the cultivation of facultative and obligatory anaerobes because its growth requires oxygen consumption from the environment.

The ovostatic action of Escherichia coli was much reduced (Table 3) because Escherichia coli can grow well in the presence of a trace of free oxygen. Therefore, motile larvae were formed in a concentration of 20 x 10^3 organisms per ml., the highest concentration to support the development of Ascaris lumbricoides eggs to larvae in this investigation. Tables (5 and 6) show that the organisms used were capable of growing both in the presence and absence of oxygen. Therefore, their ovostatic action was more than that of E.coli. There is a good reason to suggest that the marked inhibitory action exhibited by Pseudomonas aeruginosa and Bacillus cereus in Tables 7 and 9 might be due to the production of certain enzymes and antibiotic substances by the two organisms. In addition, to the fact that Pseudomonas aeruginosa is a strict aerobe which requires all the available oxxgen for its growth, thus depriving the eggs of Ascaris lumbricoides of necessary oxygen requirement for further development,

this organism produces an antibiotic substance called bacteriocine which acts ovostatically on Ascaris ova (Grzyb &Szydlowska, 1964). In the case of Bacillus species, there are many antibiotic substances elaborated by this organism. Some of these antibiotics are bacitracin, polymyxinB and colistin. All these substances act ovostatically on the eggs of the ascarids. Furthermore, Bacillus species produce certain enzymes especially proteases which are capable of inhibiting Ascaridae in-vitro (Emanuilov, 1958).

The lack of inhibitory action or phenomenon in the control tubes containing only nutrient broth and distilled water was therefore due to the absence of organism which made the abundant oxygen present in the tubes to be available for the use of the Ascaris egg development.

The ovostatic phenomenon is explained by the fact that the Ascaris eggs cannot start cleavage or continue further development without oxygen. The oxygen present in a liquid medium is consumed rapidly by actively feeding and

multiplying bacteria which have a shorter generation time than Ascaris eggs. Bacteria growth rate is expressed in terms of generation per hour (Jawetz et al, 1970). Since all the organisms used in this study reproduce by binary fission and their generation time is 40 minutes, the growth rate of the organisms is therefore 1.5 generation per hour. Table 4, shows that there was no development of the eggs during the first day and that the eggs developed only to 2-cells stage on the second day. Comparing this development with that of the bacteria used, the bacteria is at a considerable advantage over the eggs because of its enormous population available (1.5 x 48 generations) by the commencement of egg cleavage. During this period almost all the available oxygen would have been used up by the large population of organisms, leaving little or no oxygen for the egg development. The oxidation-reduction potential of Ascaris agg which is related to its oxygen consumption, therefore, becomes very low.

The actively multiplying bacteria in a liquid medium therefore act ovostatically by completely consuming the oxygen which is necessary for the development of Ascaris eggs.

The observations in this study confirm the work of Grzyb & Szydlowska (1964), which showed that bacteriostatic doses of antibiotic on Ascaris suum eggs in cultures of E.coli and Proteus acted ovostatically. The results of the study also agree with the findings of Iwanczuk & Dozanska (1957) who showed that there was inhibition of cleavage of Ascaris lumbricoides eggs in a town sewage, but that after disinfection of the sewage with chlorine at a bacteriostatic concentration, the helminth ova started their cleavage and further development.

The use of Clorox has been shown in this study to be safe for sterilising the surface of Ascaris eggs because it had no deleterious effect on the protoplasm of the eggs.

This observation agrees with that of Hansen *et al*(1954, 1956).

Finally, the results of this study show that the development of *Ascaris* eggs could be controlled in bacterial culture and this phenomenon could be used to advantage especially in storage and in transportation of *Ascaris* eggs.

CHAPTER 3

3.3 BACTERIAL FLORA OF ASCARIS SUUM (GOEZE, 1782) AND ITS RELATIONSHIP TO THE HOST FLORA.

INTRODUCTION

The earliest work on the bacterial flora of the intestine of a parasitic nematode was done by Weinberg in 1907. He found 33 Sclerostomes positive for bacteria when he examined the intestine of 97 Sclerostomes (Strongylus) from 25 horses. McCoy (1929 a & b) found that living bacteria constituted the food of hookworm larvae, Ancylostoma caninum, developing to the infective stage. He did not attempt to isolate bacteria from the intestine of the worm. Li (1933a), studied the intestinal flora of the following nematodes: Spirocerca lupi, Physaloptera clausa, Cheilospirura hamulosa, Bunostomum trigonocephalum, and Enterobius vermicularis. Plain agar was used as the isolation medium and cultures were incubated

under aerobic conditions. The dominant bacteria in the intestine of about half of the nematode belonged to the "coli group".

A series of feeding experiments was conducted by Li (1933b) on certain roundworms belonging to the Ascaroidea and Oxyuroidea. He concluded that Ascaris lumbricoides, Toxocara canis, Toxoscaris leonina, Ascaridia galli, and Heterakis gallinarum fed on the intestinal contents of their host. According to Ackert and Whitlock (1941) ascarids and oxyurids normally feed on mucus, desquamated mucosal cells, and blood elements that are free in the lumen of the intestine. The intestinal flora of chicken has been under investigation for a long time. Kern (1897) studied the intestinal flora of 24 birds and isolated 88 species of bacteria. He concluded from direct preparations of the stomach and intestinal contents that a large number of species did not grow when submitted to ordinary culture methods. Kern reported that the following species were

obligate intestinal forms: Bacterium coli, Bacillus vegatus, Pseudomonas granulota, Bacillus defessus, and Bacterium verruscosum. The last four species are not listed in the seventh edition of Bergey's Manual of Determinative Bacteriology (1957).

Rahner (1901), King (1905), Gage (1911), and Emmel (1930) reported that Escherichia coli was the predominating type of bacterium in the chicken intestine. It was observed that the microflora was most abundant in the caecum and colon and gradually decreased in numbers towards the duodenum. They concluded that diet and extrinsic factors associated with management practices could influence intestinal flora.

Shapiro and Sarles (1949), quantitatively studied the microflora of the intestine of chicks of different ages and found that newly hatched chicks had few micro-organisms in the intestinal tract. They noted that normal

flora became established in the contents of the duodenum, ileum, caecal pouches, and colon after the chicks were fed for 16 hours within 40 hours after hatching. Total numbers of bacteria were highest in the contents of the colon, ileum, and duodenum. Their results indicated that Lactobacillus species composed the most numerous group of bacteria in all the regions of the intestinal tract. Escherichia coli was found to be the predominant coliform organism and the predominant enterococci, (Streptococcus faecalis), was present in large numbers only in the caecal pouches and colon. The principal obligate anaerobe present in the intestinal tract of the chicken studied was Clostridium perfringens.

A quantitative study of the intestinal flora of chickens parasitised with Ascaridia galli and of uninfected control chickens was carried out by Bhear (1957). He reported that the numbers of bacteria increase from the gizzard toward the cloaca. The lactic acid bacteria were the most numerous group and the predominating bacteria in this group were

Lactobacillus species. The probable predominating coliform organism was Escherichia coli and the predominating enterococcus form was Streptococcus faecalis. The probable predominating obligate anaerobe was Clostridium perfringens.

Przyjalkowski (1961) studied the bacteria flora of the intestine of Parascaris equorum and the small intestine of a horse and reported that mostly Staphylococcus and Escherichia coli were isolated both from the contents of the parasite and that of their host. He did not isolate any anaerobic organism.

The present study was carried out in view of the paucity of information on the microflora of Ascaris suum, Goeze, 1782 of pigs and their relation to the flora of the hosts. Because of the problems of relationship between bacteria and parasites, the study also attempted to verify and eventually define the group of the bacteria on the body

surface, inside the eggs, and in the internal organs of the adult worms. Furthermore, the experiment attempted to establish the type of relationship that exists between the parasite and the bacterial flora with the hope that such information could be useful in culturing parasites in-vitro and in understanding the potential role of nematodes in transmitting pathogenic bacteria in human and animal gut.

MATERIALS AND METHODS

Experiment 1:

Adult worms were collected from abattoir. The worms were washed several times in sterile distilled water until clean. With the aid of a pair of sterile forceps, each worm, after identifying the sex, was rubbed onto selective and differential agar plates and also rinsed in Nutrient broth medium, in Brewer's thioglycollate broth and in Robertson's cooked meat broth to ensure the recovery of all bacteria adhering firmly on the body surface of the worms. The specimens of faeces were cultured onto and into selective media for the recovery of the bacteria present in them.

The adult worms were killed by dipping some of them in hot water which also helped to sterilise the body surface of the worms as was done by Przyjalkowski (1961). Some of the worms were dipped in 1 : 500 dilution of mercuric chloride. The worms were kept in the mecuric chloride disinfectant for 5 minutes as was done by Li (1933a). The worms were dissected to extract the peritoneal fluid, abdominal fluid, the intestine and the internal organs. The eggs were also recovered from the uteri.

Experiment 2:

Dissection of Adult Worms:

Dissection of female Ascaris suum:

Female Ascaris suum was laid on a sterile dissecting tray. From the mid-region of the lateral line, the worm was opened towards the anterior end using sterile dissecting instruments. Using a sterile pasteur pipette with rubber teat, the peritoneal fluid was taken and cultured onto enriched, differential and selcetive media and also into

selective liquid media; the abdominal fluid was taken and cultured accordingly. The intestine was removed and washed several times to remove the adhering fluid and it was then cut into fore-gut, mid-gut and hind-gut. Each part was then washed and opened and the contents cultured. The ovary, the uterus, the oviduct and the vagina were extracted separately, washed in sterile water, crushed and then cultured separately on various media. The cultures were incubated at 37°C for 24-72 hours aerobically, anaerobically and under carbon dioxide (CO_2).

Dissection of Male Ascaris suum:

The worm was opened from the posterior end. The peritoneal fluid and the abdominal fluid were taken and cultured. The intestine was removed and treated as in the case of the female worms. The testes, the vas deferens and the seminal vesicle were extracted separately, washed, crushed and cultured separately. All the cultures were treated as in the case of female worms.

Experiment 3:

Eggs were obtained from the uteri of adult worms. After the uteri were removed from the female worms and washed in several changes of tap water, they were placed in petridishes and the eggs teased out of each uterus. The eggs were washed by centrifugation in several changes of water. The surface of the eggs were then sterilised using equal parts of 3% NaOH and 3% solution of commercial sodium hypochlorite (5.25% NaOCl according to the formular of Elliot, 1954). The eggs in this solution were incubated at 30-33°C for 24 hours. The eggs were then washed free of clorox solution by repeated centrifugation using sterile distilled water. The eggs were cultured onto different agar and into different media to find out the sterility of their surface. The plates and liquid cultures were incubated at different atmospheres for 24-72 hours at 37°C.

Using sterile Griffith grinding tubes, the eggs were grounded in batches to release the internal contents which were cultured as before.

To determine whether the solution used to sterilise the eggs surface would kill the eggs materials, some of the clorox solution and some of the sterilised eggs were cultured together in sterile tubes according to the method of Hansen et al (1954)and (1956). The cultures were placed in an incubator with a temperature of 30°-33°C for 14 days. At the end of the 14 days period, the eggs were examined under the light microscope for the development of motile larvae inside them.

Experiment 4:

Faeces cultures

Samples of the intestinal contents of the pigs harbouring the adult worms were collected into sterile screw-capped containers at the abattoir. The samples were cultured onto different media and into various liquid

media. The cultures were incubated at 37°C in different atmospheres for 24-72 hours. Cultures were examined macroscopically and microscopically to determine the species of bacteria present.

Biochemical tests were performed and each species of bacteria identified.

Bacteriological Examination

Bacteria on the body surface, in the contents of the intestine and other parts of the worms and those from different parts of the intestine of the pigs were classified into groups according to the normal flora of the pigs and also the normal flora obtained from the stool specimens of man. The following groups were used:

(1) Coliform under aerobic condition
(2) Staphylococci under " "
(3) Enterococci " " "
(4) Proteus " " "
(5) Pseudomonas " " "
(6) Bacillus " " "
(7) Lactobacilli " Microaerophilic condition.
(8) Clostridium welchii (Cl. perfringens) under anaerobic condition.

The Clostridium welchii serves as a representative of the anaerobes of the intestine.

This method of group classification was based on the example of Baron, Hansen & Lord (1960). The workers discerned in their qualitative studies only coliforms, Enterococci (Streptococcus faecalis) and a genus of Lactobacilli. The choice of the proper selective media was based on preliminary tests.

(1) Coliforms group, Proteus and other Gram-negative bacilli of the gut - an agar medium after MacConkey (1908) was used.

(2) Staphylococcus - Chapman's (1946) medium with 7.5% and 10% contents of sodium chloride and mannitol salt agar was used.

(3) Enterococci - Agar medium after MacConkey was used because on this medium the colonies of Enterococci (Streptococcus faecalis) are small usually magenta-coloured.

Studies of the anaerobic flora were conducted separately and both the worm body surface and the contents of the intestine and internal organs, the contents of the eggs and

the specimen of faeces from the intestine of pigs and man were examined. Gaspak method of anaerobiosis, using sterile blood agar plates and necessary controls was employed. Similarly, bacteriological method for isolating Clostridium perfringens, Bacteriodes fragilis using Robertson's cooked meat medium, Brewer's anaerobic thioglycollate medium and Nagler's egg-yolk medium were employed.

Controlled Investigations:

Controlled investigations were set up for the determination of the degree of selectivity of the media. At first, the quality control of the batch of media used was carried out by inoculating the determined bacteria on separate selective media. After necessary incubation period, at the right atmosphere and temperature, the test were successful and the corresponding bacteria showed good growth only on the appropriate selective media.

Present Investigation:

After the incubation of the cultures overnight, at 37°C, the culture plates were examined both macroscopically to study the colonial appearance and microscopically to study the morphology of the bacteria. Microscopical preparations stained with Gram's method were made from the colonies characteristic of coliforms, Proteus, Pseudomonas and other groups under investigation. Spore staining methods were done on all characteristic colonies on anaerobic plates. So also was Nagler's reaction for rapid detection of Cl.welchii was carried out. Biochemical and carbohydrate fermentation tests were carried out by inoculating various carbohydrate sugars such as glucose, lactose, maltose, mannitole, dulcitol, urea slope, sucrose and citrate medium. All the sugars were incubated at 37°C for 24 hours. Glucose, mannitol and lactose were fermented with the production of acid plus gas; indole was produced. Escherichia coli and Proteus organisms were motile. Proteus mirabilis

did not produce indole and citrate was not utilized by E.coli. Pseudomonas aeruginosa was oxidase positive. The colonies on MacConkey's medium that resembled Enterococci (Streptococcus faecalis) were further tested using aesculin test, and heat-resistant test. Staphylococci pathogenicity test was determined by using fresh human citrate plasma for both the slide and the tube techniques. Using the methods of Baltimore (1957), Christensen (1946), Kovacs (1956), Baker (1967), Cruickshank (1965) and Cowan & Steel (1974), the systematic positions of the other groups of the organisms were verified.

RESULTS

Table 1 shows the results of the bacteriological examination of the body surface of adult worms of Ascaris suum. From the body surface of the worms were isolated, Escherichia coli, Streptococcus faecalis (Enterococci), Staphylococcus aureus, Staphylococcus albus, Proteus vulgaris, Proteus mirabilis, Pseudomonas aeruginosa, Lactobacillus acidophilus.

Candida albicans was also isolated. There was no anaerobic organism and aerobic spore bearer isolated.

Tables 2 and 3 show that both the male and the female Ascaris suum carry in their gut and internal organs different general of bacteria in different percentages. The microscopic preparations from the materials stained for the presence of spores to demonstrate anaerobic spore-forming bacilli were negative.

Table 4 shows that the contents of the eggs of Ascaris suum harbour different genera of bacteria in various percentages. No anaerobic organism nor Lactobacillus species was isolated from the protoplasm of the eggs.

TABLE 1.

BACTERIAL FLORA OF THE BODY SURFACE OF ADULT ASCARIS SUUM

	NUMBER OF ADULT WORMS EXAMINED	NUMBER AND PERCENTAGE OF POSITIVE RESULTS													
		SPECIMEN POSITIVE FOR E. COLI		SPECIMEN POSITIVE FOR ENTEROCOCCI		SPECIMEN POSITIVE FOR STAPHYLOCOCCI		SPECIMEN POSITIVE FOR PROTEUS		SPECIMEN POSITIVE FOR PSEUDOMONAS		SPECIMEN POSITIVE FOR LACTOBACILLI		SPECIMEN POSITIVE FOR AEROBIC SPOREBEARER	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
MALE MALE ASCARIS SUUM.	26	26	100	13	50	5	19	7	27	4	15	3	12	-	-
FEMALE ASCARIS SUUM	25	25	100	14	56	4	16	7	28	3	12	1	4	-	-

N.D. CANDIDA ALBICANS WAS ISOLATED FROM THE BODY OF ONE MALE ASCARID (1(4%)

TABLE 2

EXPERIMENT 2:

BACTERIAL FLORA OF THE GUT AND INTERNAL ORGANS OF MALE *ASCARIS SUUM*

TYPE OF SPECIMEN	NUMBER OF SPECIMEN EXAMINED	SPECIMEN POSITIVE FOR E.COLI		SPECIMEN POSITIVE FOR ENTEROCOCCI		SPECIMEN POSITIVE FOR STAPHYLOCOCCI		SPECIMEN POSITIVE FOR PROTEUS		SPECIMEN POSITIVE FOR PSEUDOMONAS		SPECIMEN POSITIVE FOR LACTOBACILLI		SPECIMEN POSITIVE FOR AEROBIC SPORE BEARER	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Peritoneum fluid	26	20	76	8	31	4	15	4	15	2	8	2	8	1	4
Abdominal fluid	26	16	62	8	31	4	15	10	35	2	8	2	8	1	4
Fore-gut	26	26	100	9	34	8	31	8	31	3	12	2	8	1	4
Mid-gut	26	26	100	9	34	8	31	8	31	3	12	2	8	1	4
Hind-gut	26	26	100	9	34	8	31	8	31	3	12	2	8	1	4
Testes	26	8	31	7	27	4	15	4	15	1	4	4	15	-	-
Vas deferens	26	10	38	10	38	6	23	4	15	2	8	2	8	-	-
Seminal vesicle	26	8	31	10	38	6	23	2	8	1	4	2	8	-	--

TABLE 3

BACTERIAL FLORA OF THE GUT AND INTERNAL ORGANS OF FEMALE ASCARIS SUUM

TYPE OF SPECIMEN	NUMBER OF SPECIMEN EXAMINED	SPECIMEN POSITIVE FOR E.COLI		SPECIMEN POSITIVE FOR ENTEROCOCCI		SPECIMEN POSITIVE FOR STAPHYLOCOCCI		SPECIMEN POSITIVE FOR PROTEUS		SPECIMEN POSITIVE FOR PSEUDOMONAS		SPECIMEN POSITIVE FOR LACTO-BACILLI		SPECIMEN POSITIVE FOR AEROBIC SPORE BEARER	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Peritoneum fluid	24	18	75	8	33	4	17	4	17	3	13	2	8	-	-
Abdominal fluid	24	14	58	6	25	4	17	5	21	3	13	-	-	-	-
Fore-gut	24	24	100	8	33	5	21	6	25	4	17	6	25	2	8
Mid-gut	24	24	100	8	33	5	21	6	25	4	17	6	25	2	8
Hind-gut	24	24	100	8	33	5	21	6	25	4	17	6	25	2	8
Ovary	24	10	42	6	25	4	17	7	29	4	17	3	13	1	4
Oviduct	24	12	50	6	25	4	17	6	25	3	13	4	17	-	-
Uterus	24	12	50	8	33	5	21	6	25	1	4	3	13	-	-
Vagina	24	14	58	10	42	8	33	8	33	2	8	3	13	2	8

TABLE 4:

BACTERIAL FLORA OF THE CRUSHED EGGS OF *ASCARIS SUUM*

TYPE OF SPECIMEN EXAMINED	NUMBER OF EGG CULTURES IN BATCHES	SPECIMEN POSITIVE FOR E.COLI		SPECIMEN POSITIVE FOR ENTEROCOCCI		SPECIMEN POSITIVE FOR STAPHYLOCOCCI		SPECIMEN POSITIVE FOR PROTEUS		SPECIMEN POSITIVE FOR PSEUDOMONAS		SPECIMEN POSITIVE FOR LACTOBACILLI		SPECIMEN POSITIVE FOR AEROBIC SPORE BEARER	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
BATCHES OF CRUSHED EGGS.	32	30	94	21	66	18	56	13	41	9	28	-	-	-	-

The study on the bacterial flora of the intestinal contents of the pigs showed that all the organisms identified from the worm materials were also isolated and identified from the pig hosts. Hence from the intestine of pigs in Experiment 4 were isolated Clostridium welchii, Lactobacilli, Escherichia coli, Streptococcus faecalis (Enterococci) Staphylococcus aureus, Staphylococcus albus, Proteus vulgaris, Proteus mirabilis, Pseudomonas aeruginosa, Bacillus subtilis and Candida albicans. This shows that the specific flora of Ascaris suum and its host were similar, but unlike in the case of Ascaris suum, Clostridum welchii was isolated from the host.

Before the microflora of the eggs of Ascaris suum could be investigated, it was necessary to determine whether the clorox solution used to sterilise the egg surface would kill or injure the eggs protoplasm. Table 4 shows that the disinfectant had no deleterous effect on the contents of the eggs.

The result of the egg development at 30°C - 33°C after the use of the disinfectant (Clorox) to sterilise the egg surface showed that the solution had no lethal effect on the protoplasm as many of the eggs developed into larvae after 14 days incubation period.

DISCUSSION

The use of hot water to kill the adult Ascaris suum worms had no damaging effect on the contents of the worms. The use of 1: 500 dilution of mercuric chloride to sterilise the body surface of the adult worms also did not have any deleterous effect on the bacteria contained in the internal organs of the worms. From the results obtained, it was concluded that mercuric chloride at the concentration at which it was used and for the period of 5 minutes had a germicidal effect on the bacterial flora of the cuticle of Ascaris suum because no bacteria was isolated afterwards from the cuticle after sterilisation. Both the male and the

female Ascaris suum carry on their cuticle many genera of bacteria at various percentages. Aerobic spore bearers were, however, not isolated from the surface cuticle although this organism was isolated from the gut. The finding of different genera of bacteria on the surface cuticle of the worms is in accordance with the work of Przyljalkowski (1961) who isolated from the nutrient broth cultures different genera of bacteria from the body surface of Ascaris spp. with E.coli having the highest incidence of recovery.

The results of the dissection of adult worms are shown in Tables 3 and 4. The results show that the gut and the internal tissues of the worms harbour many bacterial species at various percentages. These results when compared with the results of the intestinal contents of the pigs, show that both the adult worms and the pigs harbour almost the same species of bacteria. The only slight difference was the isolation of anaerobic organism in the pigs but not in the ascarids. Although the flora of the intestine of the ascarids and their hosts were similar, there were increased percentage yields of the isolates especially the Enterococci and the Staphylococci from the pigs than from the ascarids. This type of results were not unexpected since Ackert & Whitlock (1941) had shown that ascarids and oxyurids normally feed on mucus, desquamated mucosal cells, and blood elemests that are free in the lumen of the intestine of the host. Furthermore, the relative size of the ascarids to the pigs could probably explain this because the

bacterial flora have more space and more nutrients in the pigs than in ascarids. Therefore, the results strongly suggest that Ascaris suum feeds on the intestinal contents including the microflora of their host. The results of this study therefore confirms the findings of Hoeppli (1927) who recorded an experiment carried out by Dr.Vogel in the Tropen-Institute at Hamburg in which a patient, positive for Ascaris was fed with powdered animal charcoal three times a day for three days. On the fourth day, a female Ascaris was expelled by an ascaricide and numerous charcoal particles were found in the intestine of the worm. The results also agree with the findings of Archer & Peterson (1929), who made an interesting observation that soon after the ingestion of a barium cereal meal by some patients, a cylindrical filling defect showing the displacement by the Ascaris specimen could be seen in the jejunum. Later, after the contrast meal had entirely passed out of the jejunum, stringlike shadows,

representing the barium-filled enteric canal of the parasite remained still for some time in the jejunum. Furthermore, the results show that the parasites possess their own bacterial flora usually similar to that of the host intestine. The results of the study are in accordance with the findings of McCoy (1929) and Li (1933a) who isolated many Escherichia coli from the intestine of some nematodes, and Li (1933b) who in a series of feeding experiments concluded that Ascarioidea and Oxyuroidea feed on the intestinal contents of their hosts. The results of this study are made plausible by the work of Tolslova (1951) who isolated from the intestine of Ascaris species 76 bacterial strains completely analagous to the microflora of the host in which these parasites lived, and Jettmar (1952) who isolated certain Gram-negative bacteria from the intestine of Parascaris equorum and Baron et al.(1960) who, comparing the bacterial flora of the small intestine of hens with that of the intestine of Ascaridia galli isolated Escherichia coli and Lactobacilli

species from both the host and the parasites.

From the results of this study it is clear that there are very good relationships between the parasite and the environment containing bacteria and this is confirmed by the observation of Liebmann (1953), that Lamblia intestinalis occurs in human intestine in large number when Escherichia coli is present in the small intestine.

The most interesting finding in this study is the presence of some species of bacteria in both the host and the parasite. This is interesting because bacterial species are highly pathogenic when once they leave their normal habitat, and Ascaris larvae and adults alike, are noted for wondering up and down the intestine of their host and into other organs off their usual route. For instance, Sprent (1955) & Mochizuki et al (1954) have shown in mice experimentally infected with Toxocara canis that the larvae of Ascaris migrated to the brain of the mice. Beautyman & Woolf(1951) had demonstrated Ascaris larva in the human brain, Woodruff & Thacker (1964). Woodruff et al(1966)&Woodruff(1968) showed tha

larvae in their migration may be capable of carrying virus and bacteria from the intestine to the brain and other tissues of their host. The wondering Ascaris larvae and adults carry with them the bacteria on their cuticle, in their gut and other tissues and they could be excreting these bacteria from time to time as long as they are feeding and developing. One good explanation for this type of association may be that both the larvae and the adult worms produce certain antibiotic substances in their intestine and other organs which render the bacteria inside them non-pathogenic to them and probably to their hosts too. It is therefore necessary to confirm conclusively the type of the antibiotic substances present inside the Ascaris suum because data recorded strongly suggest that there are some inhibitory substances inside the worms which render the harboured bacteria non-pathogenic.

Studies on the effect of Clorox disinfectant solution on Ascaris eggs revealed that the solution did not penetrate the vitelline membrane of the eggs in sufficient quantity to kill or injure the protoplasm of the egg cell. It can be assumed that the bacteria present on the protoplasm of the eggs were likewise not injured since different species of bacteria were isolated from the eggs, thus Table 4 shows that the eggs of Ascaris suum harbour different species of bacteria with E. coli on the lead; followed by Enterococci, Staphylococci, Proteus and Pseudomonas in that order. Lactobacilli and Aerobic spore bearers were not, however, isolated from the contents of the eggs. The finding of bacteria in egg contents, in the uterus, and in the ovary strongly suggests that transovarian transmission of some bacteria with Ascaris ova can not be ruled out. Although the finding of bacteria inside the Ascaris eggs is not in total agreement with the report of Baron et al (1960) who recorded very scanty growth of bacteria

from the eggs of Ascaridia galli which he examined, it supports the work of Hutchinson (1965) who established a relationship between Neoascaris egg and Toxoplasma transmission in which Toxoplasma organism were incorporated into the eggs of Neoascaris worm inbabiting the intestinal lumen of infected individuals. The finding is also in accordance with the work of Adedeji (1981) who isolated different species of bacteria from inside the eggs of Ascaris lumbricoides of man which he studied.

With all these findings, it is, therefore necessary to confirm conclusively, whether or not there is trans-ovarian transmission of some of the bacterial species recorded because data both in this study and in previous work recorded strongly suggest that this may be the case.

Finally, the findings in this study indicate that the flora of the worm is similar to that of the host in kind and in relative frequency. These data confirm those of Li (1933b) and Ackert & Whitlock (1941), who reported that ascarid parasites normally feed on the host's intestinal contents.

The data therefore demonstrate that there are mutual relationships which exist between the flora of the parasite on one hand and the host on the other. These relationships start from the early development of the parasite. The relationships that exist between the parasite and the host seem to be a mutual one as long as the worms are not many in number and as long as the worms do not wander off their usual route.

CHAPTER 4

3.4 THE EFFECT OF INTESTINAL FLORA ON THE DEVELOPMENT, INFECTIVITY AND PATHOGENICITY OF ASCARIS SUUM LARVAE IN PIGLETS

INTRODUCTION

Stefanski & Przyjalkowski (1965 & 1966) reported that normal intestinal flora favours the development of Trichinella spiralis in mice, as do also some Gram-negative bacteria: Escherichia coli, Pseudomonas aeruginosa and Proteus species given to conventional mice orally in monocultures. In subsequent studies, Stefanski & Przyjalkowski (1967), carried out some experiments with chickens infected with Ascaridia galli, mice infected with Hymenolepis nana and Aspicularis tetraptera, and rats infected with H.nana. In all these experiments, the authors observed a favourable influence of the total intestinal flora, as well as of all used Gram-negative monocultures of bacteria, viz: E.coli,

P.aeruginosa, and Proteus species. Gram-positive Bacillus subtilis also had an action like all the above mentioned Gram-negative bacteria.

In this study, apart from finding out the effect of monoaultures of some intestinal bacteria on the development, infectivity and pathogenicity of Ascaris suum larvae in piglet, the study also attempted to determine if Ascaris suum larvae are capable of disseminating bacteria that adhere firmly on the surface cuticle during their migration, thus transmitting bacteria mechanically to the host. The study also found out the synergistic effect of Ascaris suum migration and various different species of bacteria.

MATERIALS AND METHODS

Piglets, matured eggs of Ascaris suum and monocultures of different species of intestinal flora were used.

The piglets used were supplied by the University of Ibadan Teaching and Research Farm. The animals were kept

on a good standard ration throughout the period of the experiment. The pigs were kept in clean pens first for one week to find out whether or not they were harbouring helminth ova. Examination of their faeces during this period showed that they were free of any helminth infection. The twenty piglets, weaned at 6-8 weeks of age were divided into 4 groups and each group was placed in concrete pens that were cleaned and washed with water daily. The test animals were fed with ascarid larvae in overnight broth culture of a particular species of intestinal flora, the second group were fed with only ascarid larvae, the third group received overnight broth culture of the bacteria in use; while the last group had nothing other than their normal ration. This last group served as the control animals. Each pig was weighed at the beginning of the experiment and at the end of the experiment. Ascarid eggs were removed from the uteri of gravid female. Ascaris suum collected from abattoir.

The eggs were washed in normal saline (0.85% NaCl) and were kept in this solution for three days to remove from them the sticky material which causes clumping of the eggs in aqueous cultures. Eggs were cultured in petridishes and the tap water environment was replaced with fresh tap water every two days; some eggs were cultured in tap water to which a few drops of 5% formalin was added and others were cultured in 2% formalin. The cultures were kept at room temperature and agitated once or twice daily for 21 days. At the end of this period, a quantity of the matured Ascaris suum eggs from each culture dish was fed to a white mouse. All the mice died of ascarid pneumonia and ascarid larvae were recovered from their lungs thus showing that the eggs were viable. The various cultures were combined, the eggs were concentrated and washed 4-6 times in sterile distilled water. They were then deshelled and sterilised in equal volumes of 3% sodium hydroxide and 3% sodim hypochlorite overnight according to the method of Hansen et al (1954 & 1956). The eggs were subsequently washed 4-6 times with

sterile saline to remove the deshelling solution; they were checked bacteriologically for their sterility by culturing them into and onto enriched, differential and selective media. The cultures were incubated aerobically and anaerobically at 37°C for 48 hours. The eggs were later concentrated and stored in sterile tap water for feeding to the experimental piglets.

The species of bacteria used were Escherichia coli type 0128, Pseudomonas aeruginosa, Proteus mirabilis, Staphylococcus aureus, Streptococcus faecalis (Enterococci), Bacillus subtilis and Bacillus cereus. Approximately 6,000 embryonated eggs were added to an overnight broth culture of a particular species of bacteria. Some sterile sand was added. The mixture was shaken gently to release the larvae from the shell into the broth. The infective larvae were kept in contact with the bacteria broth culture for thirty minutes to enable the bacteria to adhere firmly onto the cuticle of the larvae as was done

by Taylor & Purchase (1931). The pigs were fed by mouth each day before their breakfast of breeder's grain ration. Three other piglets were fed by mouth with ascarid larvae only. Seven piglets were fed each with overnight broth culture of bacteria only. Three piglets were not given anything other than their normal ration. The first set of seven piglets served as the test animals, the second set of three piglets served as the Ascaris control, the next set of seven piglets served as bacterial control while the last set of three piglets with only normal ration served as the negative controls. The experimental infections were carried out for eleven days. Towards the end of the first week of the beginning of the experiment, the infected pigs with Ascaris larvae only and with Ascaris larvae plus bacterial species were thumping badly. Some of them became very uneasy and they showed no desire for food. These symptoms are indicative of the passage of ascarid larvae through the lungs and

the symptoms were not observed in the pigs that received bacteria only and those that served as negative controls. At the end of the eleventh day of the experiment, the infected animals showed signs of pneumonia, cough with respiratory difficulties. Later, the symptoms began to moderate and the infected pigs ceased thumping and regained their appetites. Both the infected and uninfected pigs were then left on their normal ration for about 70 days. The faeces of the infected animals were examined at the end of the sixtieth day and they were found to contain Ascaris ova. At this time the piglet that received Escherichia coli type 0128 and one piglet that received only Ascaris larvae were given fresh larvae plus E. coli type 0128 and fresh larvae respectively. During the first 48 hours that followed this dose, the two piglets become very uneasy and they showed no desire for food. Within a week, the two piglets became moribund. Both the two piglets, the pig earlier infected with only Ascaris

and a negative control pig were killed for Postmortem examination. The pigs were weighed before they were killed. All the other pigs including the controls were also weighed. Smears were taken from the samples of lungs, spleen, liver, intestine and kidney and were stained by Gram's stain method. Samples of the liver, lungs, intestine, spleen and kidney were collected and preserved in 10%buffered neutral formalin,(BNF) for histological examinations. Sections were cut at 6 microns (/u) and stained with Haematoxylin and Eosin (H & E) stain and also with Gram's stain. Samples of liver, lungs, spleen and kidney were cultured for bacterial growth. The isolates were identified both macroscopically and microscopically. Crush preparations of the lungs and the liver were made and examined for larvae under the microscope. The packed cell volume)(PCV), total white blood cells and differential leucocytes counts were performed on all the animals including those that were killed.

All the remaining piglets were not killed for post-mortem examination for economic reasons. The University of Ibadan Teaching and Research Farm supplied the piglets on the understanding that the experiments were not terminal and that the animals were to be returned to the farm after the end of the experiments, thus the use of microscopic method to show whether or not the pigs were harbouring Ascaris ova.

RESULTS

The infected animals became very unthrifty compared with uninfected ones as shown in Figs 1 & 2. The infected animals weighed less than the uninfected animals. Table 1 shows the PCV, WBC, and differential leucocytes counts of the pigs infected with Ascaris larvae and bacteria while Table 2 shows the PCV, WBC, and differential leycocytes counts of the pigs infected with Ascaris larvae only. Table 3 shows the PCV, WBC and differential leucocyte counts of control animals. The summary of data on weight gains of pigs experimentally infected with Ascaris larvae plus bacteria, Ascaris larvae only and of uninfected control animals is shown in Table 4.

TABLE 1: MEAN PACKED CELL VOLUMES, TOTAL WHITE BLOOD CELLS COUNTS AND DIFFERENTIAL LEUCOCYTE COUNTS OF PIGLETS INFECTED WITH ASCARIS LARVAE AND BACTERIAL SPECIES

Animal infected with	Packed cell volume (PCV)	Total WBC	Eosinophils %	Lymphocytes %	Neutrophils %	Monocytes %
Ascaris plus Proteus	36	21,800	9	51	38	2
Ascaris plus Pseudo.	42	17,200	8	54	34	4
Ascaris plus B.cereus	36	18,100	11	50	38	1
Ascaris plus Staph.	36	24,600	8	49	42	1
Ascaris plus Faecal strept.	37	26,000	10	40	38	2
Ascaris plus B.subtilis	40	22,300	8	46	42	4
Ascaris plus E.coli type 0128	36	27,500	9	40	46	5

TABLE 2:

MEAN PACKED CELL VOLUMES, TOTAL WHITE BLOOD CELL COUNTS AND DIFFERENTIAL LEUCOCYTE COUNTS OF PIGLETS, INFECTED WITH ASCARIS ONLY

Animal infected with	Packed cell volume (PCV)	Total WBC	Eosinophils %	Lymphocytes %	Neutrophils %	Monocytes %
Ascaris only	42	22,600	2	80	18	-
Ascaris only	37	28,100	2	82	16	2
Ascaris only	44	21,300	1	81	16	2

TABLE 3: MEAN PACKED CELL VOLUMES, TOTAL WHITE BLOOD CELLS COUNTS AND DIFFERENTIAL LEUCOCYTE COUNTS OF NEGATIVE CONTROL PIGLETS

Control Animals	Packed cell volume (PCV)	Total WBC.	Eosinophils %	Lymphocytes %	Neutrophils %	Monocytes %
1	38	15,300	2	67	28	3
2	44	13,900	2	69	26	3
3	40	14,550	2	70	24	4

TABLE 4: SUMMARY OF DATA ON WEIGHT GAINS OF PIGLETS EXPERIMENTALLY INFECTED WITH ASCARIS AND BACTERIA

Piglet infected with Ascaris and Bacteria	Weight at beginning	Weight at end of experiment	Weight Gained	Percentage Weight Gained
Proteus	26lbs	34lbs	8lbs	24
Pseudomonas	24 "	35 "	11 "	31
B. cereus	21 "	31 "	10 "	32
Staphylococci	23 "	26 "	3 "	12
B.subtilis	20 "	28 "	8 "	28
Enterococci	20 "	30 "	10 "	33
E.coli 0128	25 "	33 "	8 "	24

TABLE 5: SUMMARY OF DATA ON WEIGHT GAINS OF PIGLETS EXPERIMENTALLY INFECTED WITH ASCARIS ONLY

Piglet infected with Ascaris only	Weight at beginning	Weight at end of experiment	Weight Gained	Percentage Weight Gained
1	24lbs	31lbs	7lbs	23
2	23 "	35 "	12 "	34
3	9 "	12 "	3 "	25

TABLE 6: SUMMARY OF DATA ON WEIGHT GAINS OF CONTROL PIGLETS

Control Piglets	Weight at beginning	Weight at end of experiment	Weight Gained	Percentage Weight Gained
1	18lbs	46lbs	28lbs	61%
2	13 "	34 "	21 "	62%
3	20 "	46 "	26 "	57%

The control pigs were worm-free. In the infected pigs no helminth parasite other than ascarids were found. As can be seen in Tables 4,5 & 6, the total amount of weight gained by the infected animals was lower than the weight gained by the uninfected pigs. The Gram's stained slides of the piglet infected with Ascaris plus E.coli 0128 showed Gram-negative bacilli. There was no bacteria seen in the other slides. The crush preparation showed larvae in the lungs.

Gross Post-mortem findings

Four piglets (P149/83, P150/83, P161/83 and P162/83) that were slaughtered had the following necropsy findings: Piglet P149/83 which was infected with only Ascaris larvae initially had generalised serous atrophy of body fat. There was also a patch of congestion at a point between the apical (anterior) and cardiac lobes of the right lung. The stomach contained normal ingesta and the small intestine was virtually empty but there were some adult ascarid worms in

the intestine. There were multifocal areas of milky-white slightly depressed spots measuring 1-2cm in diameter in the liver, this is the so-called milk-spots. There was no significant gross findings in other organs.

Piglet P150/83 which was infected with Ascaris larvae plus Escherichia coli 0128 had a generalised serous atrophy of body fat. The pericardial and perirenal fats were gelatinous. There was a slight firm and nodular area of grey hepatisation with abscess pocket in the intermediate lobe and another one on the anterior $\frac{1}{3}$ of the diaphragmatic lobe of the right lung. There was also a patch of red hepatisation on the antero-ventral part of the left apical lobe. The greyish area contained a cream-coloured purulent exudate in a fibrous tissue capsule which on culture yielded E.coli serotype 0128. The liver contained 1-2cm greyish-white and depressed focus immediately dorsal to the area of attachment to the gall bladder. There were multifocal areas of 2-3cm sized pale necrotic, slightly firm (Fibrotic) spots on the parietal and visceral surfaces of the liver.

There were two 2 x3cm sized circumscribed firm whitish foci on the liver. When incised, they contained fibrous connective tissue surrounding some dialated hyperaemic tunnels. There was enlargement of the hepatic lymph node (Fig.3). The small intestine contained a lot of greyish watery material. There were some adult worms in the intestine. There was no significant gross lesion in other organs.

Piglet P161/83, the control piglet that was uninfected showed no gross lesion. The stomach and intestinal contents appeared normal and there was no significant gross lesion in the organs.

Piglet P162/83, that was infected with Ascaris and later on reinfected with fresh dose of Ascaris larvae towards the end of the experiment had multifocal areas of petechial to ecchymotic haemorrhages on the parietal and visceral surfaces of the cardiac and anterior half of the diaphragmatic lobe of the right lung. These areas were slightly firm and rubbery. The intestinal contents

appeared normal but there were some adult ascarid worms in the intestine. Ascaris larvae were found in the crush preparation of the lungs. There were numerous milk-spots in the liver and a few nodular foci which consisted of fibrous connective tissue surrounding some hyperaemia tunnels. There were no significant gross lessions in other organs.

Histological Examination

Microscopical examinations of the organs of the infected and the uninfected control animals revealed the following findings: In Piglet P149/83 there were a few haemorrhagic foci in the lung. There were also a few atelectatic areas with congested alveolar capillaries and haemosiderosis (Fig.4). There was no parasite section seen. The liver showed some degree of fatty degeneration and a few haemorrhagic tracts in some lobules. There were also a few foci of fibrous connective tissue proliferation in some lobules (Fig.5). There was no significant finding in the spleen and intestine. There was no bacterium seen in the Gram's stained section.

In Piglet P150/83, the lungs sections showed areas of diffuse neutrophilic infiltration into the alveoli and some bronchioles and marked fibroplasia of the interlobular connective tissue (Fig.6). There were a few large focal areas of caseous necrosis with numerous bacterial colonies within the necrotic debris. There was also a fibrous capsule around the necrotic mass and layer of inflammatory cells. The necrotic centre was surrounded by a zone of marked cellular infiltration consisting of neutrophils and eosinophils (Figs 7 & 8). There was hyperplasia of the bronchial epithelial cells in areas of the lung adjoining the necrotic mass as well as diffuse alveoli and peribronchiolar infiltration marked by neutrophils (Fig.9). Some areas of the alveolar tissue had been replaced by fibrous connective tissue. The liver showed several foci of granulomatous nodules with central haemorrhagic areas with dying and dead cells. The central area of each nodule was surrounded by large areas of mononuclear cell infiltration predominantly lymphocytes

and eosinophils (Fig.10). There were also a few foci of lymphoid follicle formation within the area of cellular infiltration which surrounded the necrotic centre. The area was surrounded by a fibrous tissue capsule. There are a few areas with bile-duct hyperplasia and fibrous tissue proliferation in other parts of the liver. In the hepatic lymph node, the germinal centres of the lymphoid follicles were very prominent and there was also increased cellularity in the paracortical and medullary areas. Most of the cells were lymphocytes. There was no significant finding in the kidneys, spleen, and intestine. The Gram-stained section of the lungs and liver showed Gram-negative bacilli within the necrotic debris.

In piglet P161/83, most areas of the lungs were normal (Fig.11) but there were a few focal areass of peribronchial lymphoid hyperplasia and alveolar haemorrhages. There were no significant findings in the spleen, and kidneys. No bacterial colonies were seen in the section stained by Gram's method.

In Piglet P162/83 many bronchial and bronchiolar lumina contained sections of helminth parasite which are Ascaris species (Figs 12, 13 & 14). There was usually an accompanying peribronchial and peribronchiolar infiltration by mononuclear cells which were predominantly lymphocytes and eosinophils(Figs. 15,16 & 17). There was also fibrous connective tissue infiltration in the area of peribronchial cellular infiltration (Fig.16) and hyperplasia of the bronchial epithelium (Fig.18). Some portions of the bronchi had been destroyed and replaced by infiltrating cells and sections of the parasite (Figs. 14 & 17). The peribronchial areas contained normal alveolar inspite of parasite sections within the lumen of bronchi in such areas (Fig.19). The histological picture of the liver revealed a few large-sized granulomatous nodules with central areas containing sections of a parasite (Ascaris spp); and necrotic cells and surrounded by a zone of marked mononuclear cell infiltration, mainly lymphocytes and eosinophils bounded by a fibrous tissue

capsule (Figs. 20 & 21). There were a few areas of bile-duct hyperplasia and pseudolobulation in the liver tissue adjoining the parasitic granulomata (Fig. 22). There was marked reactive hyperplasia in the hepatic lymph node. There were no significant findings in the kidneys, spleen and intestine.

FIG.1: Showing unthriftiness and retarded growth of the infected piglets at the end of the esperiment. (16 - 18 weeks)

FIG.2: Showing control piglets at the end of the experiment. Note the difference. (16-18 weeks)

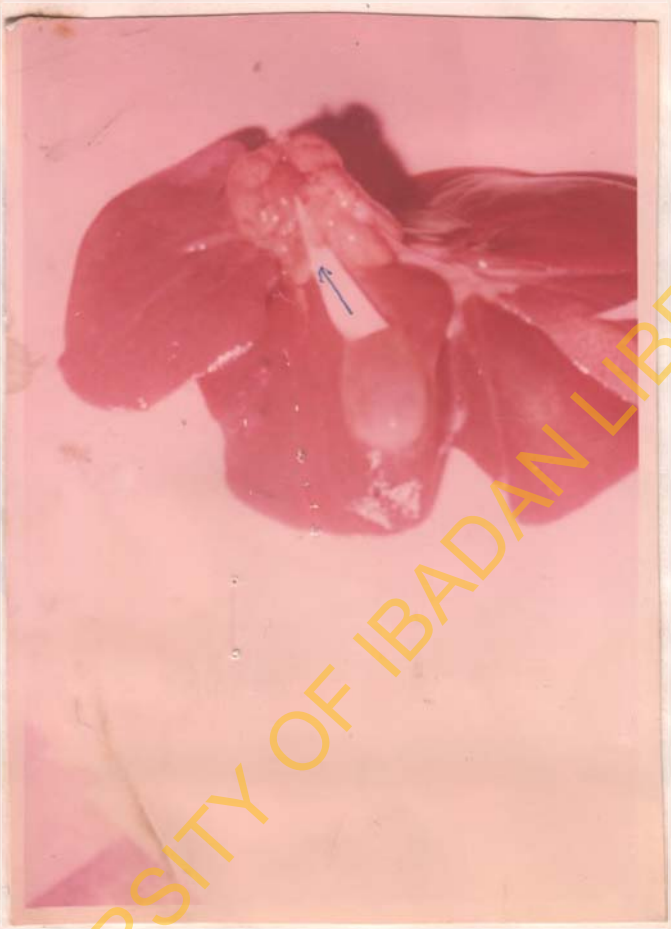

FIG.3: Enlarged hepatic lymph node (arrowed) due to synergistic effect of Ascaris plus E.coli infection.

P150/83

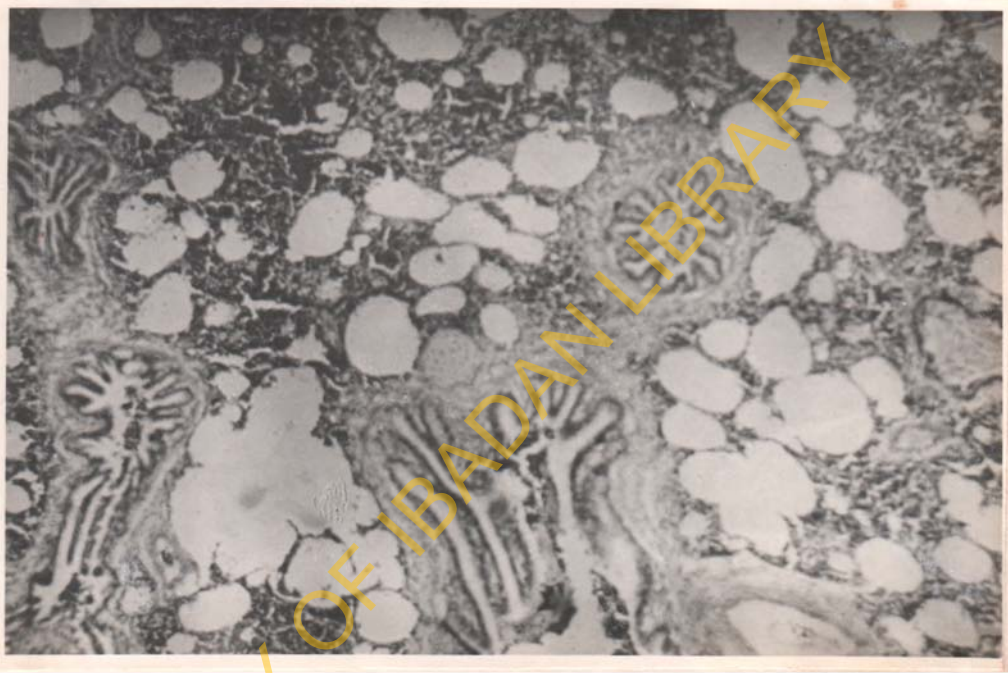

FIG. 4: Lung showing area of slight alveolar haemorrhage and congestion of alveolar capillaries. Many alveoli are normal. (H & E) x 160

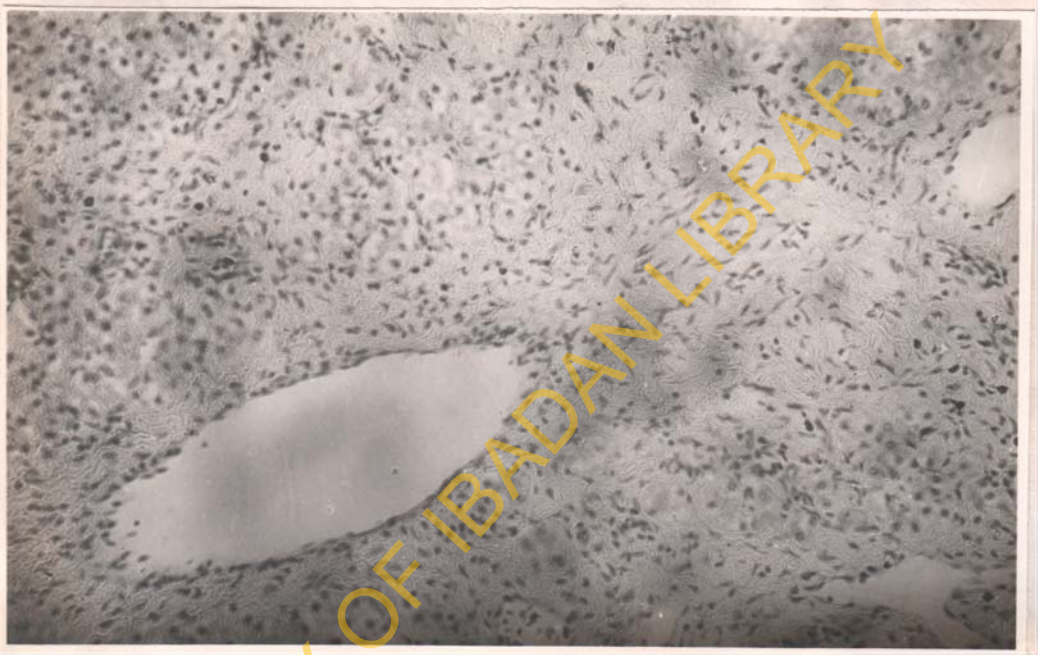

FIG.5: Liver showing areas of fibrous tissue proliferation (repair) of the liver sequel to parasitic infection.
(H & E) x 160.

FIG. 6: Lung showing diffuse infiltration by some mononuclear cells indicating that the animal is progressing towards pneumonia (H & E) x 62

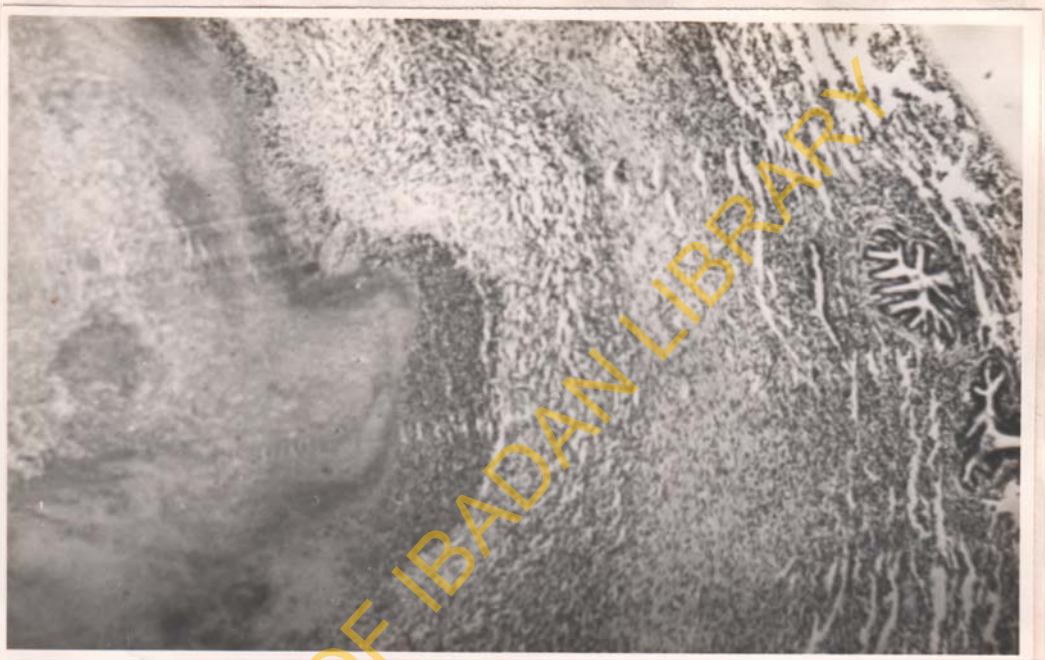

FIG.7: Lung showing area of necrosis with bacteria colonies and surrounding thickened wall. Notice the bacteria granuloma formed following initial parasitic infection (H & E) x 62.

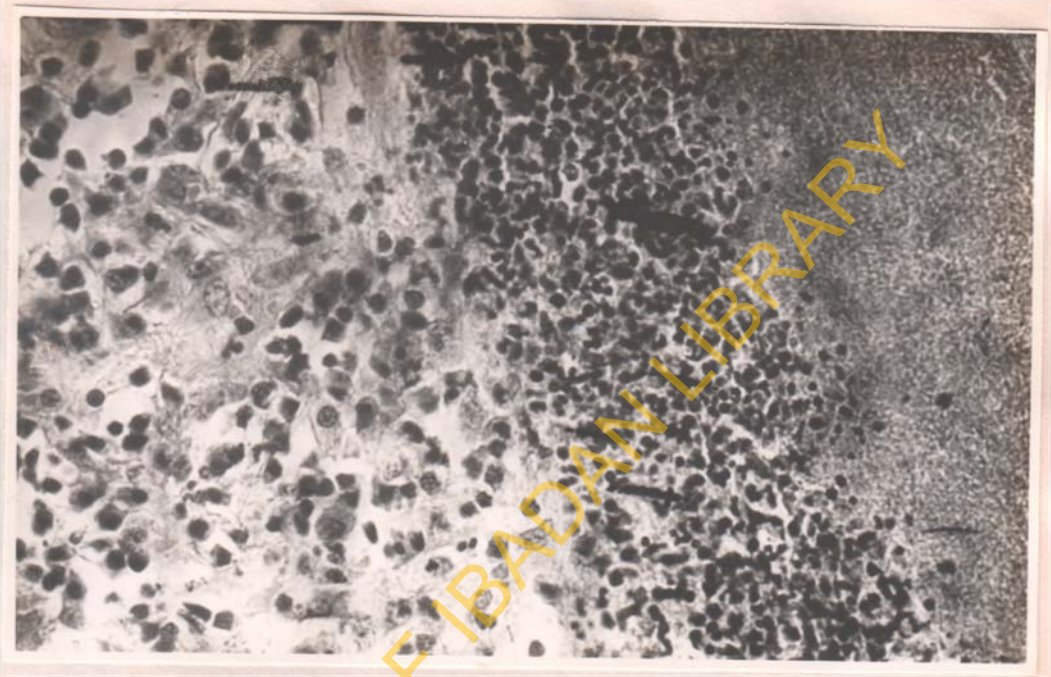

FIG: 8:

Lung showing area of necrosis containing bacteria on the right with surrounding areas of neutrophilic infiltration and an outer zone of mononuclear cells infiltration which are predominantly eosinophils and macrophages - evidence of parasitic infection.
(H & E) x 400

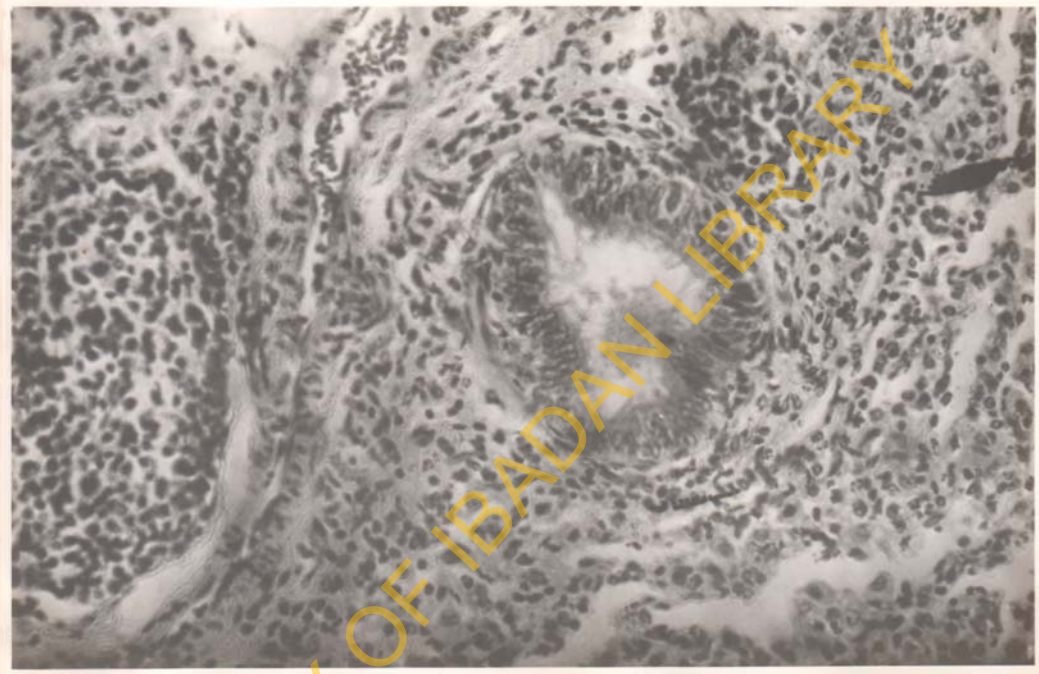

FIG.9: Lung showing peribronciolar and alveolar neutrophilic infiltration due to secondary bacterial infection. Notice the bronchiole which is intact and no infection inside it except around it (H & E) x 250.

FIG.10: Liver showing central area of necrosis and mononuclear infiltration around the necrotic areas - evidence of bacterial infection. (H & E) x 62.

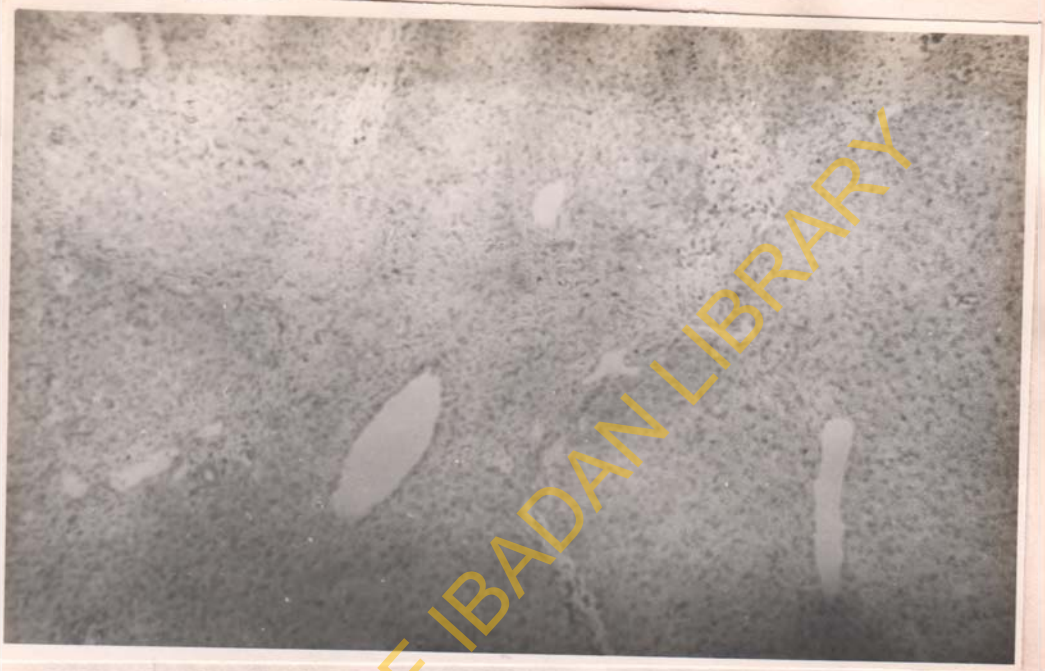

FIG. 11: Normal Liver of P161/83

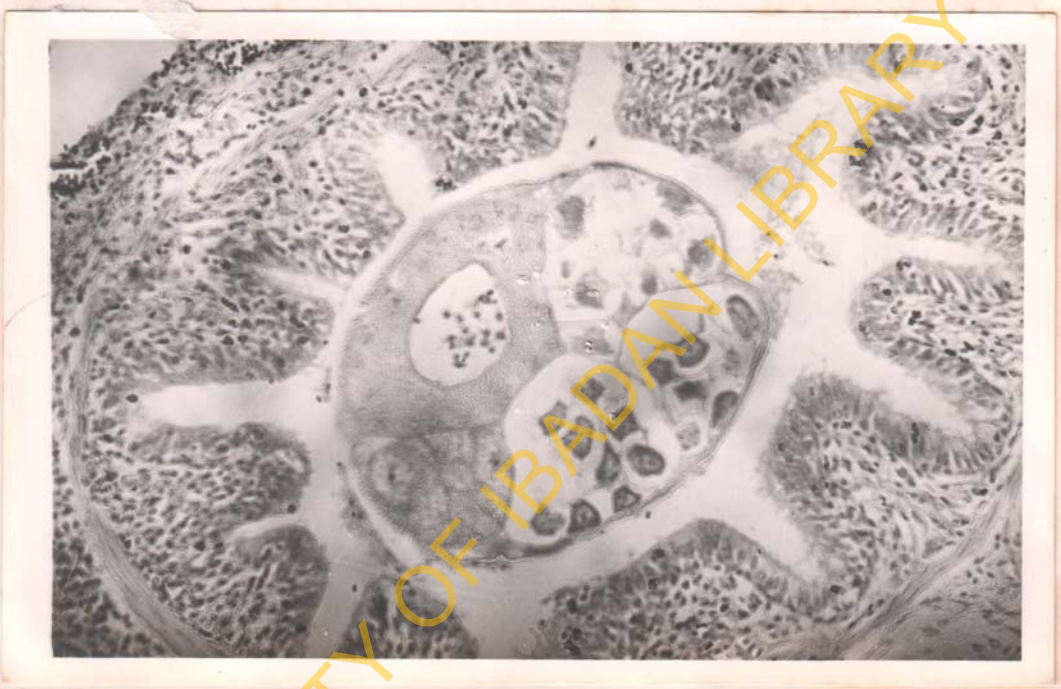

FIG. 12: Lung showing cross-section of parasite in a bronchiole. There is slight peri-bronchiolar mononuclear cells infiltration around the cross-section of the parasite predominantly eosinophils - indicating parasitic infection.

(H & E) x 160.

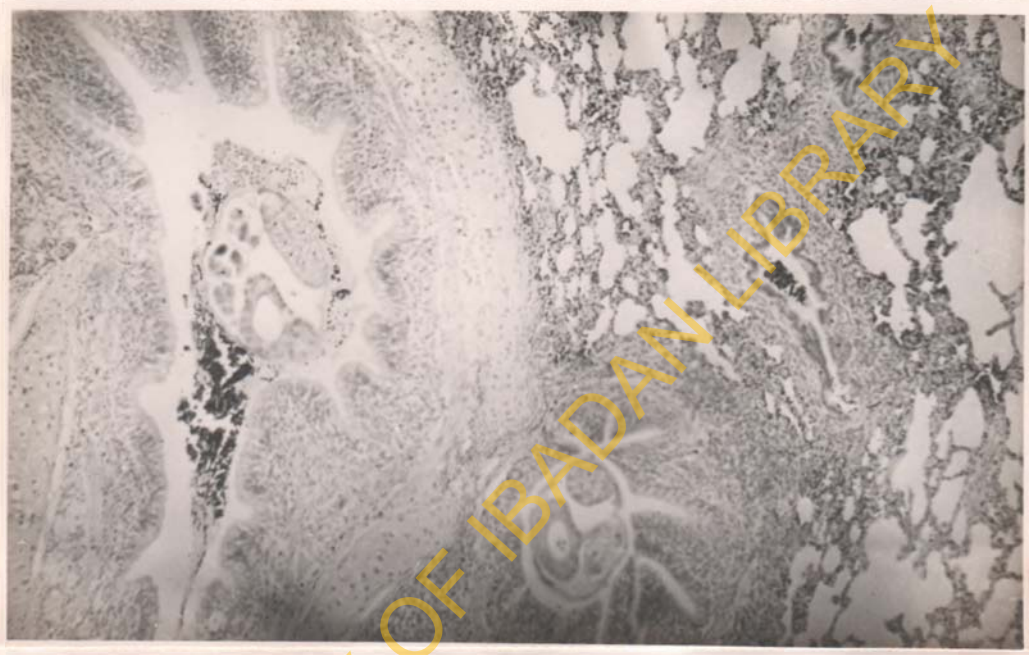

FIG.13: Lung showing parasite sections in bronchi with peribronchial mononuclear cells infiltration.

The intervening alveoli are normal.

(H & E) x 62

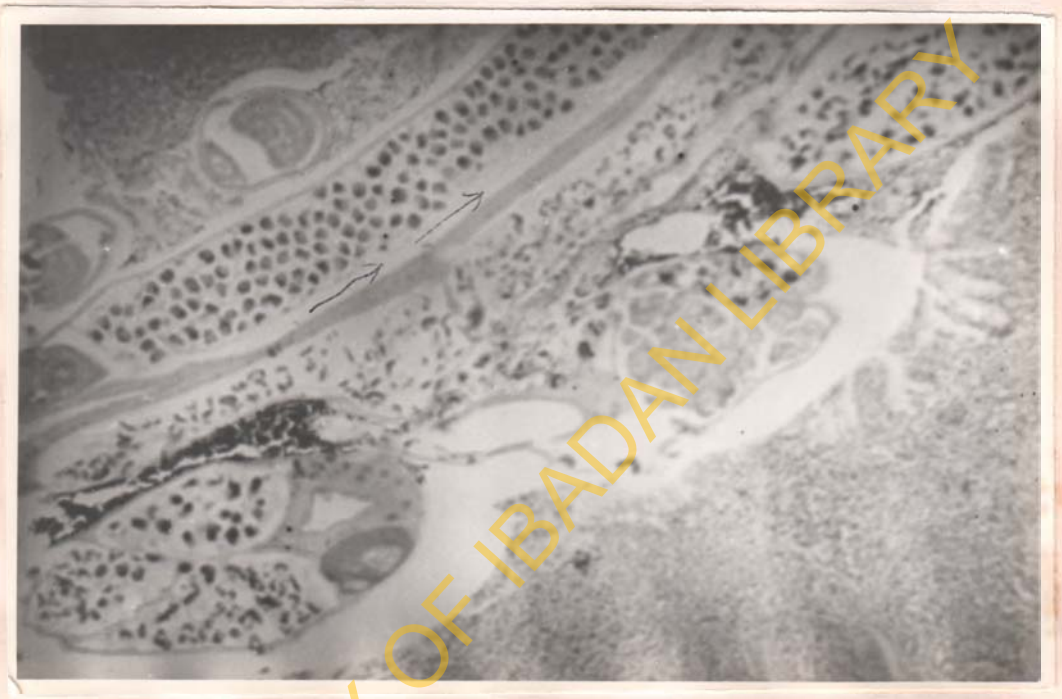

FIG.14: Lung showing lungitudinal section of parasite in the bronchus.(arrowed)
(H & E) x 62

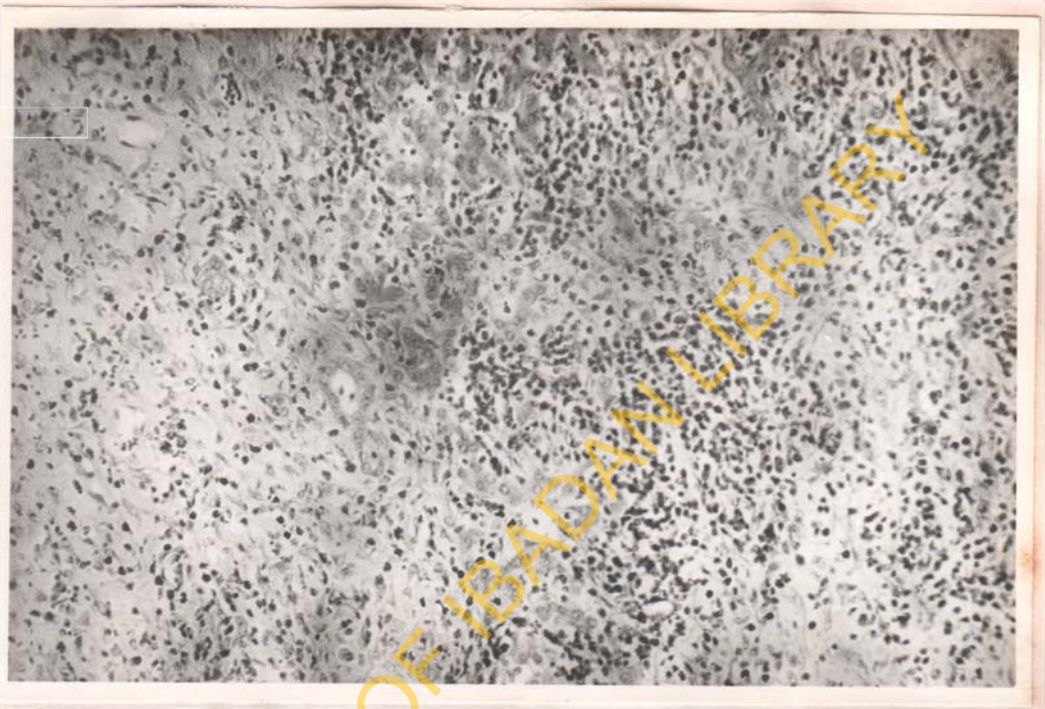

FIG.15: Liver showing area surrounding parasitic granuloma; Hepatocellular necrosis, infiltration by eosinophils and few neutrophils (H & E) x 160.

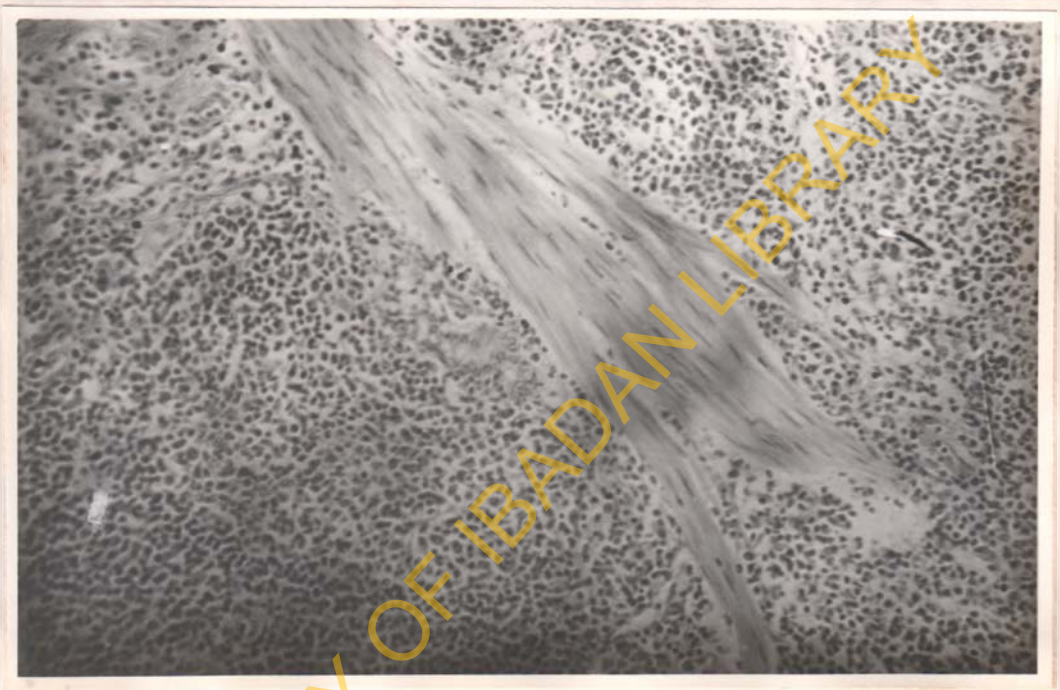

FIG. 16: Lung showing area of marked mononuclear cell infiltration in an area where normal bronchial tissue has been lost.

Notice the formation of fibrous connective tissue. (H & E) x 160.

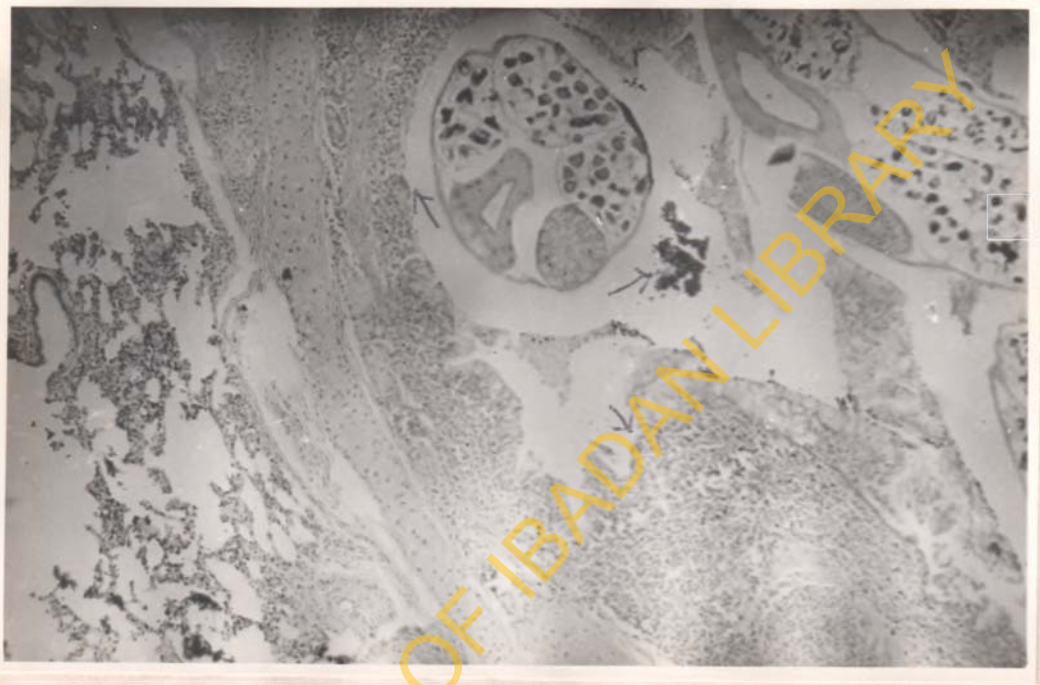

FIG. 17: Lung showing T.S. of parasite in a bronchus. Note loss of bronchial epithelium (arrowed), haemorrhages (arrowed) and marked mononuclear cell infiltration into the bronchial wall and around the parasite section.

(H & E) x 62.

FIG. 18: Lung showing hyperplastic bronchial epithelium with parasite section in the lumen. This is an evidence of respiratory embarrassment caused by the parasite. The area will secrete more mucus to wash out the irritant (parasite). (H & E) x 160.

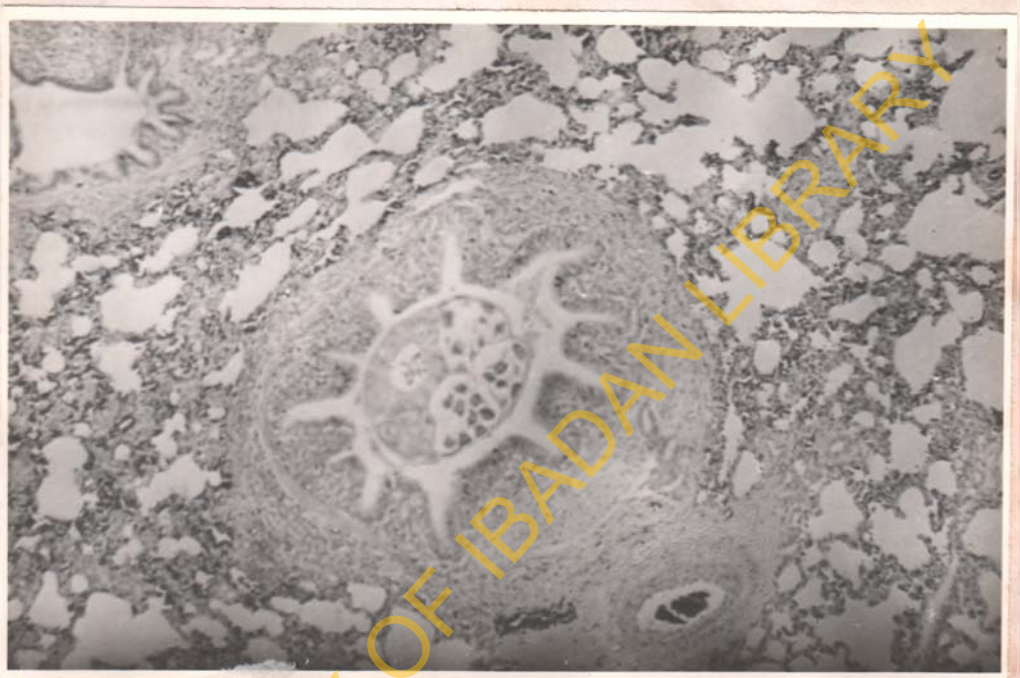

FIG. 19: Lung showing cross-section of the parasite in bronchus with normal surrounding alveoli - indicating that the parasite was just passing through the bronchus and has not destroyed the wall of the bronchus

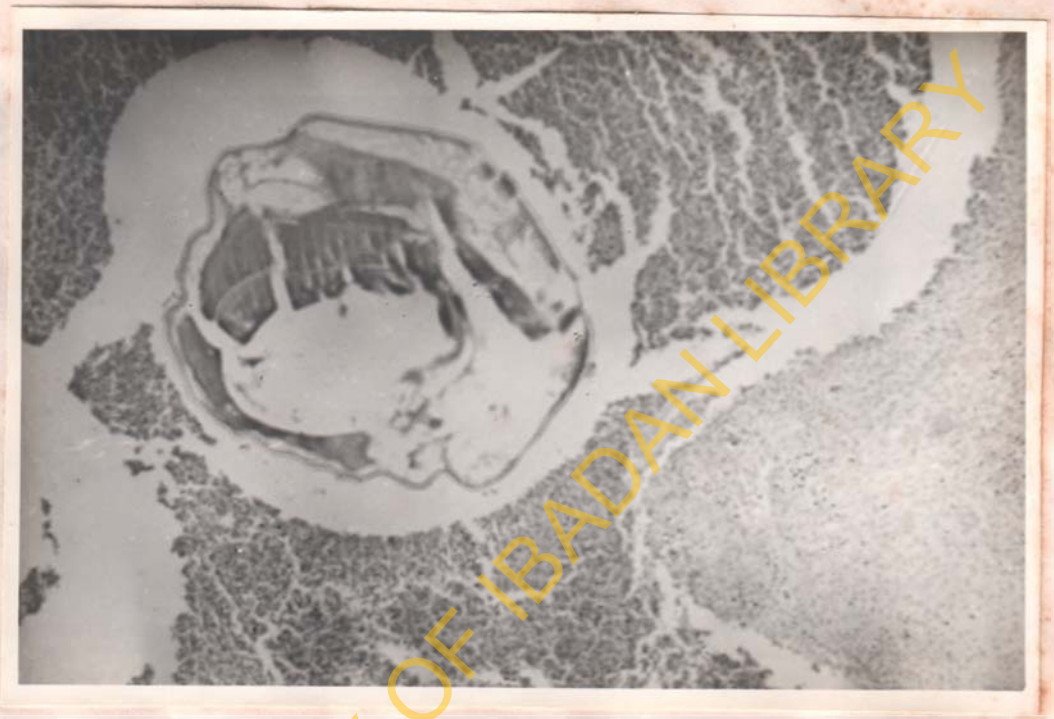

FIG. 20: Liver showing parasitic granuloma with fibrous tissue capsule surround and mononuclear cell (eosinophils) infiltration around section of parasite in centre of the lesion (H & E) x 62.

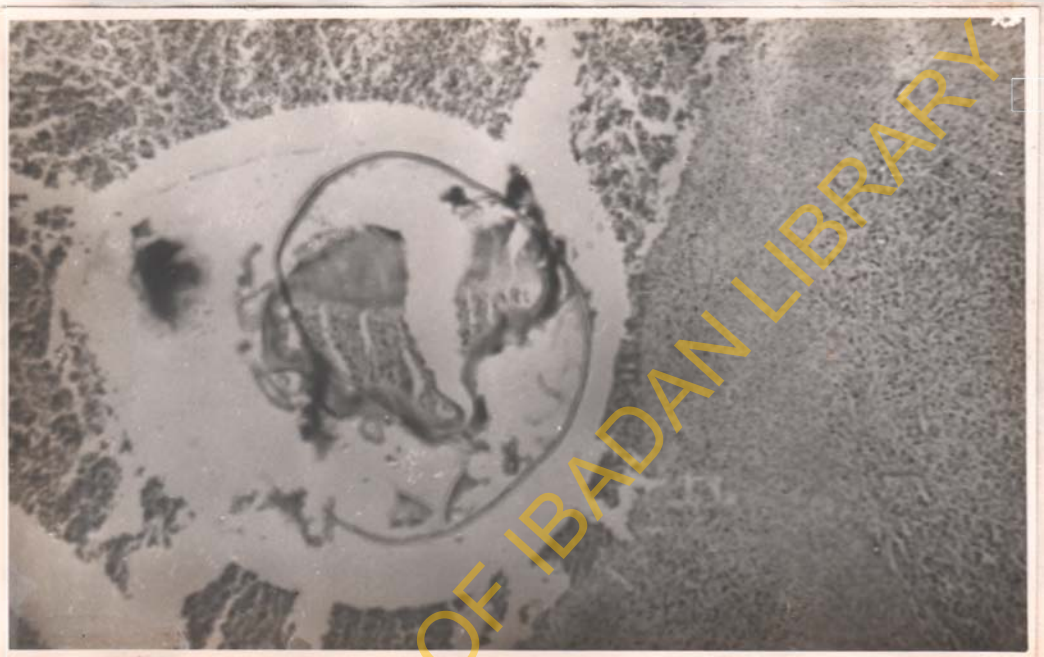

FIG: 21.

Liver showing parasitic granuloma surrounded by a zone of cellular infiltration and a fibrous connective tissue capsule. (H & E) x 62

FIG.22:

Liver tissue in vicinity of parasitic granuloma showing pseudolobulation. (H & E) x 62

DISCUSSION

Although the literature on the interrelationship of bacteria and intestinal helminths in animals is scanty Newton et al,(1959), Wescott & Todd (1964), and Wescottt (1968) have studied Nematospiroides dubius infection in germfree mice and Przyjalkowski (1967) has worked with Trichinella spiralis in free and mono-contaminated mice.

In the present study, Ascaris suum larvae and monocultures of intestinal bacteria were used. The data presented in this study provide clearly data on the extent to which infections of ascarid only and ascarid plus bacteria can affect adversely the growth rate of pigs in an environment of heavy bacterial contamination and in an environment with little or no bacterial contamination. Infections with large intestinal roundworms (Ascaris suum) constitute a problem in swine production that is of considerable economic importance.

These worms are among the most widespread and injurious of the intestinal helminth parasite that infect pigs (Ramsom, 1927, Burch, 1930 and Roberts, 1934). Light infections may produce some degree of unthriftiness and retarded growth where severe infections may result in death of the affected animal (Schwatz, 1937). Fig.1 shows the effect of Ascaris suum infection in piglets. The infected animals were very unthrifty and showed retarded growth. These animals can be compared with the control animals in Fig.2 which appeared healthy and robust. It is reasonable to assume that apart from the damage caused by the migrating larvae of ascarids to the pigs' internal organs, sensitisation is caused during larvae migration and is intensified by the repeated infection (Jung, 1953). Furthermore, the presence of worms in the small intestine, their growth, metabolism, and secretions might excert an adverse effect on the nutrition of the host. Many worms in the small bowel of the host undoubtedly rob the pigs of nourishment by

utilizing the semi-digested food. Tables 1,2 and 3 show that the packed cell volumes of the pigs infected with Ascarids only, those infected with ascarids plus bacteria and the control pigs are

within the normal range (normal packed cell volume (PCV) of pig 32-50%), but the differential leucocyte counts are quite interesting. In the pigs infected with ascarids plus bacteria, the neutrophils are somehow higher in count than those of animals with ascarid only. This high count of neutrophils is an evidence of bacterial infection whereas the animals infected with ascarids only gave very high count of lymphocytes in response to ascarids infection. This is characteristic of helminth infections and the results obtained in this study agree with the findings of Przyjalkowski and Wescott (1969), who recorded very high percentage (71.1% - 82.4%) of lymphocytes in animals infected with Trichinella spiralis. There is also a big difference in the percentage counts of eosinophils of the parasitised and unparasitised piglets. The

high eosinophil counts recorded in the parasitised pigs is not surprising as Strafuss and Zimmerman (1967) found that peaks of eosinophils appeared in pigs infected with nematodes. Tables 4,5 & 6 summarised the data on weight gains of both infected and control animals. The results show that all the infected animals harbouring adult worms had their growth retarded. For example, the presence of adult worms was sufficient to destroy the health of the pigs to the extent that progressive loss of weight occurred so that the infected animals weighed less at the termination of the experiment than they did at the beginning. The maximum weight gains in infected animals was 34% whereas in the uninfected control animals it was 62%. These results agree with the findings of Spindler (1947) who found that pigs infected with 20 or more worms at 8 weeks of age failed in gain of weight in proportion to the number of worms they harboured. He also reported that one pig with 109 worms gained no weight at all, whereas uninfected pigs gained an average weight of 100lbs. Because of the

presence of adult worms in the pigs, there is bound to be loss results from condemnation of carcases for jaundice owing to blockage of bile-ducts. In this study, the number of adult worms recorded from each pig killed was relatively small compared with the number of larvae administered. This is difficult to explain, this is probably due to cellular response.

The results of the study also show that Ascaris larvae and Ascaris larvae plus bacteria species were infective for the pigs. The Ascaris larvae developed to maturity both in the presence of bacteria species as shown by the presence of Ascaris eggs in the faeces of all the infected animals. The findings in this study are in accordance with the works of Wescott & Todd (1964), Stefanski & Przyjalkowski (1965 & 1966), Przyjalkowski (1967) and Stefanski (1965) who found that intestinal microflora of the host favoured the establishment of intestinal nematodes.

In the animal infected with Ascaris larvae only, the histological findings show patchial haemorrhages which is an evidence of traumatic damage probably due to the larval migration of the parasite. There was no major .. .. lesions observed on this animal possibly because of tissue repair sequel to initial parasite damage. Furthermore, there was no parasitic section in the tissue probably because the parasite had already left the lungs. The liver shows fibrous tissie repair sequel to previous damage. In Piglet P149/83, it did appear that the animal had recovered from the infection. The parasite had completed its developmental and migratory phases in the liver and lungs with minimal damage. Such damage was not serious enough to leave any major lesions in the organs. However, the areas of fibrous tissue proliferation in the liver will suggest a repair process sequel to a previous parenchymal damage. This damage was severe enough to have caused extensive tissue damage which could not be replaced by regenerated hepatocytes but by fibrous

connective tissue.

The lung lesion in Piglet P150/83 suggests a significant secondary bacterial infection of a pre-existing wound. The pneumonia is suppurative and of a long duration in view of connective tissue proliferation which led to a fibrous adhesive pleuritis in the lung. The inflammatory reaction which seemed to be mainly alveolar, peribronchial, and peribronchiolar will suggest that the bacteria most probably reached the lung by other routes other than the aerogenous route. This finding suggests that the bacteria were carried to the lung by the migrating Ascaris larvae since the animals were infected with both bacteria and larvae together. The results of the study show that Ascaris larvae are capable of transmitting bacteria mechanically, because, after infection during the necropsy, bacteria was isolated from the organs both in direct smears and in cultures. These findings agree with the work of Weinberg, (1907) who concluded that by biting and boring into the intestinal wall, or by wounding it with

hooks and suckers, the majority of helminths favour the penetration of microbes, these being carried on the exterior and in the intestine of the worms. The increased pathogenicity caused by both the Ascaris larvae and Escherichia coli shows that the two agents work together to produce a disease condition more severe than the sum total of effects produced by each independently. The observation in this study is in accordance with the findings of Nayak & Kelley (1965) who showed that Ascaris suum increased the severity of swine influenza in pig. There are several ways that migrating Ascaris larvae may contribute to this synergism. Nayak & Kelly (1964) have shown thatthere is a greater amount of haemagglutination antigen produced in compounded Ascaris-influenza-bacteria infection and this material may be toxic to the lung tissue. Furthermore, the trauma produced by larvae rupturing alveoli may induce sufficient stress to lower the animals resistance to very severe infection. Capillary damage produced by penetrating

larvae may allow more viral, or bacterial antigens to gain access to the systemic circulation and thus produce viraemia, bactereamia or toxaemia.

The presence of a few eosinophils will suggest an underlying hypersensitivity or parasitic infection which was superimposed by the bacteria pneumonia. The liver lesion which showed some inflammatory response most probably suggests an initial marked damage to the liver and secondary bacterial infection. The cellular response suggests a long-standing lesion which is most probably due to both parasitic, bacterial and hypersensitivity reaction. The areas of nodule formation indicate areas of massive tissue damage with replacement by infiltrating cells reacting to an antigenic stimulation and presence of fibrous connective tissue to repair some of the damaged tissues. The bile-duct hyperplasia is also suggestive of reaction of the bile-duct system to an irritant or foreign body. The hepatic lymph node shows a remarkable

reactive hyperplasia probably as a result of inflammatory reaction occuring in the liver. The absence of any inflammatory reaction in the intestine of the infected animal suggests that the worms which were found did not attach to the mucosa.

Piglet P161/83 sections of the liver and lungs appear normal. This is an animal used as control with neither parasitic nor bacterial infection, and these sections could therefore be compared with those from infected animals for proper assessment of the damage done to the organs by both the parasite and the bacteria either individually or together.

In Piglet P162/83, the lung lesion indicates the presence of developmental stages of the Ascaris suum as they pass through the bronchioles and bronchi. There is little or no damage to the bronchiolar epithelium but the bronchial epithelial damage suggests some pressure atrophy induced by the parasites which occupied the bronchial lumen. The marked cellular reaction around the

bronchi and bronchioles and the cell types suggest an antigen-antibody reaction probably induced by the parasites, their metabolic by-products or the antigenic coat released into the lung tissue during moulting. The presence of fibrous connective tissue capsule indicates that the lesion is a parasitic nodule being walled off from the normal lung tissue. Similar parasitic nodules are present in the liver and perhaps this suggests that the ages of the liver and lung lesions may be about the same. The pathogenesis of the liver lesion is possibly similar to the lung lesion. Reaction is mainly mononuclear cells. Enlargement of goblet cells shows increased mucus production. There is diffuse neutrophilic infiltration like in P150/83 which suggests that the major lesion is due to parasitic demage only.

In summary, the findings in this study concerning pathogenicity and pathogenesis of Ascaris suum in pigs are in total agreement with the works of Spindler (1947), Roberts (1934), and Soulsby (1967). The action of the given

monocultures of intestinal bacteria observed in this study therefore offers a promising model system for further investigation of the interaction of bacteria and intestinal helminths.

CHAPTER 5

3.5 SOURCES OF HUMAN INFECTION OF ASCARIASIS IN IBADAN

INTRODUCTION

In areas where human faeces is used as manure or where promiscuous defaecation is practised, these factors contribute significantly to the pollution of soil, and helminth eggs and larvae may be readily demonstrated on fresh vegetables purchased from the market (Chang & Chin, 1948). It is therefore reasonable to assume that in those areas, the prevalence of intestinal helminths is in part due to the ingestion of infective stages adherent to the uncooked food; plants and water. Where refrigeration and commercial canning are little used as in Nigeria, vegetables are freely bought in the market or are taken from gardens and eaten raw in order to satisfy the consumers' need and desire for

balanced diets. In such foods, the development and survival of the infective stages of helminths exert an undetermined influence on the prevalence of these parasites. Reported observations by Chang & Chin (1949) indicate that none of the common preservatives such as salt, alcohol, and vinegar in the usual concentration can be relied upon to kill the eggs of Ascaris lumbricoides. It has been noted by Fueki (1952) that vegetables, soil, dust and fingers constitute the principal sources of Ascaris infection.

Since there has been no published record in Nigeria on the importance of root and leaf vegetables and other food items consumed raw in the transmission of Ascariasis, this study was designed to find out this possible role in Ibadan.

MATERIALS AND METHODS

Different food items that are usually eaten raw or uncooked such as Gari, leaf vegetables, root vegetables and fruits were brought from different shops and open

markets in Ibadan. The food items were examined for Ascaris eggs. Each food item was washed in sterile distilled water several times. They were then centrifuged at a top speed of an international centrifuge at 2,500rpm. for 2 minutes using sterile universal containers already covered. The deposit was examined, the number of Ascaris eggs present was counted and the number of embryonated eggs noted, using light microscope.

The supernatant and the sediments of the centrifuged foods were cultured on different culture media for bacterial growth. Palm wine was centrifuged at a top speed of an international centrifuge for 2 minutes at 2500rpm. Both the deposit and the supernatant were treated as above. Gari was suspended in sterile distilled water. It was mixed very well and then allowed to sediment. The supernatant was collected. More water was added and most of the grains of gari was removed by staining through

sterile four-plygause. The whole supernatant was then centrifuged at 2500rpm for 2 minutes. The deposit was examined for Ascaris ova. Both the deposit and supernatant were cultured.

In addition, the deposits and the supernatants of all the food items were separately inoculated into nutrient broth containing 5% and 10% acetic acid and into 70% Ethyl alcohol. Both the acid and alcohol cultures were left for 15 minutes, 30 minutes before subculturing them onto nutrient media and the eggs present cultured for larval development. This egg culture was made after washing off the acid and alcohol from the eggs 4-6 times with sterile distilled water. The sediments and the supernatants were also boiled at 100°C for 5 minutes, 10 minutes and 15 minutes. They were cooled down and then subcultured onto culture media and the eggs present were cultured for larval development. Identification of the isolated bacteria was based on the Gram's stain, motility and biochemical reactions as given in Bergey's Manual of Determinative Bacteriology, Baltimore (1957).

RESULTS

TABLE 1: FREQUENCY OF ASCARIS EGGS ON EDIBLE FOODS

Kinds of Specimen	Number Examined	Positive Specimen	Percentage Positive	Total Egg Counts	Embryonated Eggs
Lettuce	43	11	25.6	27	10
Cucumber	55	9	16.4	21	6
Carrots (roots)	45	11	24.4	38	12
Mango	35	8	22.9	20	6
Tomato	52	5	9.6	13	3
Garden eggs:	63	10	15.9	15	2
Sweet pepper	32	7	21.9	10	3
Onion (bulb)	29	4	13.8	7	1
Gari	36	7	19.4	18	7
Palm-Wine	29	5	17.2	11	5

NB: The spread is shown in Appendix 1 - 10

TABLE 2: EFFECT OF ACETIC ACID AND ETHYL ALCOHOL ON BACTERIA

Type of Organism	5% Acetic Acid	10% Acetic Acid	70% Ethyl Alcohol	Time of Exposure in minutes
Staph.aureus	NG	NG	G	15
Staph.aureus	NG	NG	NG	30
Esch.coli	NG	NG	G	15
Esch.coli	NG	NG	NG	30
Proteus spp.	NG	NG	G	15
Proteus spp.	NG	NG	G	30
Aerobic sporebearer	G	G	G	15
Aerobic sporebearer	G	G	G	30

KEYS:

G = Growth on subculture
NG = No growth on subculture.

TABLE 3: EFFECT OF ACETIC ACID AND ALCOHOL ON DEVELOPMENT OF BOTH EMBRYONATED AND NON-EMBRYONATED ASCARIS LUMBRICOIDES EGGS

Type of Specimen	5% Acetic Acid	10% Acetic Acid	70% Ethyl Alcohol	Incubation period in Days
Ascaris lumbricoides eggs plus Bacteria	++	++	++	21
Ascaris lumbricoides eggs only	++	++	++	21

KEYS:

++ = Development of eggs to infective larval stage

NOTE: Acetic acid and ethyl alcohol had no effect on either the embryonated or developmental stages.

Table 1 shows the recovery rate of Ascaris eggs on the food items examined. The bacteriological examination of the food items yielded some bacterial species. The species recovered from the cultures of the water used to wash the food items were Staphylococcus aureus, Staphylococcus albus, Escherichia coli, Proteus mirabilis Proteus morgani and Proteus vulgaris. No anaerobic organism was isolated.

Tables 2 and 3 show the effect of acetic acid and ethyl alcohol on bacteria and Ascaris lumbricoides eggs. The 5% acetic acid killed all the bacteria isolated from the food items but had no effect on the eggs of Ascaris lumbricoides. The acetic acid and ethyl alcohol appeared to be essentially without effect on either the embryonated (infective) or developmental stages (Table 3).

Boiling the eggs and bacteria at 100°C is effective in killing them and this is confirmed by the failure of eggs to develop in culture.

DISCUSSION

In Nigeria, intestinal nematodes abound both in the rural and urban environments because our tropical climate offers excellent opportunities for easy and rapid development of the different stages of these parasites which are rapidly disseminated in our soil through our gross and indiscriminate defaecation habits. The prevalence of Ascaris infection among the people therefore has been at a level of 5.81% (Ramsay, (1934) in the North and 73.4% (Okpala, 1956) in the South.

Table 1 confirms that various food items that are eaten raw can serve as vehicles of Ascaris infection to man. Fueki (1952) found Ascaris eggs on many vegetable specimens. The finding of Ascaris eggs on edible vegetable fruits therefore emphasises the need for strict observation of simple hygiene methods aimed at eliminating the Ascaris eggs before consumption of the uncooked fruits and vegatables.

There are many ways by which these food items can be contaminated with Ascaris lumbricoides eggs. The eggs of Ascaris could be transferred from objects to fingers and from fingers to the mouth or directly from the objects to the mouth. In countris where Ascaris eggs are abundant in the environment they have been recovered from any object including paper money (Dolt & Themme, (1949) and Gonzalez-Castro (1951).

Infection with Ascaris eggs can be soil-borne in the case of vegetable and roots, water-borne in the case of palm-wine. The contamination of food items is very common in materially less favoured countries like Nigeria where the absence of community services result in low sanitary standards and greater opportunities for the common spread of infection in water supplies or foods contaminated by cases and carriers. This contamination is also common in areas where there are overcrowding and careless droppings of small children in the gardens and the open ditches around

the farms and houses. In areas where human faeces are used as fertilizer of garden crops, all the raw vegetables, including roots, stem, leaves and fruits which ripen in or near the ground are especially exposed and can be easily contaminated and be sources of infection when improperly washed or cooked before consumption. Furthermore, Ascaris eggs remain viable for a long time. Davaine (1863) found that the ova of Ascaris lumbricoides remained infective after storage for five years and Bailliet (1866) observed that the eggs of Ascaris remained viable after twelve months exposure to heat of summer and to the cold of winter. Other studies (Brown, 1927a, Cort, 1931, Cort & & Otto, 1933, Otto & Cort, 1934, and Headlee, 1936 have shown that Ascariasis is essentially a household infection and the infectivity of the soil is maintained for the most part by promiscuous defaecation. For the reasons given above, all fruits getting in touch with contaminated soil would likely carry Ascaris eggs on their surface. Ascaris eggs

are adhesive, therefore vegetables and other food items that are not properly washed or cooked serve as sources of infection. The results of this study confirm the work of Fueki (1952), Kobayashi (1954), Nishimura (1957) and Fujii (1957) who carried out a survey on the soil of farming land for Ascaris eggs and found that the soil of green leavy vegetable fields was heavily contaminated with Ascaris eggs.

Dust is also known to play an important part in the dispersal of Ascaris eggs Bogojawlenski & Demidowa (1928). Nakayama (1956) has shown that Ascaris eggs provide a physical characteristic of aerosol. This fact indicates that the eggs can be regarded as the dust in nature and therefore can be air-borne. Ascaris eggs adhering to small particles may be carried away by the wind from the surface of cultivated land (Kobayaski, 1955 and Morishita et al, 1959) and adhere to vegetable, enter into house and contaminate food items like gari and others. Ascaris

eggs in the faeces used as fertilizer in agricultural farms or in faecal deposit in farms and gardens are scattered on the soil and occasionally onto vegetable. Attachment of viable eggs onto vertical surfaces up to 30cm above soil surface has been observed by Beaver (1932). The eggs on the surface of the soil may be splattered onto vegetables when it rains. The eggs also flow out of farming land during heavy rainfall and may be carried away to other places by the running water.

Although acetic acid has not been studied as an Ascaris ovicidal agent, as the principal active constituent of natural vinegars, it is a well known food preservative, and its toxicity for bacteria, yeasts and moulds has been the subject of investigations which have demonstrated that at room temperature, 15 minutes contact (or less) with 5% concentration (or less) kills several strains of food poisoning Staphylococci, Salmonella typhi, Eschericia coli and others (McCulloch, 1945). In this study, the use of 5%

acetic acid failed to kill Ascaris lumbricoides eggs. These findings agree with the results of Soh (1960) and Faust (1970) who showed that chemical sterilisation of Ascaris lumbricoides eggs is impractical, since the eggs thrived and matured when immersed in strong chemical solutions. The use of 5% acetic acid was, however, capable of killing all the species of bacteria isolated from both the supernatant and the sediment of water used to wash the vegetables. The results of this study confirm the work of Beaver & Deschamps (1949) who showed that 5% acetic acid kills several strains of food poisoning - Staphylococci, Salmonella typhi and E.coli. This finding clearly shows that the use of acetic acid would not be effective as a prophylactic measure against Ascaris infection.

The use of 70% Ethyl alcohol cannot be relied upon as a prophylactic measure as it failed to kill the eggs of Ascaris. The use of boiling water to destroy both the bacteria and the Ascaris eggs present seems to be the only way to get out of Ascaris infection in edible fruits. This

finding is in accordance with the observation of Ogata (1925) who studied the effect of heat in the destruction of the ova of Ascaris lumbricoides and found that eggs of Ascaris exposed to 70°C-100°C for one minute were killed. Boiling treatment could however, not be used on fruits and vegetables that are normally eaten raw.

Of interest is the finding of Ascaris eggs in palm wine which is one of the country's local wines and which is usually taken uncooked or unfiltered. Palmwine is tapped from palmtree and naturally one would expect such wine coming out of tall trees of palmwine to be free from both bacteria and parasites contamination. The finding of Ascaris eggs in palmwine therefore, suggests that the contamination could be due to the use of contaminated water for the dilution of the wine. It could also be due to the use of contaminated containers and the activities of flies and dust.

One of the most interesting findings of this study is the presence of Ascaris eggs in gari which is one of the staple foods in Nigeria. The preparation of gari involves frying the gari at such a high temperature that is capable of destroying both bacteria, viruses, protozoa cysts and helminth ova that may be present in it during fermentation period. The presence of Ascaris eggs in gari could probably be due to the use of dirty or contaminated containers and utensils for preparation, selling, buying or for drinking the food. Alternatively, it could be due to careless handling of gari by the gari sellers who probably are excreting Ascaris eggs in their faeces and thereby contaminate the food with the eggs in their fingers. Most importantly is the role of flies in contaminating gari in open markets. The gari sellers sell their gari in containers and not in polythelene bags or glass cased containers. The sellers stay in open markets where flies are always abundant and feeding freely. Flies

like Musca domestica and other species of Musca possess both external and internal structures particularly well adapted for picking up and carrying of living bacteria and parasites including Ascaris eggs from contaminated faeces to gari in the open markets, especially since markets are near refuse dumps where people defaecate freely because there are no public toilets in our markets. In the direct feeding position of the fly, cestode, trematode and helminth eggs including Ascaris eggs, can enter the gut. In this way, Ascaris eggs may be transmitted to gari while the fly is feeding. Ascaris eggs may be conveyed onto gari through the agency of the vomit, the so-called vomit-drop which is often used by the fly when feeding on solid food items. The fly may also carry Ascaris eggs on any part of the body particularly the proboscis, or on the glandular hairs of the pulvilli. These flies are for ever in search of food and because of their high intake of nourishment, they defaecate very often. This constant discharge of

excreta is one of the many factors which make housefly a very dangerous carrier of Ascaris infection. Finally, the role of carriers engaged in gari handling either in preparation or distribution of the food stuff can be incriminated as sources of infection. Vegetable salad, fruits, roots, palmwine and gari contaminated with human excreta are vehicles of infection and as long as our food items are sold in open containers in open markets, the food items will continue to be some of the sources of Ascaris infection to individuals who eat these food items raw.

4.0 CONCLUSIONS AND RECOMMENDATIONS

4.1 CONCLUSIONS

Arising from the observations and data, obtained during this study on Ascaris-bacteria relationship, the following conclusions have therefore been made:

(1) The time required for culturing Ascaris eggs to the infective larval stages could be shortened to the barest minimum by using thermostatically controlled incubators in the Laboratory;

(2) The use of formalin has no effect whatsoever on the development of Ascaris eggs, but formalin can be used to prevent bacterial contamination of the eggs;

(3) When Ascaris eggs are inoculated in broth culture of intestinal bacteria, the bacteria species used up all the available oxygen and thereby inhibited the cleavage and development of the eggs;

(4) The gut of both man and piglets is inhabited by both Ascaris and bacteria which probably live happily as individuals independently of the other. However, the larvae of Ascaris have been found to act as vehicles of bacterial transportation from the intestine;

(5) The adult worms of Ascaris harbour bacterial species and could therefore be important agents of transportation of bacteria into the tissues. Such an association was found to increase the chances of invasion of tissues by the harboured intestinal bacteria which turned out to be even more virulent than their like in the intestine. This close association was found to increase the pathogenicity of Ascaris in piglets;

(6) The results confirmed the work of Bradley et al (1966), Philips et al (1959), Wescott (1970), Brown & Perna (1958) and Woodruff (1968), who observed that close association of protozoa and microflora increased the pathogenicity of the protozoa;

(7) The results of this study have confirmed that a close attention is required to prevent an indirect cause of ill-health in man and piglets. From the results of this study, it is now very definite that some of the bacterial species especially E.coli, which are regularly associated with Ascaris worm can be easily transported by the worms to places outside the intestinal lumen;

(8) The results have also established that certain food items like carrots, vegetables and fruits that are eaten raw by many people in Ibadan population serve as sources of Ascaris infection to man.

4.2 RECOMMENDATIONS

The examination of faecal samples for Ascaris infection should be mamdatory to further elucidate possible causes of ill-health which may not be unconnected with normally commensal bacteria of intestine being transported to the tissues where they become invasive and pathogenic. The microscopic examination of stool from man and animal who show retarded growth (especially in children and young animals) and with pyrexia of unknown origin, no doubt, will eliminate late discovery of Ascaris infections. The results of this late discovery may be fatal, because when parasitic infection is discovered late and antibiotic discontinued, the bacteria carried by migrating Ascaris larvae and adults would have been permitted to overwhelm the patient and spread via the blood to the meninges, causing fatal septicaemia and meningitis. Periodic deworming of children and piglets

are strongly recommended.

Finally, the observance of the simple rules of personal hygiene and the sanitary disposal of faecal wastes should be taught to all levels in the population in order to prevent this constant contamination of food items with Ascaris eggs with a view to reducing the exposure level of the population to Ascaris infection.

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APPENDIX 1: [illegible] THE SPREAD OF ASCARIS LUMBRICOIDES EGGS ON LETTUCE LEAVES

Batch number	Total No. of specimens examined.	Number Positive	Total egg Counts	Embryonated Eggs.
1	4	1	2	-
2	2	-	-	-
3	8	2	3	1
4	4	2	7	3
5	2	1	2	-
6	10	2	6	4
7	3	1	3	1
8	2	-	-	-
9	4	1	2	2
10	4	1	2	-
TOTAL	43	11	27	11

APPENDIX 2: [redacted] THE SPREAD OF *ASCARIS LUMBRICOIDES* EGGS ON CUCUMBER FRUIT

Batch Number	Total No. of specimens examined.	Number Positive	Total egg Counts	Embryonated Eggs
1	7	1	3	1
2	2	-	-	-
3	5	1	3	1
4	2	-	-	-
5	3	-	-	-
6	4	-	-	-
7	6	2	5	2
8	6	1	2	--
9	7	2	3	2
10	5	1	2	-
11	3	-	-	-
12	5	1	3	-
TOTAL	55	9	21	6

APPENDIX 3: THE SPREAD OF *ASCARIS LUMBRICOIDES* EGGS ON CARROTS (ROOT)

Batch Number	Total No. of specimens examined	Number Positive	Total egg Counts	Embryonated Eggs
1	3	-	-	-
2	4	1	3	1
3	2	-	-	-
4	3	1	4	1
5	4	1	4	1
6	5	2	6	2
7	7	2	8	2
8	3	-	-	-
9	2	-	-	--
10	5	2	5	2
11	7	2	8	3
TOTAL	45	11	38	12

APPENDIX 4: [illegible]F THE SPREAD OF ASCARIS LUMBRICOIDES EGGS ON MANGO FRUIT

Batch Number	Total No. of specimens examined	Number Positive	Total egg Counts	Embryonated Eggs
1	4	1	3	1
2	3	-	-	-
3	5	2	7	2
4	4	1	2	-
5	5	3	5	2
6	4	-	-	-
7	3	-	-	-
8	4	1	3	1
9	3	-	-	-
TOTAL	35	8	20	6

APPENDIX 5: [illegible] THE SPREAD OF ASCARIS LUMBRICOIDES EGGS ON TOMATO FRUIT

Batch Number	Total No. of specimens examined.	Number Positive	Total egg Counts	Embryonated Eggs
1	5	-	-	-
2	6	-	-	-
3	10	1	2	-
4	9	1	4	1
5	12	2	5	2
6	10	1	2	-
TOTAL	52	5	13	3

APPENDIX 6: THE SPREAD OF ASCARIS LUMBRICOIDES EGGS ON GARDEN EGGS

Batch Number	Total No. of specimens examined	Number Positive	Total egg Counts	Embryonated Eggs
1	12	2	3	-
2	10	2	4	1
3	10	1	2	-
4	11	2	2	-
5	10	2	3	1
6	10	1	1	-
TOTAL	63	10	15	2

APPENDIX 7: [redacted] THE SPREAD OF ASCARIS LUMBRICOIDES EGGS ON SWEET PEPPER

Batch Number	Total No. of specimens examined	Number Positive	Total egg Counts	Embryonated Eggs
1	2	-	-	-
2	2	-	-	-
3	4	1	2	-
4	10	3	4	1
5	6	1	1	1
6	8	2	3	1
TOTAL	32	7	10	3

APPENDIX 8: THE SPREAD OF ASCARIS LUMBRICOIDES EGGS ON ONIONS (BULB)

Batch Number	Total No. of specimens examined	Number Positive	Total egg Counts	Embryonated Eggs
1	5	1	1	-
2	3	-	-	-
3	5	1	1	-
4	6	1	2	-
5	3	-	-	-
6	7	1	3	1
TOTAL	29	4	7	1

APPENDIX 9: THE SPREAD OF ASCARIS LUMBRICOIDES EGGS ON GARI

Batch Number	Total No. of specimens examined	Number Positive	Total egg Counts	Embryonated Eggs
1	7	2	5	2
2	5	1	2	1
3	6	1	2	1
4	8	2	7	2
5	6	1	2	1
6	4	-	-	-
TOTAL	36	7	18	7

APPENDIX 10: THE SPREAD OF ASCARIS LUMBRICOIDES EGGS ON PALM WINE

Batch Number	Total No. of specimens examined.	Number Positive	Total Egg Counts	Embryonated Eggs
1	5	1	2	-
2	7	1	3	2
3	6	1	3	2
4	4	1	1	-
5	5	1	2	1
6	2	-	-	-
TOTAL	29	5	11	5

APPENDIX 11:
Direct Smear Method

Procedure - Place a drop of saline on a clean slide and take as much or slightly less than a match head of faecal material by means of a disposable applicator such as a swab stick or toothpick. Mix with saline thoroughly and examine microscopically after placing a coverslip on it.

Note (1) Smear on the slide should be thin enough to read newspaper print through. Properly prepared faecal film is grey in colour.

(2) Saline may be replaced with glycerine-water (1:2) solution to prevent drying.

(3) The amount of saline used for mounting faecal material should be enough to fill all space beneath the coverslip without any saline flowing beyond theedges of the coverslip.

(4) Ascaris infection with a female worm may be theoretically detected by observation of three faecal films prepared by this method. But this is not true in

the case of human infections with other species of helminths because of much lower oviposition as compared with that of Ascaris.

APPENDIX 12:

Kato Cellophane Thick Smear Method

Procedure:

(1) Cellophane coverslips, 40 /um in thickness and 26 x 28mm in size.

(2) Cellophane staining medium containing 100 parts of distilled water (6% phenol), 100 parts of glycerine and one part of 3% malachite green solution

(3) Soak the coverslips individually in the medium for more than 24 hours.

Procedure:

(1) Place 60-70mg (as much as a red bean) of faecal sample on a slide.

(2) Cover it with the cellophane coverslip after removing the excess of the medium by shaking.

(3) Press the coverslip by means of rubber stopper or finger to spread faecal material evenly to the edge of the slip. Examine it microscopically.

Note -

(i) In slides standing for 60 minutes at atmospheric temperature of 25°C and relative humidity of 75% deformation of eggs on these slides may occur, presumably due to overdrying.

(ii) Some eggs on the slides prepared by this method are observed as somewhat different from those prepared by the direct smear method. The thin-shelled eggs such as those of hookworms are seen flattened and rather roundish in shape in the slides used in this method. Both in thick-shelled eggs such as those of Ascaris and whipworm, no morphological deformation occurs in the direct film and cellophane thick-smear method.

APPENDIX 13:

Brine Floatation Method

Preparation:

Saturated Nacl medium with a specific gravity of 1.200, which is prepared by adding more than 400gm of NaCl into 1ooml of water and dissolving well by slight heating and thorough stirring. In practice the supernatant fluid from the saturated NaCl solution with NaCl undissolved at the bottom of a vessel after standing for a while is preferably used as a floatation medium.

Procedure:

(a) Place 0.5g of faecal sample taken from various parts of a faecal specimen into a small sized test tube containing a previously prepared small amount of the floatation medium.

(b) Stir vigorously with an appropriate applicator.

(c) Place the test tube virtically and add the medium up to the brim of the tube so as to form a convex surface of the medium at the month of the tube. Then let it stand for 30-45 minutes.

(d) Carefully superimpose a grease-free coverslip on the convex surface in contact with the medium without overflow and carefully lift the coverslip by a straight upward pull and then place it on the slide for microscopic observation.

Note (a) Incomplete stirring may result in no egg floatation to the surface of the fluid due to incomplete separation of eggs from the faecal debris.

(b) Egg floatation may be incomplete when the floatation period is too short (less than 30 minutes) or too long (more than 60 minutes). In the latter case, the eggs floating at the surface may descend again.

(c) This method is suitable for detection of eggs with low specific gravity such as those of hookworm but not for heavy eggs such as trematode, whipworm eggs and Ascaris infertile eggs.

APPENDIX 14:

Magnesium Sulphate Method

Preparation - Prepare $MgSO_4$-NaCl floatation medium by adding 290gm of NaCl and 185gm of $MgCl_2$ into 1,000ml of previously warmed water. Then stir thoroughly.

Procedure: - Follow the same procedure as in brine floatation method using $MgSO_4$ - NaCl medium instead of saturaded NaCl as in the former method.

APPENDIX 15:

Formalin-Ether Method:

Procedure: (a) Place 0.5g of faecal sample with 2-3ml of water in a small-sized test tube and stir thoroughly.

(b) Filter the faecal suspension through gauze into a centrifuge tube after increasing its volume to 15ml with water.

(c) Centrifuge the tube at 2,000rpm. for 1-2 minutes and decant the supernatant.

(d) Add 7-10ml of 10% formalin and fix the sediment for 10 minutes. Add 3ml Ether.

(e) Shake the stoppered tube vigorously for 20-30 seconds by hand.

(f) Centrifuge the tube at 2,000r.p.m. for a minute after removal of the stopper from the tube. Decant the supernatant with the faecal scum which is separated previously from the tube wall by means of an applicator

(g) Place the sediment on a clean slide by tilting the tube or by sucking up the sediment by a long capillary pipette and discharging onto the slide. After placing a coverslip examine microscopically.

Note:

(a) Formalin-fixed sediment may be stored for long periods of time and sent anywhere for microscopic examination.

(b) Egg recovery rate by this method is somewhat lower than that by AMS 111 and Tween 80-Citrate buffer method and the amount of the final sediment obtained by this method is somewhat larger than those previously mentioned.

APPENDIX 16:

McMaster Egg Count Method

Procedure:

(1) Weigh out 2gm. faeces from sample.

(2) Thoroughly mix sample with 60ml of saturated salt solution, and pass through sieve into evaporating dish or glass jar.

(3) Wash remaining debris in sieve with further 30ml saturated salt solution.

(4) Agitate, fill both counting chambers by means of pipette, count all eggs in both chambers. In practice, faeces can be diluted at once through sieve with 60ml saturated salt.

Note: Eggs of Ascaris, hookworm, strongyloides and trichostrongyles being higher than the salt solution float to upper surface of fluid and are easily seen, free from debris which is on lower plane. Trematode eggs collapsed and do not float

Each chamber 1sq cm., divided by a grid into 6 areas each equal to diameter of microscope field with x 6 eyepiece, ⅔rds objective. Depth 1.5mm. Volume of each chamber therefore, 15ml. 2gm. faeces in 60ml = 1:30 dilution. Volume counted 0.3ml = 1/100th of 30ml. Therefore eggs counted x 100 = Eggs/per gm of faeces.

APPENDIX 17:

Stoll's Egg Count Method

Procedure:

(1) Place faeces until the surface of aqueous fluid reaches the 60ml mark in Stoll's graduated Erlenmeyer flask containing previously prepared 0.1N NaOH up to the 56ml mark.

(2) Add 10-20 glass beads (3-4mm in diameter) to the flask and stand it with a stopper overnight.

(3) Shake the flask vigorously, pipette out an aliquot of 0.15ml onto a clean slide and place a coverslip on it.

(4) Count the total number of eggs concerned microscopically. Then multiply the number by 100 to obtain number of eggs per gram (E.P.G.).

Note:

(1) Two ml of faecal sample are added to 58ml od 0.1N NaOH. E.P.G. may be obtained by multiplying the total number of eggs in 0.15ml aliquot of the faecal suspension by 200.

(2) Water content of faeces may vary with the nature of faeces. To convert to the normally formed faeces basis, the following factors should be used according to the nature of faeces used: normally formed, 1; mushyformed, 1.5; mushy 2.0; mushy diarrhoeic 3.0; obviously diarrhoeic 4.0 and watery 5.0. But no correction is made on values obtained from many people in the field-survey.

APPENDIX 18:

Beaver's Egg Count Method.

This method involves the use of a photometer in the preparation of a faecal smear of a known standard density. This photometer is difficult to standardise and the facility is difficult to maintain in a place like Nigeria.