STUDIES OF FLUKE INFECTIONS IN CATTLE

With special reference to the Enzyme-linked Immunosorbent Assay (ELISA) as a diagnostic tool for bovine fascioliasis and shistosomiasis

A dissertation submitted in partial fulfilment of the requirements for the degree of Master of Science of the University of Zambia in Biology

by

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APPROVAL

This dissertation of LILY NG'AMBI is approved as fulfilling part of the requirements for the award of the degree of Master of Science in Biology by the University of Zambia.

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SUMMARY

Studies on fluke infections in cattle with special reference to diagnostic methods were carried out. A suitable diagnostic method was necessary for use to investigate the prevalence and distribution of Fasciola spp. and Schistosoma spp. in cattle

The Enzyme-linked Immunosorbent Assay (ELISA), based on the antibody-antigen reaction was developed and standardised for sero-diagnosis of fascioliasis and schistosomiasis in both experimentally and naturally infected cattle.

Using crude adult worm antigen preparation, the ELISA test was able to detect antibodies at four weeks post infection in animals experimentally infected with F. hepatica and S. bovis. This is several weeks earlier than infection could be detected by presence of eggs in the faeces. Moreover the ELISA test was specific for either Fasciola or Schistosoma infections. There were no cross-reacting antibodies between the two parasites.

Samples from an abattoir survey which were naturally infected with F. gigantica and S. mattheei were examined by the ELISA test sedimentation technique. The ELISA test detected 47 percent as positive, while sedimentation technique 29 percent. No schistosome egges were detected by sedimentation, but the ELISA test detected antibodies in 48 out of 564 samples.

DECLARATION

I declare that the work presented in this dissertation is my own, unless otherwise referred to and acknowledged in the appropriate place in the text; it has not been published previously or submitted to this or any other University for the award of any degree.

L. Ng'ambi

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ABBREVIATIONS USED

S.	*Schistosoma*
F.	*Fasciola*
Spp.	Species
FHA	*Fasciola hepatica* antigen
SBA	*Schistosoma bovis* antigen
ELISA	Enzyme-Linked Immunosorbent Assay
L.	*Lymnaea*

INTRODUCTION

The two important diseases in Zambian cattle caused by the flukes Fasciola gigantica (liver fluke) and Schistosoma mattheei (blood fluke) are termed "fascioliasis" and "schistosomiasis" respectively. While reputedly widespread, the distribution of these diseases is not well documented in Zambia. A report by Silangwa (1973) mentioned fascioliasis in Western Province while schistosomiasis was recorded by Dinnik and Dinnik (1965) in the region. These diseases are important, however, as they cause ill-thriftiness, loss of milk production (and so poorer mothering), reduction of growth rates and significant economic losses at slaughter due to liver condemnation.

In order to study these two conditions in Zambia, it is necessary to first elaborate methods that can be used to investigate the prevalence and distribution of Fasciola spp. and Schistosoma spp. in cattle so that infested herds can be identified and studied in depth. Diagnostic methods suitable for mass screening must be of high sensitivity, and specificity, as well as cheap and easy to perform.

The tests which have been used traditionally are sedimentation, floatation and serodiagnosis. The sedimentation and floatation methods though easy to establish, are rather tedious when examining large numbers of samples. They are not very sensitive and can only detect infections when adult worms start producing eggs in significant numbers.

The detection of schistosome infections in cattle by parasitological methods like the sedimentation technique presents several problems. Apart from being tedious, such a method often fails to demonstrate the presence of the infection, particularly in long standing cases.

Moreover not only are the eggs usually not seen because of their smaller size and therefore difficult to find among the debris, but also because of their ability to hatch out immediately on contact with fresh water. These could be probably some of the reasons for the scarcity of data on the incidence and the distribution of bovine schistosomiasis in Zambia.

Similarly assuming that chances of detecting liver-fluke eggs in the faeces of an infected animal mainly depend upon the adult burden in the liver, the lower incidence usually obtained from examination of the faecal samples could be due to the infection being in the prepatent period, or to the presence of very light infections which could not be easily detected by examining one faecal sample from each animal.

Several serological tests have been developed recently for use in the diagnosis of bovine fascioliasis and schistosomiasis. (Doyle, 1971; Hillyer, 1975; Van Tiggele and Over, 1976; Hanna and Jura, 1977; Lawrence, 1977; Schillhorn Van Veen and Buys, 1979). Of these the Enzyme-linked Immunosorbent Assay (ELISA) has shown promise (Burden and Hammet, 1978;

Farrel et al., 1981; Oldam, 1983; Wescott et al.,1984) and could serve as a routine diagnostic test if procedures were standardized.

The ELISA test has many advantages over other diagnostic methods. This test has been proved to be sensitive, using only small amounts of reagents for large numbers of samples which can be handled with ease. The ELISA test can be used as a sero-epidemiologic tool. Sero-epidemiologic methods measure the "period prevalence", that is, infections current during a period of time, irrespective of when the infections occurred. Whereas parasitologic tests measure the "point prevalence", that is infections existing at a specific point in time (Kagan, 1979).

The economic significance of bovine fascioliasis in Zambian cattle can be illustrated by the high rate at which the livers are condemned at the commercial abattoirs. Silangwa (1973) estimated the yearly average loss due to fascioliasis to be approximately 37,000 Kg of liver meat.

The extent to which liver flukes are directly responsible for actual mortality among cattle in Zambia is yet to be determined. However, the observed extensive damage to the liver tissue gave the impression that fascioliasis could contribute to the apparent high rate at which cattle lose condition under stress of age, poor grazing and other diseases.

Bovine schistosomiasis in Zambia, although not very common, has been reported. Three species have been identified in domestic and wild ruminants. S. mattheei, S. leiperi and S. magrebowiei. S. mattheei is said to be a common parasite of cattle in Southern Africa and can be pathogenic in large numbers (Lawrence, 1978). However, in most circumstances the host-parasite relationship limits the level of infection to one which can be tolerated by the host and thus the parasite does not present a problem. But with the intensification of livestock production and increased conservation of water in some parts of the country, the potential exists for periodic explosive increases in schistosome population which could cause serious disease.

Thus, there is a need for a better method for diagnosing these flukes diseases and moreover very little information is available on the epizootiology of the liverfluke in Zambia. With the use of the ELISA test such information can be available.

This dissertation presents a review of appropriate literature followed by accounts of three studies of the ELISA test for the diagnosis of bovine fascioliasis and schistosomiasis. The study concludes with a discussion of the applicability of this diagnostic method for field conditions in Zambia.

II. Literature Review

1. Bovine Fascioliasis

Bovine fascioliasis is an economically important, trematode disease of cattle caused by the liverfluke, *Fasciola spp*. The life cycle of the fluke involves a freshwater snail intermediate host, *Lymnaea spp* and so is associated with watering places and swamps. The disease causes considerable financial and meat loss through both condemnation of livers at abattoirs and because of unthriftness produced by the parasites in young cattle (Magzoub and Adams, 1977).

High prevalences of liverfluke infestation are characteristically associated with communal grazing and common watering sites. This is especially pronounced in the dry season when cattle concentrate on available grazing and utilize permanent watering sites. These waters become foci for the transmission of the parasites by virtue of the concentration of hosts together with enhanced access of infective stages. Cattle in enzootic areas become infected as calves and continued exposure produces a progressive chronic disease.

The clinical disease which is most frequently seen in cattle under two years (Armour, 1975) is characterised by weight loss, anaemia, eosinophilia and hypoalbuminaema and is due to the haematophagic activities of adult flukes in the bile ducts. In severe cases, submandibular oedema is prominent

and terminal diarrhoea may occur (Armour 1975).

Most commonly, and particularly in adult cattle, the liverfluke is responsible for a sub-clinical disease with only minor haematogical changes, but a lowered productivity reflected by inadequate food conversion rates, poor carcase formation and reduced milk yields, particularly solids (Armour 1975).

There are three forms of fascioliasis - acute, subacute and chronic (El-Azay and Schillhorn Van Veen, 1983). The acute form is rarely reported in cattle, but does occasionally cause considerable losses. Acute fascioliasis is due to the immature stage passing through the liver (Sewel, 1966). Soliman (1960) and Zaki (1960) described acute fascioliasis but noted this form required infection with a large number of metacecariae under field conditions.

The subacute form is more common in cattle and is caused by young adult flukes emerging from the liver into the bile ducts. This clinical syndrome is characterized by anaemia and is mainly observed during the beginning of the wet season (Schillhorn Van Veen, 1980a)

Chronic fascioliasis is the most common form of parasitosis and is associated with a relatively small number of flukes in the bile ducts (Schillhorn Van Veen, 1980a) It is frequently seen in young animals during the rainy season as a result of newly acquired infections. The chronic condition also appears in the

dry season in older animals experiencing loss of bodily condition due to poor fodder at that time of the year. Together the chronic state in young and old animals appears to be of prime economic importance (Schillhorn Van Veen, 1980a)

Bovine fascioliasis in Africa

The geographical distribution of Fasciola hepatica and F. gigantica is presented in Figs. 1 and 2. (pp.44,45).

These species are exclusively transmitted by snails of the family Lymnaeidae F. gigantica is a tropical parasite and in Africa is transmitted by Lymnaea natalensis. F. hepatica is transmitted by L. truncatula.

Data available concerning the distribution of fascioliasis in Africa is rather limited. In the north, F. gigantica infections were observed in cattle from Egypt and Sudan (El-Azay and Schillhorn Van Veen 1983). In West Africa, bovine fascioliasis due to F. gigantica has been reported to be common by several authors (Coyle, 1956; Ogambo-Ongoma, 1972; Schillhorn Van Veen, 1980a and El-Azay and Schillhorn Van Veen, 1983)

Fascioliasis as a disease of livestock has been recognised in East Africa for a long time. Ogambo-Ongoma (1971) observed fascioliasis in cattle in Uganda. In an extensive field survey carried out, infection rates between 4.9 per cent and 78.8 per cent were confirmed. Fascioliasis was also found to be common

in Kenya and Tanzania (Bitakaramire, 1967; Hammond,1956).

Bovine fascioliasis in Kenya was mainly due to F. gigantica although the presence of F. hepatica infection has been reported, but in very low incidences and confined to the highlands (Ogambo-Ongoma, 1969).

In East Africa, cattle seem to be susceptible to both species of Fasciola, but the distribution of the flukes is related to the distribution of appropriate snail hosts. (Figs 1 & 2).

Bovine Fascioliasis in Zambia

Bovine fascioliasis has been reported to be common in two major wetland areas of traditional cattle keeping in Zambia, namely the Zambezi flood plain in Western Province and the Kafue flats in the Southern part of the country (Jorgensen and Awan, 1977).

Between 1944 and 1964, 65,000 livers of Barotse cattle from the Zambezi flood plain were examined at the Livingstone abattoir. Silangwa (1973) found that an average of 89.6 per cent of these were condemned due to fascioliasis. This represents a loss of 37,000 Kg of liver meat per year and illustrates the seriousness of this disease in wetland cattle.

Jorgensen and Awan (1977) reported high incidences of fascioliasis in cattle in the Mazabuka District in Southern Province. Of the 200 animals examined, 44.5 per cent was found to be infested.

As far as is known, fascioliasis in Zambia's livestock is caused by F. gigantica. The distribution of this fluke is limited by the presence of the snail intermediate host L. natalensis. The wet conditions prevailing on the flood plains and other wetlands of Zambia are ideal for this snail.

F. gigantica has been recorded from many species of wild herbivores inhabiting these wetlands (Le Roux, 1933) and is a common parasite of ungulates inhabiting swampy areas.

Fasciola Spp. Life Cycle

The adult flukes lay eggs in the bile ducts. Eggs then pass into the intestine and out in the faeces. Under optimum conditions for development the miracidia hatch out in two weeks. However, the eggs must be free of faeces and surrounded by water before any development can take place. Light and hypertonicity of the egg contents are essential components of the hatching stimulus (Dunn, 1969).

The ciliated miracidia have a strictly limited infective life. They can survive for about 24 hours but the infective span is much shorter and unless they find a snail within an hour or so of hatching, they are unable to penetrate it (Dunn, 1969). During penetration the miracidia shed their cilia and enter the snail as young sporocysts.

The sporocysts develop in the digestive tract of the snail producing a number of rediae, which migrate to the large digestive

organ, where the cercariae develop. Shedding of cercariae from the snail begins at about five weeks from the entry of the miracidium and continues at intervals for several weeks, the peak out-put being at about seven weeks. In dry conditions, snails may retain their mature cercariae for long periods and shed them as soon as they become immersed in water. Under certain adverse conditions, the redia may produce within itself a second generation of "daughter rediae", thus postponing the production of the cercariae and adding yet another generation. Each of the second generation rediae ultimately produces its own family of cercariae. The second generation is more common at the end of the summer (Taylor, 1964). The shed cercariae swim to any firm surface, of a plant, where they attach and secrete a cyst wall around themselves. Before encystment, however, they lose their tails and become metacercariae. The site of attachment is always near the surface of the water and never more than 5 centimetres (cm) deep. A period of two to three days must elapse before encystment is complete and infection of the final host can take place by ingestion of the metacercariae with herbage.

After ingestion the metacercariae excyst in the small intestine of the definitive host and migrate through the gut wall into the peritoneal cavity and to the liver. They migrate in the liver tissue for a period of about 9 to 12 weeks before entering a bile duct in which they grow to adults. The prepatent period for F. gigantica is 13 to 16 weeks and for F. hepatica, 10 to 11 weeks (Dunn, 1969).

effects of moderate and long standing chronic infections (Dargie, 1980; Lawrence, 1980; Saad et al., 1980). In addition, changing methods of husbandry and water conservation will in the future, create environments very favourable for the transmission of schistosomiasis (Lawrence and Condy, 1970) and the disease may therefore become a major veterinary problem over large parts of Africa.

Bovine schistosomiasis in Africa is caused by a number of species of Schistosoma; S. bovis, S. mattheei, S. margrebowiei and S. leiperi. These inhabit the mesenteric veins causing disease. These blood flukes require an intermediate host snail of the genus Bulinus which often occupy similar areas to those inhabited by Lymnaea. Thus, bovine schistosomiasis and fascioliasis may be enzootic in the same areas.

The intermediate snail host spectra of S. mattheei and S. leiperi only comprise members of the Bulinus africanus species complex. S. bovis is transmitted by members of B. truncatus, B. africanus and B. forskalii. S. margrebowiei is transmitted by members of B. forskalii and possibly also by members of B. tropicus and B. truncatus (Christensen et al., 1983)

Bovine Schistosomiasis in Africa

The information available on the distribution of bovine species of schistosomiasis in Africa has been reviewed by Dinnik and Dinnik (1965) and by Pitchford (1977).

Data available concerning the distribution of S. bovis outside East Africa are rather limited, but it appears that the

species is widely distributed all over the northern (except Egypt) and western parts of Africa. In Central Africa, it extends southwards to central Angola, southern Zaire and northern Zambia. S. bovis occurs in East Africa in a clearly defined zone from Sudan, Ethiopia and Somalia southwards through Uganda, Rwanda-Burundi, Kenya and Tanzania into northern Zambia. S. mattheei occurs in a zone from eastern South Africa northwards through north-eastern Botswana, Zimbabwe, Mozambique, eastern and northern Zambia and Malawi into southern Zaire in Central Africa and to Rwanda-Burundi and Tanzania in East Africa. S. bovis and S. mattheei thus co-exist in Southern Zaire, northern Zambia, Rwanda-Burundi and Tanzania, but studies by Dinnik and Dinnik (1965) have shown that S. bovis is normally the dominant species in areas where the two species co-exist (Fig 3).

S. margrebowiei is mainly known from a small area extending from north-east Botswana to south-western Zambia and southern Zaire. The distribution of S. leiperi corresponds to that of S. margrebowiei in southern Central Africa including Zimbabwe, northern Zambia and central and southern Tanzania (Pitchford, 1976). In areas where the conditions are favourable for schistosome transmission, prevalence rates of S. bovis and S. mattheei in cattle may occur at a level of 40-70% and commonly higher (Christensen et al., 1983). Prevalence rates in this range have been found in Sudan (Eisa, 1966; Majid et al., 1980), Kenya and Tanzania (Kinoti, 1961, Dinnik and Dinnik, 1965), Chad .

(Graber, 1969), South Africa (Pitchford, 1961; Pitchford and Visser, 1969; Pitchford et al., 1973), Zimbabwe (Condy, 1960) and Nigeria (Schillhorn Van Veen, 1980b).

According to Dinnik and Dinnik (1965), S. mattheei is an indigenous parasite of wild african bovines. Naturally acquired infestation have been recorded in baboon and vervet monkey, in various species of rodents, in zebra, as well as in a large number of wild bovine species. From the comprehensive studies conducted by Pitchford and Visser (1975) and Pitchford et al., (1973, 1974) it appears that water buck (Kobus ellipsiprymnus), puku (K. vardoni lechwe (K. leche)and presumably also sable antelope (Hippotragus niger), wildebeest (Connochaetes taurinus)and buffalo (Syncerus caffer) are primary definitive hosts for S. mattheei. The wild bovines may provide a reservoir for S. mattheei in domestic animals by sharing watering points and pastures.

S. margrebowiei and S. leiperi both have been recorded in wild bovines, among which lechwe, puku and waterbuck serve as primary definitive hosts (Pitchford 1976, 1977; Pitchford and Wolsternhormme 1977 While these schistosomes have been found in cattle at times (Le Roux, 1957) they are essentially wild animal parasites and not normally associated with domestic bovine schistosomiasis.

Bovine Schistosomiasis in Zambia

The occurrence of bovine schistosomiasis in Zambia has been implied by several workers (Buckely, 1946; Le Roux, 1957;

Nelson et al., 1962; Dinnik and Dinnik, 1965; WHO, 1967 Hira, 1975), but no figures for the prevalence of Schistosomiasis among domestic ruminants are presently available.

Le Roux (1957) recorded three schistosome species, S. mattheei, S. leiperi and S. margrebowiei in domestic and wild ruminants in Zambia. He encountered a very heavy infestation with S. mattheei in man and disoussed its presence in cattle.

Schistosome infections have been reported in wild animals, the lechwe and waterbucks by Howard et al.,(1982). These wild animals are closely associated with permanent water and the lechwe in particular associated with cattle. Lechwe were found to be parasitized by S. margrebowiei and S. leiperi, while waterbuck contained these species as well as S. mattheei.

Epizootiology of bovine schistosomiasis

The epizootiology of schistosomiasis involves a vertebrate definitive host, a molluscan intermediate host and their common presence in an environment in which transmission can take place. Each of these components is subjected to considerable variability as the epizootiology of the disease presents perplexing aspects not encountered in many other infectious diseases. In order to facilitate an understanding of the epizootiology of schistosomiasis the life cycle is summarised as follows:-

Eggs laid by the female worms in the intestinal veins of cattle are passed out in the faeces and hatch after contact with water to release miracidae. Optimal hatching occurs when the faeces are diluted and agitated. Eggs remain viable only so long as the faeces remain moist and will survive for at least four days (Pitchford et al., 1973)

The emerging miracidia seek-out and penetrate a suitable snail. Daughter sporocysts develop within mother sporocysts. Cercariae develop within the daughter sporocysts and begin to emerge after about five weeks. Transmission of the parasite from the snail to the final host can occur throughout the year with a marked increase in the dry season, and a low level during the cold season. Numbers of cercariae shed are greatly reduced in the cold season and infections acquired by the snail during this season have a prolonged development period.

With the beginning of warm weather, shedding increases and delayed infections mature to give a sharp increase in the number of cercariae in the water. The periodicity of shedding is thought to be an adaptation to the drinking habits of the natural wild hosts (Lawrence, 1978). Cercariae recovery from natural waters is at its greatest at the point where faecal contamination of the water occurs and it decreases rapidly down-stream (Lawrence, 1978). Infection of cattle by the

cercariae is usually by penetration of the skin. The habit of cattle of standing in water during the heat of the day makes them liable to very heavy exposure to cercariae (Reinedie, 1970). After penetration the cercariae transform into schistosomulae and migrate to the lungs and enter the circulating system.

The parasites mature in the hepatic portal veins, mate and migrate to their final site, the mesenteric veins. Female worms penetrate the smaller veins of the intestine to lay their eggs. The eggs escape from the blood vessels and move through the intestinal wall, the embryo developing into a miracidium during its progress, until finally the eggs are liberated into the lumen of the intestine. The prepatent period is usually six to seven weeks (Lawrence, 1977) but may be as long as nine weeks.

Temperature and rainfall are the most important elements that influence the size of the snail populations, the development of the parasite within the snail host and as well as the final host, (Cowper, 1971).

All snails that transmit bovine schistosomiasis show a high degree of tolerance to variation in the temperature of their habitat (Cowper, 1971).

Seasonal and climatic factors have a profound effect upon the life-cycle of intermediate snail hosts.

Peak snail densities in most habitats occur at the end of the rainy season and at the beginning of the dry season (Appleton, 1978). This seasonality of occurrence with most intensive transmission at the beginning of and during the dry season, induced by variation in snail numbers is furthermore enforced by the fact that other influential factors also provide optimum conditions for transmission during the same period. Such a seasonal pattern of transmission has been demonstrated for S. bovis in cattle in the Sudan (Malek, 1969; Majid et al., 1980) and for S. mattheei in wild bovines in South Africa (Pitchford et al., 1974).

The length of the prepatent period of all four bovine species is in the range of 4-5 weeks when at optimal temperature conditions (Southgate et al., 1981). Pitchford and Visser (1969) have shown that the prepatent period of S. mattheei at less optimum temperature conditions may be prolonged for up to 20 weeks. The effect of temperature on intramolluscan development may therefore, if other influential factors are kept constant, induce the following transmission patterns:

(1) intensive transmission all year round,

(2) a pronounced season transmission with no transmission during the cold period and a more or less intensive transmission during the hot period and

(3) a less pronounced seasonal pattern with intensive transmission during the hot season and a less intensive

transmission during the cold season.

3. Diagnosis of Fascioliasis and Schistosomiasis

In the past the diagnosis of bovine fascioliasis and schistosomiasis has been based mainly on clinical signs, necropsy and faecal examination (Dunn, 1969). The clinical signs of schistosomiasis may be confused with those of fascioliasis and so the diagnosis has been confirmed by faecal examination with the detection of terminally-spined eggs. Fascioliasis in cattle generally occurs in a chronic form which does not manifest itself clinically by specific and clearly perceptive symptoms. Therefore the diagnosis has to be verified through laboratory technique (Henricksen, 1974)

Conventional diagnostic methods such as faecal sedimentation aim at the demonstration of eggs passed with the faeces of the host animal. The sedimentation method utilizes the fact that owing to their gravity, the eggs in aqueous suspension will quickly sink to the bottom if the liquid is not stirred.

However the demonstration of fluke infections through faecal examination will be limited to the patent phase of the parasite life cycle, during which eggs are excreted with faeces. As the excretion of eggs does not start until 6 and 11 weeks post infections for Schistosoma spp and Fasciola spp

respectively (Henricksen, 1974, Lawrence, 1978) efforts have been made through several years to apply other diagnostic methods at an earlier stage of infestation. In this respect attention has been focussed on those immunological methods, which could demonstrate circulating antibodies as early as 4-5 weeks post infection(Mclaren et al., 1978; Farrel et al., 1981).

However the reliability of immunodiagnostic techniques is often limited when cross-reacting antibodies from other related or non-related infections are present. Cross-reactions are especially a problem in many parts of the tropics, where multiple infections are more a rule than an exception (Schillhorn Van Veen and Buys, 1979). Efforts to circumvent this problem of cross-reactions are mainly concentrated on the purification of antigens. The specificity and sensitivity of an immunodiagnostic technique depend on the quality of the antigen employed (Fife, 1971).

Several immunodiagnostic techniques have been developed for the dignosis of Fasciola spp and Schistosoma spp infections (Doyle, 1971; Hillyer, 1975; Van Tiggele and Over, 1976; Hillyer and de Weil, 1977, 1979; Burden and Hammet, 1978 and Farrel et al., 1981). Each of these methods has particular advantages and disadvantages. For example, Immunoelectrophoresis is highly specific, but has a low sensitivity; Indirect Immunofluorescence is sensitive but only group

Specific; Complement fixation test is easily performed but is insufficiently specific.

However, recent developments in methodology for detection of anibodies, particularly Radio Immunoassay and Enzyme-linked assay, have overcome earlier difficulties in respect of the insensitivity of serodiagnostic techniques (Voller et al., 1976).

The Enzyme-linked Immunosorbent Assay (ELISA) was first introduced in 1971 by Engvall and Perlmann. ELISA is an Immuno Enzyme method in which specific antibodies can be measured by Enzyme-labelled anti-immunoglobulins in antigen-coated tubes.

The ELISA may be compared directly with Radio immunoassay (RIA) and Immunofluorescent antibody, (IFA) (Voller et al., 1974). The antigen-antibody reactions upon which these tests depend, require the use of labels to visualise whether the test is positive or negative. RIA uses a radioactive isotope and IFA uses an ultra violet fluorescent dye attached to antibody. Both of these have disadvantages:-

RIA has greater sensitivity and specificity, but the isotopic lables may decay. Complex and expensive equipment is required for quantitation of activity and safety requirements indicate that highly trained staff carry out the test procedures.

Disposal of the used reagents is governed by radiation safety regulations. Most developing countries do not have a radiation centre, access to radioactive reagent or a safe disposal system.

IFA tests are difficult to standardize and to quantify. The assessment of results is very subjective and can result in an unacceptable degree of variation between workers. However, the use of enzyme labels overcomes many of these disadvantages.

Specificity and sensitivity are retained, while the use of micro-volumes of sample and reagents reduce the cost of the procedure further. Simple equipment can be used to perform the test and it can be automated to a significant degree. The reagents are stable and easy to standardize.

The general principle for the ELISA technique is as follows:-

(i) An antigen or antibody is linked to a solid-phase carrier surface, usually the inside of the walls of a microelisa plate.

(ii) The sensitised carrier surface "captures" the corresponding antibody/antigen from a test solution.

(iii) an enzyme labelled specific antibody attaches to the antigen/antibody complex.

(iv) the attachment of the enzyme label is detected by the colour change resulting from the hydrolysis of an added substrate by the enzyme.

(v) the optical density of the final colour is directly proportional to the amount of unknown antibody or antigen in the test solution. The result is usually expressed as a quantitative optical density measurement.

Since 1974, ELISA has been tried for the diagnosis of various parasitic disease. Huldt et al., (1975) used ELISA for detecting Schistosoma antibodies in humans and found that it has potential for epidemiological use. Mclaren et al. (1978) found ELISA to be better than other immunodiagnostic methods for detecting Schistosoma antibodies. Farrel et al. (1981) and Burden and Hammet (1978) using ELISA were able to detect antibodies against Fasciola hepatica in calves at 3-4 weeks post infection. Since this is several weeks earlier than diagnosis by faecal examination, the ELISA offers the clinician the advantage of early diagnosis and the opportunity to treat while immature flukes are migrating through the liver.

III. Studies on diagnosis of bovine fascioliasis schistosomiasis

Several immunological tests have been evaluated for diagnosis of fascioliasis and schistosomiasis. The Enzyme-Linked Immunosorbent Assay (ELISA) has been developed as a sero-diagnostic tool for these trematode infections.

However, previous reports have demonstrated that adult worms of Fasciola hepatica and schistosomes have cross-reactive and/or common antigens (Hillyer and Leticial Sagramoso de Ateca, 1980). Moreover the presence of at least five cross-reactive antigens between these parasites was demostrated by Capron et al (1968). Thus cross-reactive antigen between these two parasitic trematodes if evident would clearly interfere in serodiagnosis.

The objectives of the first part of the study were to establish the optimal ELISA test conditions for F. hepatica and S. bovis infections and then to use these to examine the potential of ELISA to diagnose experimentally induced infections of the parasites in cattle.

The second part of the study investigated the occurrence of cross-reacting antibodies between F. hepatica and S. bovis, using the ELISA test. These two parasites were used in both experiments because they were routinely established in experimental animals at the State Veterinary Serum Laboratory, Copenhagen, DENMARK where the work was carried out.

In the last part of the study, the ELISA test was used to detect antibodies against F. gigantica and S. mattheei infections in naturally infected cattle under field conditions. These are the common liverfluke and schistosome of cattle in Zambia.

IV STANDARDIZATION AND EVALUATION OF THE ENZYME-LIKED IMMUNOSORBENT ASSAY (ELISA) IN CALVES EXPERIMENTALLY INFECTED WITH *Fasciola hepatica* AND *Schistosoma bovis*

A INTRODUCTION

Several serological tests have been develped for use in the diagnosis of bovine fascioliasis and schistosomiasis. (Doyle, 1971; Hillyer, 1975; Van Tiggele and Over, 1976; Hanna and Jura, 1977; Lawrence, 1977; Schillhorn Van Veen and Buys, 1979). Of these the Enzyme-Linked Immunosorbent Assay (ELISA) has shown promise (Burden and Hammet, 1978; Farrel et *al.*,1981 and Oldam, 1983), and could serve as a routine diagnostic test if procedures were standardized.

The objectives of this study were to establish the optimal ELISA test conditions for these parasitic infections and then to use these to examine the potential of ELISA to diagnose experimentally induced infections of *Fasciola hepatica* and *Schistosoma bovis* in cattle. *F. hepatica* and *S. bovis* were used in this study because these parasites were routinely established in the experimental animals at the laboratory.

This part of the study was carried out in Denmark at the State Veterinary Serum Laboratory.

B. MATERIALS AND METHODS

1. Antigen preparation

1.1 Fasciola hepatica antigen (FHA)

The FHA antigen was prepared from adult flukes obtained from 50 mice orally infected with 3-5 metacercariae. The mice were from a strain of inbred albino mice, maintained at the State Veterinary Serum Laboratory, Copenhagen for 25 years. The F. hepatica metacercariae were kindly supplied by Dr. Over, Centraal Diargeneedkundig Instituut Lelystad, HOLLAND.

At 8 weeks post infection, the mice were killed. The adult liver flukes were then collected from the bile ducts and washed twice for 3 minutes in Phosphate-buffered Saline Solution (Pbs, pH 7.4). They were weighed wet, placed in a tissue homogenizer with approximately 1ml of Pbs/gram of parasite and ground by hand until no visible tissue particles remained.

The resultant mixture was kept at 4 degrees centigrade (°C) for 24 hours. To obtain the soluble antigen, the mixture was centrifuged at 12 000 x g for 30 minutes at 4°C. The supernatant was then designated FHA antigen. The protein concentration

was determined by the Lowry method (Lowry et al., 1951), and then the antigen was freeze dried and stored in vials as 100 ug/ml protein concentration.

1:2 Schistosoma bovis antigen (SBA)

The SBA antigen was prepared from S. bovis adult worms kindly supplied by the Danish Bilharziasis Laboratory, Copenhagen. The adult worms were washed in Pbs twice for 3 minutes. After weighing, they were placed in a tissue homogenizer with approximately 1ml of Pbs/gram of parasite and ground by hand until no visible tissue particles remained.

The resultant mixture was kept at 4°C for 24 hours and then centrifuged at 12 000 x g for 30 minutes at 4°C. The supernatant was then designated SBA antigen. The protein concentration was determined and then the antigen was freeze-dried and stored in vials as 100ug/ml protein concentration.

2. ELISA Conjugate and Substrate

The conjugate used was Peroxidase-conjugated IgG fraction of rabbit antibovine IgG (DAKOPATIS, DENMARK). The substrate, 2,2-azino-di (3-ethylbenathiazoline sulfonic acid) diammonium salt (ABTS), was obtained from Sigma Chemical Co. (Lot No. 19C - 0380; Sigma, St. Louis, MO).

3. Micro-ELISA plates

The Micro-ELISA plates used were obtained from DYNATECH Laboratories (M12A, Flat Bottom, Micro-ELISA Plates Immulon).

4. Serum samples

Pooled serum samples from calves experimentally infected with either F. hepatica or S. bovis were used as Positive reference serum samples. The Negative reference serum was obtained from fluke-free calves.

For the evaluation of the ELISA test, sera were collected weekly from experimentally infected calves and stored at -20°C until use.

A calf was inoculated with 400 F. hepatica metacercariae using a balling gun, orally. Another calf was infected with S. bovis cercariae by the leg immersion technique (Van Wyk, et al., 1975). The calves were bled weekly throughout the experiment from the jugular vein. Serum was extracted from the blood and stored at -20°C until use.

The faeces were collected weekly and examined for Fasciola and Schistosoma eggs, by sedimentation method.

5. The ELISA test procedure

The antigen was diluted to 5ug/ml in Sodium carbonate buffer (0.1M, pH 9.6) and 100 ul was added to each well in a micro-ELISA plate.

The coated plate was covered with another empty plate to prevent evaporation, then incubated at 37°C in a waterbath for 30 minutes. Using a washing unit devised by Henriksen (1979) the plate wells were washed with tap water into which Tween '20' was added from a flask connected to a tap. The final concentration of the Tween '20' in the water was 0.05%. Washing was done twice for 10 seconds. The wells were dried by gentle tapping while the plate was inverted.

Positive and negative reference sera were diluted 1:200 in Pbs + 0.05% Tween '20' and 100 ul was added to the appropriate wells in the plate. After covering as above the plate was incubated at 37°C for 30 minutes with slight rotation.

The plate was then washed as indicated above. A 100 ul of conjugate diluted in Pbs + 4% horse serum was added to all wells except the substrate control wells. The plate was covered and incubated as indicated for the serum incubation step. After another washing, 100 ul of substrate solution was added to each well. Incubation

under rotation at room temperature for 30 minutes followed. After adding of a stopping reagent (40% Hydroflouric acid) the optical density was determined immediately using a Titertek Multiskan Spectrophotometer (Flow Laboratories) at Wavelength 405 nm.

6. Standardization of Antigen concentration

The micro-ELISA plate wells were coated with antigen at protein concentration of 1, 5, 10, 15 and 20ug/ml. Positive and negative reference sera were diluted to 1:200 and the conjugate was diluted to 1:2000. The ELISA test was as described above. The Positive/Negative (P/N) ratios were calculated for each concentration.

7. Standardization of Serum dilution

The micro-ELISA plate wells were coated with antigen at a concentration of 5ug/ml. Positive and negative reference sera were diluted to 1:100, 1:200, 1:400 and 1:800 and 1:1600. The conjugate was diluted to 1:2000. The P/N ratios were calculated, for all dilutions.

8. Standardization of Conjugate dilution

Plates coated with 5ug/ml antigen and reference sera diluted 1:200 were used to find the optimum dilution of the conjugate. The conjugate was diluted to 1:500, 1:1000, 1:2000, 1:3000, 1:4000, 1:5000 and 1:6000.

C. *RESULTS*

1. The results of the standardization tests are presented in graphic forms in Figures 4.1 to 4.6 *(pp.47,48)*.

The antigen concentration of 5ug/ml protein concentration had the highest P/N ratio, while a dilution of 1:200 for serum had the highest ratio (Fig. 4.3 and 4.4) and for the conjugate, the optimum dilution was found to be at 1:3000 (Fig.4.5 and 4.6).

2. Evaluating the ELISA test

The results obtained are shown in Figs 4.7 and 4.8. In both the graphs the antibodies for the infected calves began to increase after the third week post infection. After this early rise, the antibodies increased continuously to reach a more or less constant level during the patent period. The optical density (O.D.) values for control calves maintained a level just below 0.2 throughout the experiment.

The F. hepatica eggs were first noted at 10 weeks post infection (Fig. 4.8) and S. bovis eggs were first noted at 6 weeks post infection (Fig. 4.8). The eggs continued to be observed until the end of the experiment.

These results are discussed in the general discussion (Section VII p.41 'et Seg)

V. DETECTION OF ANTIBODIES IN BOVINE FASCIOLIASIS AND SCHISTOSOMIASIS BY THE ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA).

A. INTRODUCTION

The ELISA test for diagnosis of Fasciola hepatica and Schistosoma bovis was developed and standardised as described in Section IV.

Previous reports have demonstrated that adult liverflukes and schistosomes have cross-reactive and/or common antigens (Hillyer et al., 1980). The presence of at least five cross-reactive antigens between these parasites was demonstrated by Capion et al. (1968). Cross-reactive antigens between F. hepatica and S. bovis if evident, would clearly interfere in serodiagnosis.

This part of the study investigates the occurrence of cross-reacting antibodies between F. hepatica and S. bovis, using the ELISA test. These two parasites were used because they were routinely established in experimental animals at the State Veterinary Serum Laboratory, Copenhagen, DENMARK, where this study was carried out.

B. MATERIALS AND METHODS

. Experimental design

Twelve calves aged 5 to 7 months were used in this study. Two weeks prior to the start of the experiment, all calves were dosed with anthelmintic (Fembendazole 5g/Kg.) to eliminate nematode infections. Throughout the experiment the calves were housed separately under warm-free conditions, indoors in milking cow pens.

The calves were divided into four groups of approximately equal total body weights. The groups were then allocated to the four treatments as shown in Table 1.

Table 1: Experimental design for detection of cross-reaction.

GROUP	NO. OF CALVES	TREATMENT
I	3	control (no treatment)
II	3	10,000 *S. bovis* cercariae per calf
III	3	900 *F. hepatica* metacercariae per calf
IV	3	10,000 *S. bovis* cercariae and 900 *F. hepatica* metacercariae per calf

2. Faecal Examination

Examination for F. hepatica eggs in faeces from infected animals commenced from week six post metacercariae inoculation, while examination for S. bovis eggs in faeces commenced from four weeks post cercariae exposure and thereafter at weekly interval throughout the experimental period.

A sedimentation method described by Henriksen (1974) was used. This consisted of three sedimentations, of 10 minutes each and staining with malachite green. A sample was considered positive when one or more eggs were observed in 2 gm of faeces.

3. Blood sampling and examination

All the calves were bled from the jugular vein weekly throughout the experiment. Serum was extracted from the 10ml blood and stored at $-20^{O}C$ until use.

The ELISA test was then performed as described in section IV..

C. RESULTS

The results of the experiments are shown in Figures 4.9 and 4.10, (pp. 55, 56) Fig 4.9 shows the results of the ELISA test using the S. bovis antigen on sera from calves experimentally infected with S. bovis and F. hepatica. The Optical Density (O.D.) values for Group II (S. bovis only) calves began to rise after the 4th week of post infection. Similarly, O.D. values for Group IV (S. bovis/F.hepatica) began to rise at week 4. While in Group II (F. hepatica only) and in Group I (Control) O.D. values remained just below 0.1 level throughout the experiment.

Fig 4.10 shows the results of ELISA test using the F. hepatica antigen on sera from calves experimentally infected with S. bovis and F. hepatica . The O.D. values for Group III (F. hepatica only) began to rise as early as 4 weeks post infection. The peak period was between 10-12 weeks followed by a gradual decline. Group IV animals had a curve similar to Group II, but the O.D. values were slightly higher. O.D. values for Control and S. bovis groups remained just below 0.1 level throughout the experiment.

S. bovis eggs were first detected at 7 weeks post infection in all calves infected with S. bovis F. hepatica eggs in infected animals were first detected in the faeces at 11 weeks post infection.

VI EVALUATING THE ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) IN CATTLE NATURALLY INFECTED WITH *F. gigantica* AND *S. mattheei* IN MAZABUKA DISTRICT

A. INTRODUCTION

In this part of the study the ELISA test was used to detect antibodies against *F. gigantica* and *S. mattheei* infections in naturally infected cattle.

The sampling was carried out at the Mazabuka District Abattoir, in the Southern Province of Zambia. The samples were processed at the Research Laboratories of the Department of Veterinary and Tsetse Control Services in Mazabuka.

The Mazabuka District was selected for the field testing of ELISA for fluke diagnosis in cattle because it is in the midst of a very important commercial and peasant cattle-keeping area, known to be affected by these flukes. In addition, Mazabuka has an abattoir which regularly slaughters up to 10 cattle a day, thus making it available to obtain necessary information on each animal slaughtered.

B. MATERIALS AND METHODS

Faecal, blood and liver samples were collected from all the cattle that were brought to the Mazabuka Abattoir for slaughter during the six month period, July to December, 1983. The cattle were of mixed breeds of Zebus, Simmentals, Freisians, Boran and Africanders. Most of the cattle brought for slaughter originated from commercial farms while the remainder were from traditional farms.

The ages of cattle ranged from 1 to 10 years. The age of each animal was estimated after examination of dentition and records of cattle owners.

The faeces and livers were processed the day of collection while serum extracted from the blood was kept at $-20^{o}C$, until the period of collection was over.

1. Faecal sampling and examination

About 10gm of faeces was extracted from the rectum of a slaughtered animal and kept in mono-containers. The samples were transported in cooler boxes to the laboratory.

The faeces were examined for *Fasciola* eggs using the method described by Henriksen (1974) (Section IV&V) A modified sedimentation technique for examining for *Schistosoma* eggs described by Bell (1963), was used. This consisted of sedimentations in 0.9% saline for 20 minutes. The sedimentations were repeated until the supernatant was clear. The sediment was transferred onto the slides and systematically examined.

2. Blood sampling and examination

About 20ml whole blood was collected in "Vacutainer" tubes from the cut jugular veins, immediately after slaughtering the animal. The tubes were transported in cooler boxes to the laboratory were the serum was extracted and kept in clean tubes at -20°C.

The serum was examined for antibodies against Fasciola and Schistosoma infections using the ELISA test as described in Section IV . The FHA and SBA antigens were used (IV , 1.1, 1.2) as these were the only ones available.

3. Recovery of F. gigantica worms from livers

The F. gigantica adult worms were recovered from all the lobes of the livers. The visible bile ducts were cut upon and flukes collected using forceps and preserved in 10% Formalin.

4. Liver digestion and examination for Schistosoma eggs

About 5 gm of liver tissue was digested by the KOH digestion method,,described by Cheever (1968). The liver tissue was cut into small pieces and transferred to a 100ml erlenmeyer flask containing 50ml 3% Potassium Hydroxide solution.

The flask was shaken and incubated at 37^{o}C overnight. When all the tissue pieces had been digested, the contents in the flask was shaken and three representative samples of 1ml of the mixture were transferred onto small petri dishes. These samples were then systematically searched for eggs under a stereo microscope at X10 magnification.

B. RESULTS

F. gigantica eggs measuring 156-197um by 90-104um and adult flukes from 25-75mm in length and up to 12mm in width were found in the faeces and in the condemned livers respectively. These adult flukes had an anterior cone smaller than that of *F. hepatica*, the shoulders were not as prominent and the body was more transparent than *F. hepatica*, thus confirming them to be *F. gigantica*. (Dunn 1969).

S. mattheei eggs as described by Dinnik and Dinnik (1965) were found in faecal samples and in the digested liver tissues (Appendix 3, 5 and 6). *F. hepatica* and *S. bovis* eggs were not present in any of the samples examined.

A total of 564 animals were sampled in six months with the majority being in November and December (Table 2,) pp.67. However most of the animals (above 50%) that were found positive for fascioliasis by ELISA test were brought for slaughter during the months of July to October. The sedimentation test was able to detect 51% positive in the month of July.

Table 3 shows the relation between ELISA O.D. values and age of *F. gigantica* infected animals. The ages ranged from 1 to 5 years above. Most of the animals 263 (47%) were aged 5 and above and of these 58% were found positive for fascioliasis by the ELISA test. Those aged 2 years and under were 170 (32%) and of these 42% were positive by ELISA.

Samples that were positive for fascioliasis by the ELISA test were 266 (47%) and positive for sedimentation 162 (29%) (Table 4). Samples positive by ELISA, sedimentation and the actual recovering of flukes from livers were 60 (11%)(Table 5) pp. 68.

Samples that were positive for schistosomiasis by ELISA were 48 (8.6%) and by sedimentation 3.

All the animals that were brought for slaughter at the abattoir originated from commercial and traditional farms, Of the 398 samples from commercial farms, 161 (41%) were positive by ELISA and 50 (32%) positive by sedimentation for fascioliasis (Table 6) pp.68

The sources of cattle were divided into four quadrats for compiling infection by area. From the North and West of Mazabuka Town, 52% and 53% samples respectively were positive for fascioliasis. While 47% and 30% samples from the East and South respectively were positive (Tables 7 and 8). pp.70.

The entire results are tabled in Appendices 1 - 6 on a monthly basis.

The results obtained indicate that the ELISA test used was highly sensitive at O.D. levels of diagnostic significance.

V.I DISCUSSION

Before an antibody-antigen system can be carried out, it is essential to establish the optimal concentration/dilution for antigen, serum and conjugate.

A too high concentration of protein during coating may give rise to high background values and if the protein concentration is too low the sensitivity of the ELISA will decrease.

As with the antigen, the optimum dilution for serum samples has to be determined by preliminary titrations. If too low a dilution is selected then many strong antibodies in serum will be in excess with insufficient antigen sites on the solid phase to bind effectively.

The objective of the addition of the enzyme-labelled antiglobulin conjugate is to link the enzyme with the antibody or antigen in such a way that each retain the maximum amount of their reactivity. Therefore the optimum dilution for the conjugate in a test system has to be determined.

The optimal antigen concentration, serum dilution and conjugate dilution was determined using the P/N ratio as the criterion. The optimal antigen concentration was 5_{ug} protein/ml, the optimal dilution for serum was 1:200 and for conjugate 1:3000.

Crude antigen obtained from adult worms was used. Adult worms were chosen as an antigen source because they were readily obtainable in substantial amounts at little cost. Crude preparation of the antigen was used to ensure that most antigenic determinants would be included. This is because the key to the success of any diagnostic test which detects antibody to a given organism is the development of a satisfactory antigen.

F. hepatica is a complex organism that exposes the host to a mosaic of antigens (Farrel et al., 1981). Each development stage of this fluke may also have unique antigenicity as well as those common to all stages of the life cycle, thus complicating the isolation of the

appropriate antigens. The use of freeze-dried antigens has the advantage that their activity is maintained for a long period.

Using the FHA and SBA antigens in the experiments to evaluate the ELISA test, sera from infected calves exhibited O.D. readings consistently higher than did the sera from control calves at 4 to 5 weeks after infection. This is several weeks earlier than diagnosis can be verified by faecal examination. Moreover the antibodies increased with time. The ELISA test therefore has the advantages of early diagnosis and the O.D. values can be used to predict the age of infection.

It is quite evident from the study that the ELISA test can be used to diagnose both Fasciola and Schistosoma infections in cattle. However, previous reports have demonstrated that adult liverflukes and schistosomes have cross-reactive and/or common antigens (Hillyer and Leticial Sagramoso de Ateca, 1980). The presence of at least five cross-reactive antigens between these parasites was demonstrated by Capron et al.(1968). Cross-reactive antigens between Fasciola spp. and Schistosoma spp. if evident, would clearly interfere in serodiagnosis.

Therefore in the second part of the study, the ELISA test was used to find out if cross-reacting antibodies occur between F. hepatica and S. bovis. The results showed that antibodies did not cross-react with antigens prepared from

respective worms, unlike Hillyer and Leticial Sagramoso de Ateca (1980), who observed sera obtained from mice infected with F. hepatica to cross-react with S. mansoni egg antigen (SEA) by 3 weeks after infection using the ELISA test.

Using the ELISA test, antibodies against S. bovis infection had increased markedly by the 8th week. Antibodies against F. hepatica were observed by 3-4 weeks pos infection and increased rapidly until a peak at 10-12 weeks. This peak period seems to coincide with the time when the adult worms are in the bile ducts and are ready to start egg production. After the peak period there was a gradual decline.

This experiment was carried out in controlled conditions with animals experimentally infected only once. However in an enzootic situation, repeated infections are common and this may result in a change in reactivity. Therefore the ELISA test was evaluated in naturally infected animals.

In the Mazabuka abattoir survey, a total of 48% samples were found positive for fascioliasis. The ELISA test was able to detect antibodies against F. gigantica infections in naturally infected cattle. However the ELISA test detected infections in many more cases that the sedimentation technique. These cases were probably still in the prepatent period, or were very light, such that one sample was not sufficient to detect eggs.

The sedimentation technique can only detect infection during the patent phase of the parasite life cycle, during which eggs are excreted with faeces. This means that all faecal samples from animals harbouring immature flukes were diagnosed as negative. ELISA test on the other hand, is able to detect infection as early as 4 weeks post infection.

The ELISA test was also able to detect antibodies against Schistosoma infection, while no positive cases were detected by sedimentation. A few positive cases were detected by the liver digestion technique. Sediment technique although easy to perform, proved to be tedious when examining 564 samples and finding the eggs was difficult due to the debris and small size of the eggs.

Most of the samples that were positive for fascioliasis were collected during the months of July to October. It is during this dry season that cattle usually pick up the infection.

More than 50% of the animals brought for slaughter originated from the North and West of Mazabuka town. This is an area near the Kafue River and Kafue flood plains. Most of the animals from the traditional farms are taken to the flood plains during the dry season. The water grass pastures have excellent grasses nutritionally, but they are also a habitat of the intermediate host of F. gigantica. and Schistosoma spp.

It is likely that the cattle become infected by liverfluke metacercariae while grazing such pastures.

Animals from commercial farms are usually watered from water troughs or small streams running on the farm. If these troughs are left uncleaned for a long period, Lymnaea snails find their way inside and multiply. In this case the animals pick up the infection while drinking water.

The economic significance of fascioliasis in cattle is illustrated by the high rate at which livers are condemned at commercial abattoirs. For instance at the Mazabuka abattoir, during the six month study, 59 livers were condemned due to the actual finding of the adult fluke in the livers. This means approximately 400 Kg of liver meat was condemned.

The extent to which liver flukes are directly responsible for actual mortality among the Zambian cattle is yet to be determined. However, the observed extensive damage to the liver tissue gave the impression that fascioliasis could contribute to the apparent high rate at which cattle lose condition under stress of age, poor grazing and other disease.

Thus the use of the ELISA test could enable the intensity and duration of infection to be known, which could lead to appropriate treatment. The ELISA test can be applicable to field work and can be used for mass screening with minimal field and laboratory effort. Further, the ELISA test can be used in the study of epizootiology of bovine fascioliasis and schistosomiasis by sampling live animals in the field and following the disease in tagged cattle.

Animals which show high optical density readings can be isolated and appropriate treatment given, leading to improved management practices.

VIII FIGURES AND TABLES

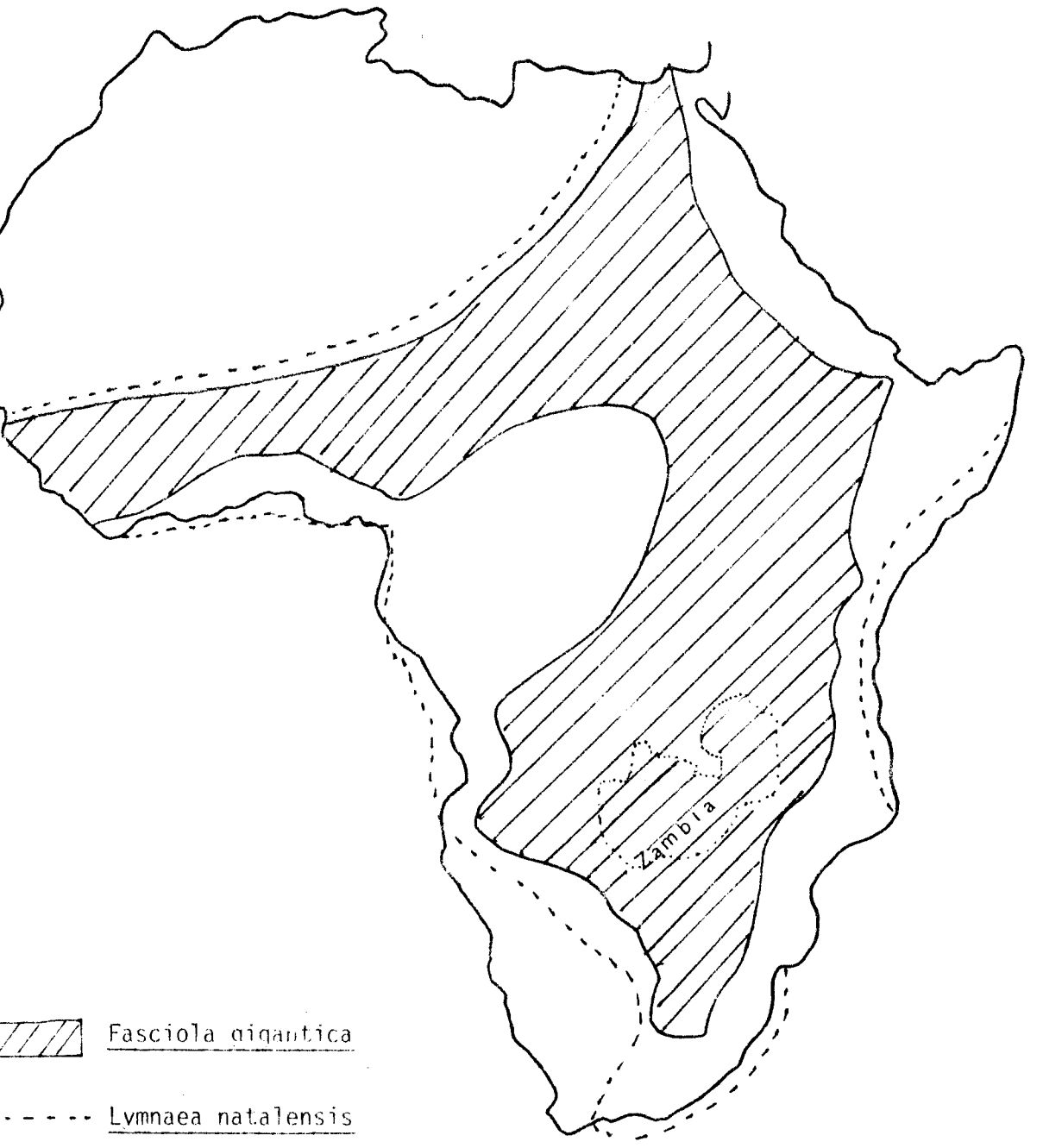

FIG. 1: Distribution of F. gigantica and the intermediate host species L. natalensis in Africa. After H.J. Over (1982)

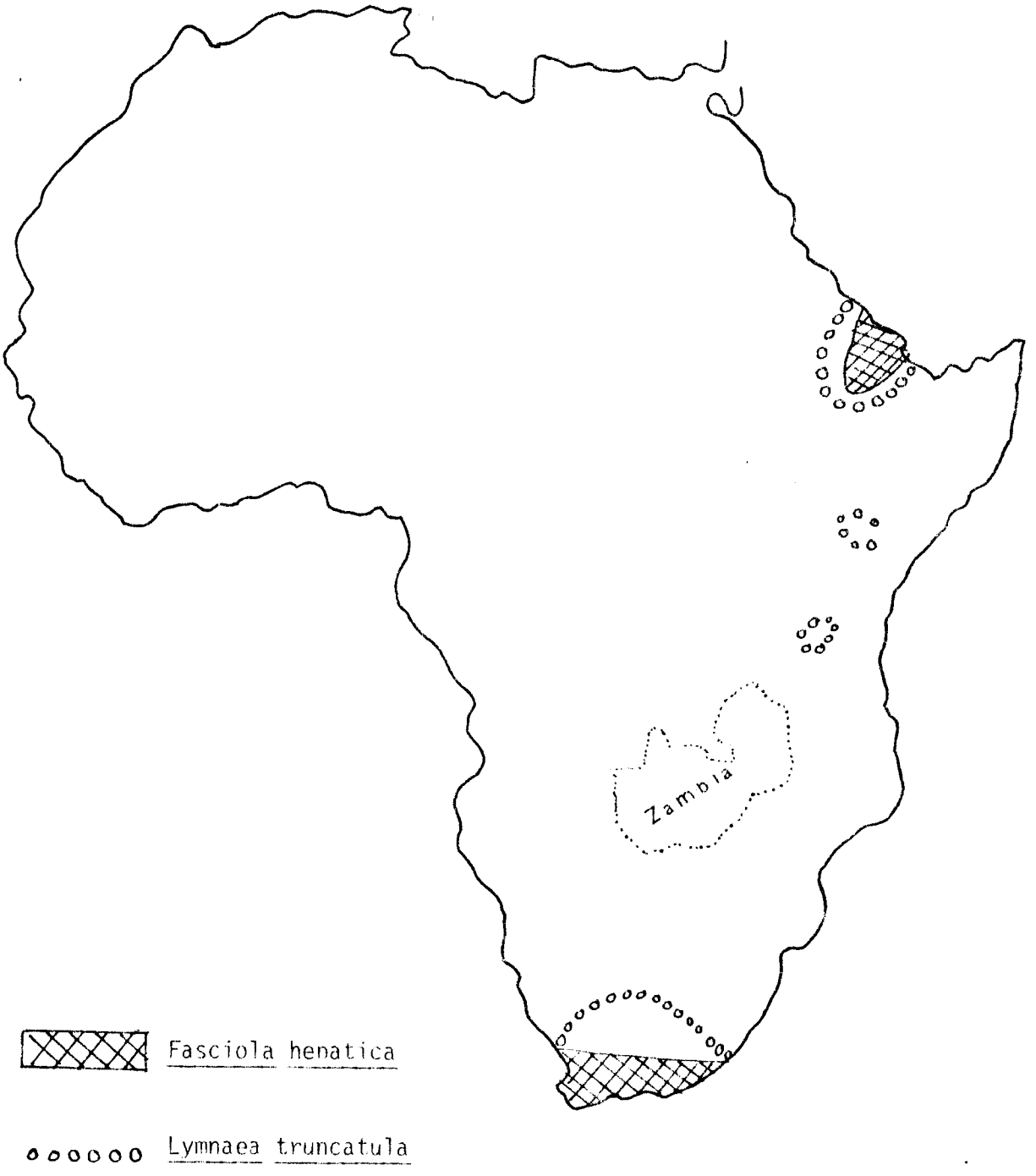

FIG. 2: Distribution of F. hepatica and the intermediate host species L. truncatula in Africa. After M.J. Over (1982)

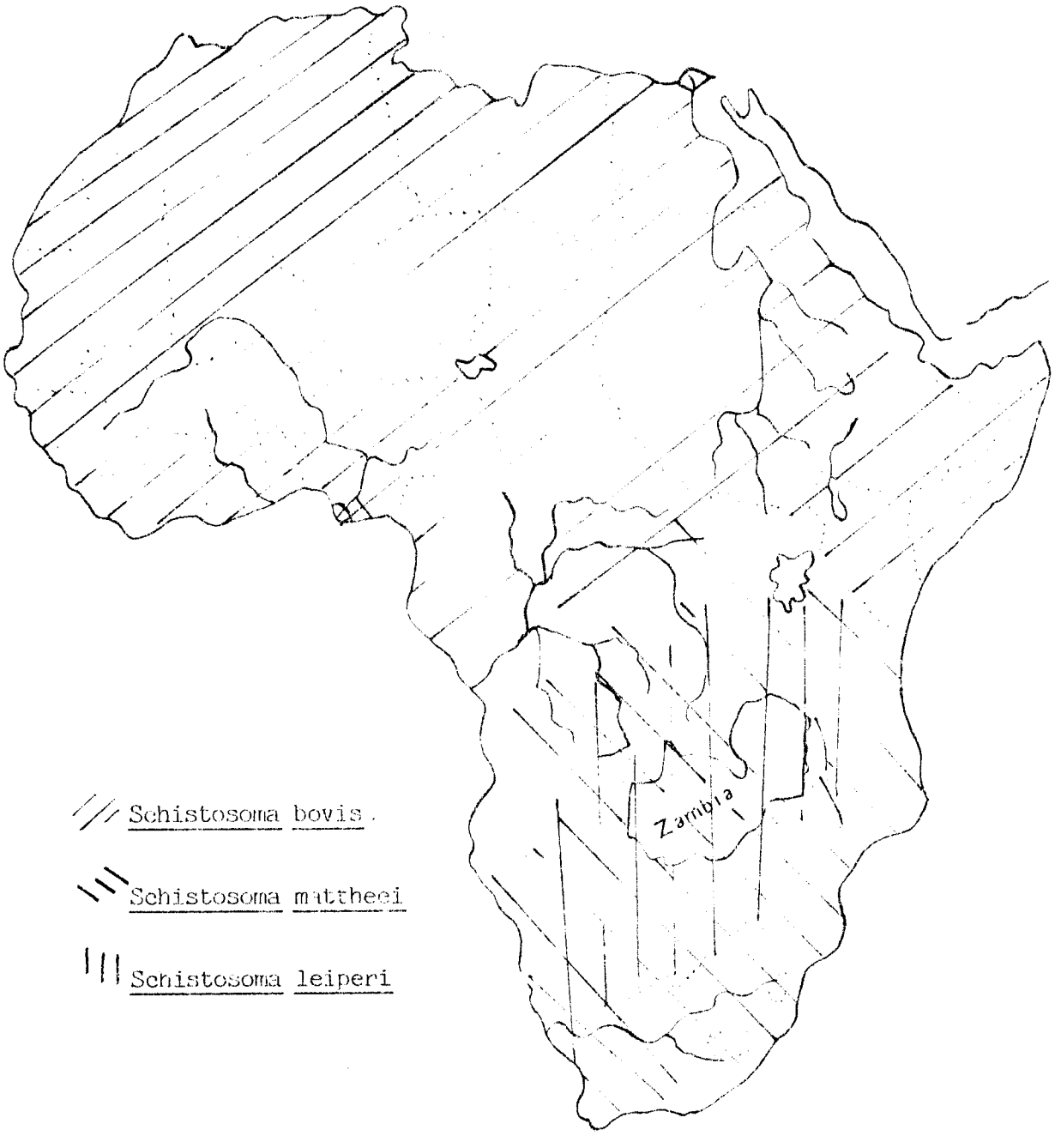

FIG. 3: Distribution of different Schistosoma species in livestock animals in Africa. After Cowper S.G. (1971)

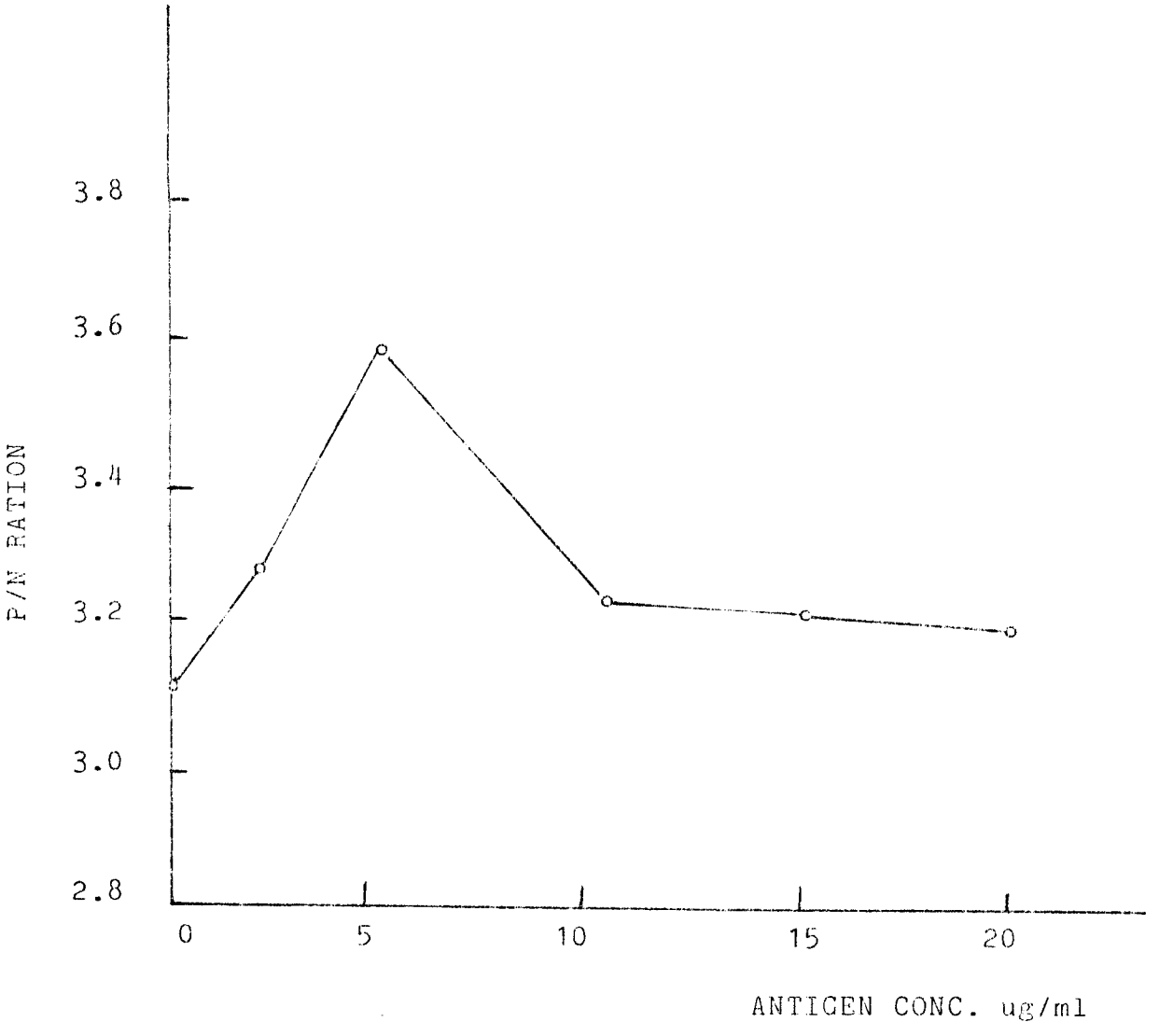

FIG. 4.1: Standardization of the FHA antigen concentration

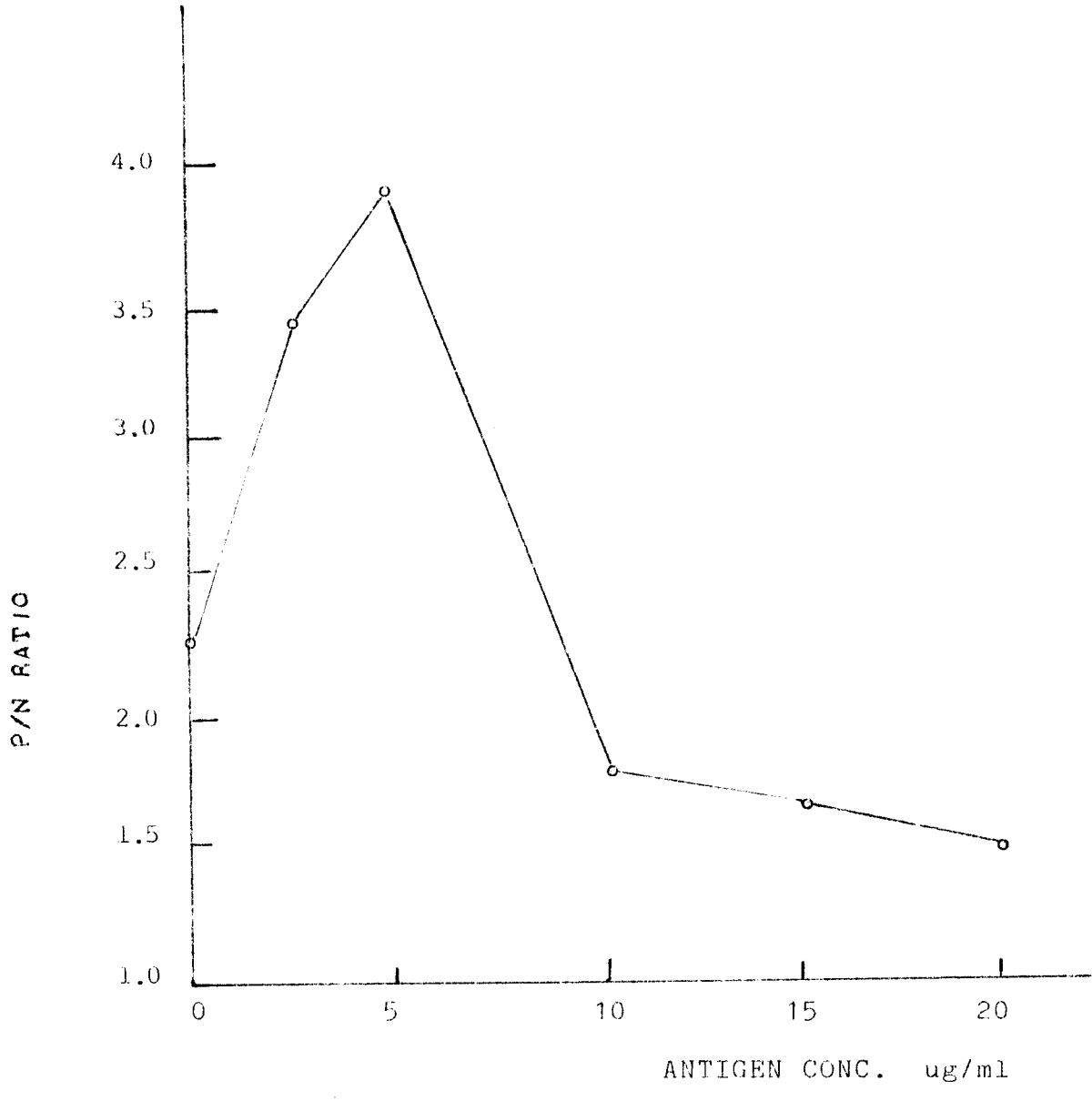

FIG. 4.2: Standardization of the SBA antigen concentration.

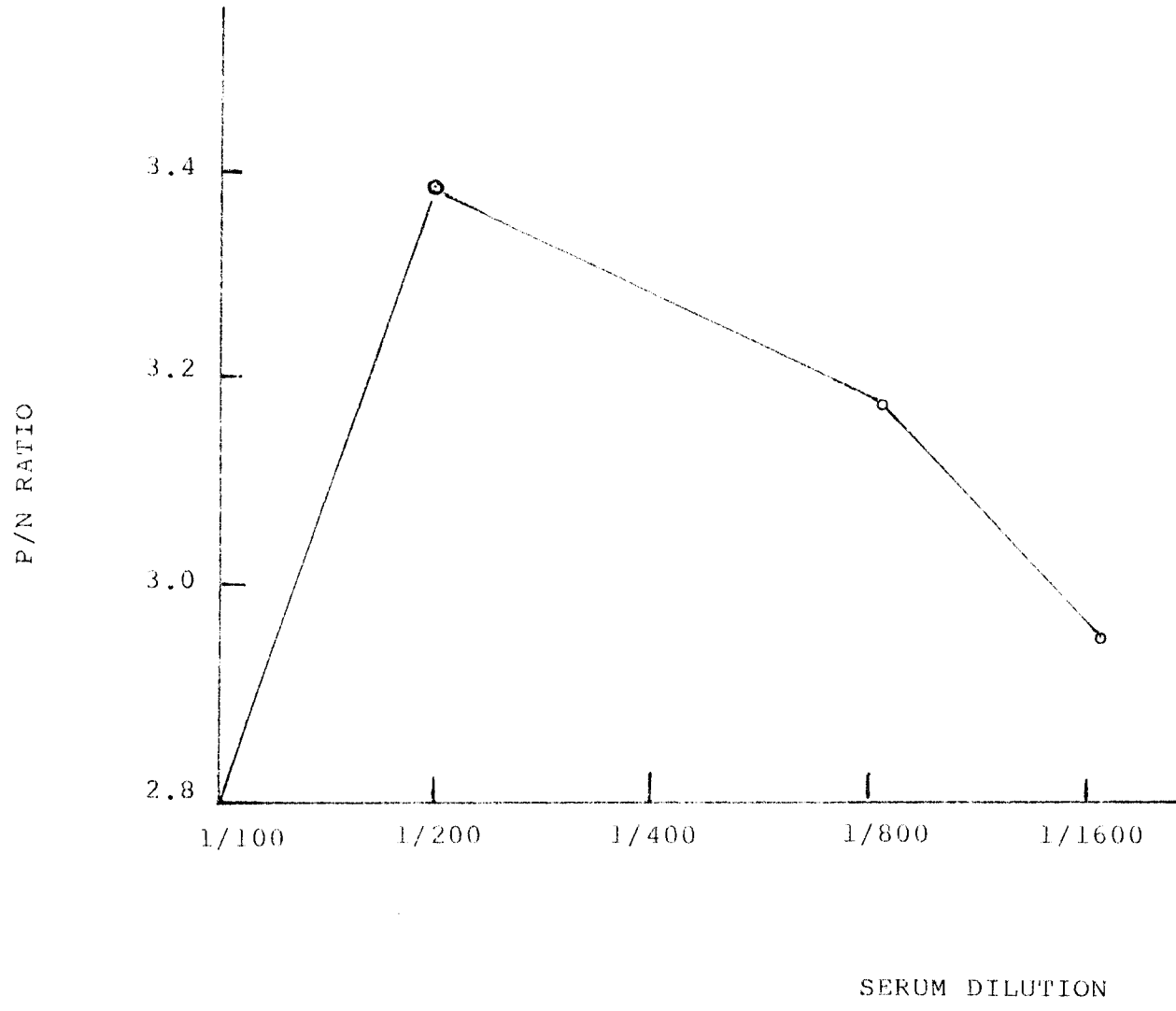

Fig. 43: Standardization of F. hepatica serum dilution

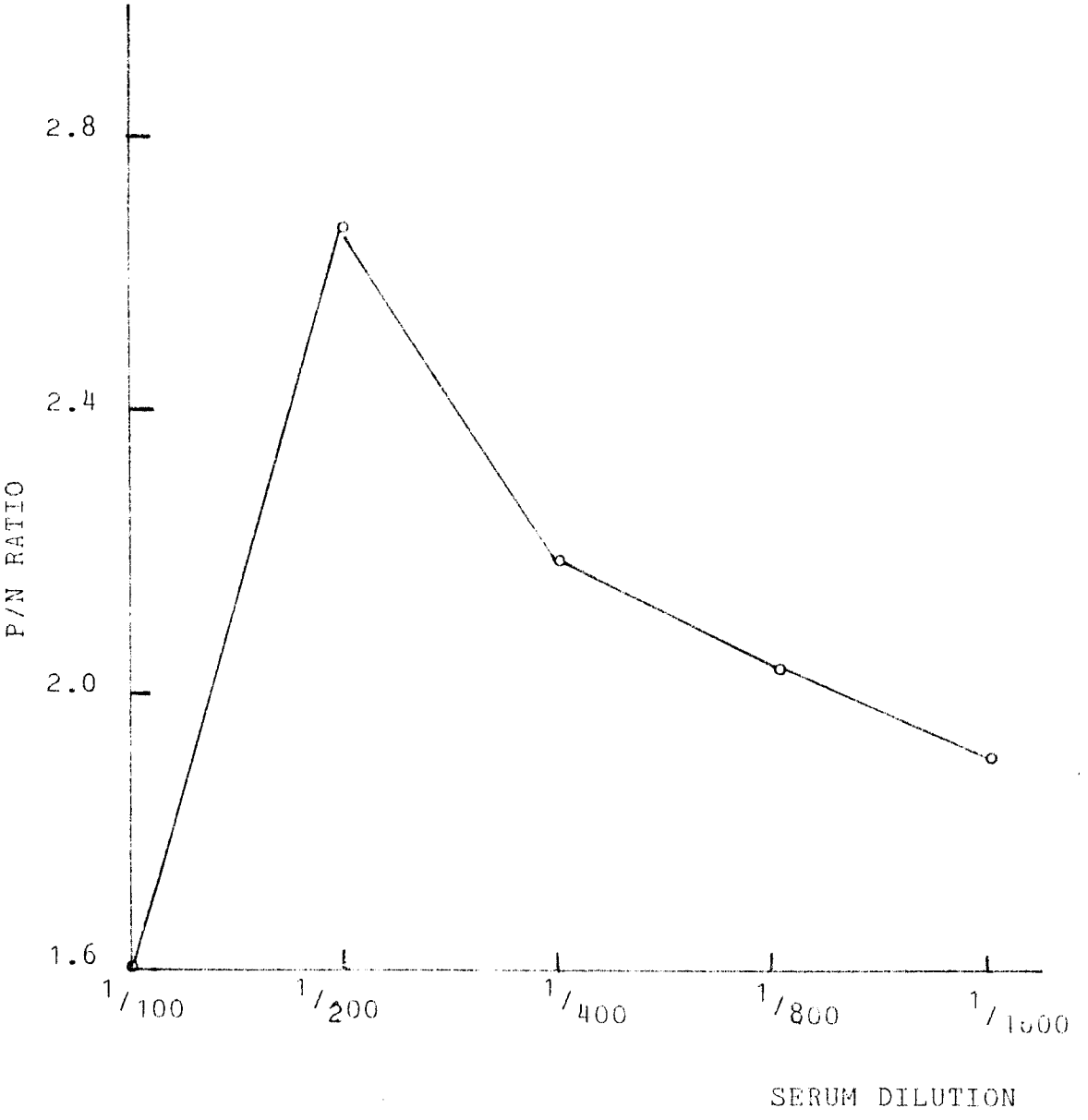

FIG. 4.4. : Standardization of S. bovis serum dilution

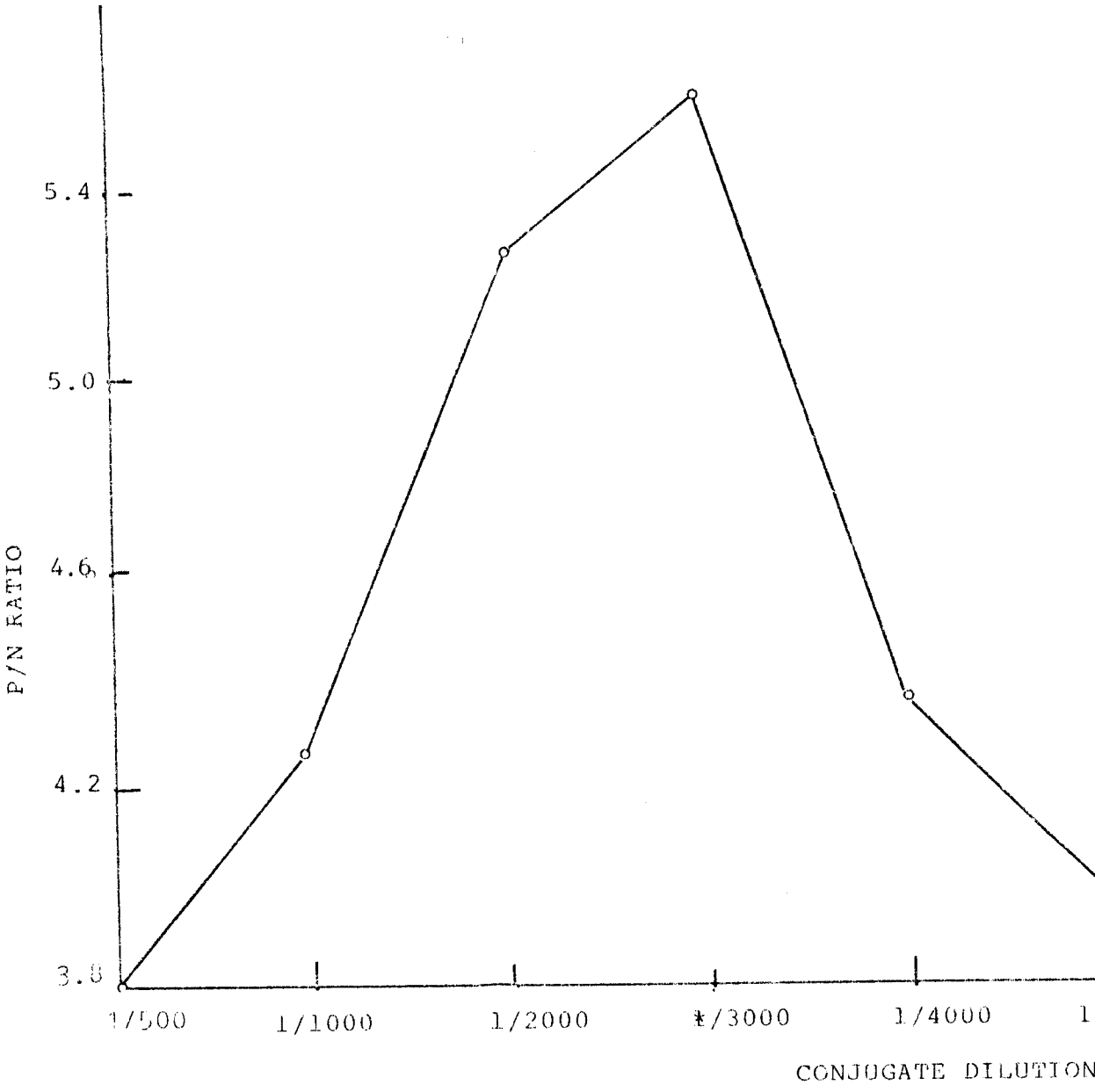

FIG. 4.5 Standardization of conjugate dilution for F. hepatica infection.

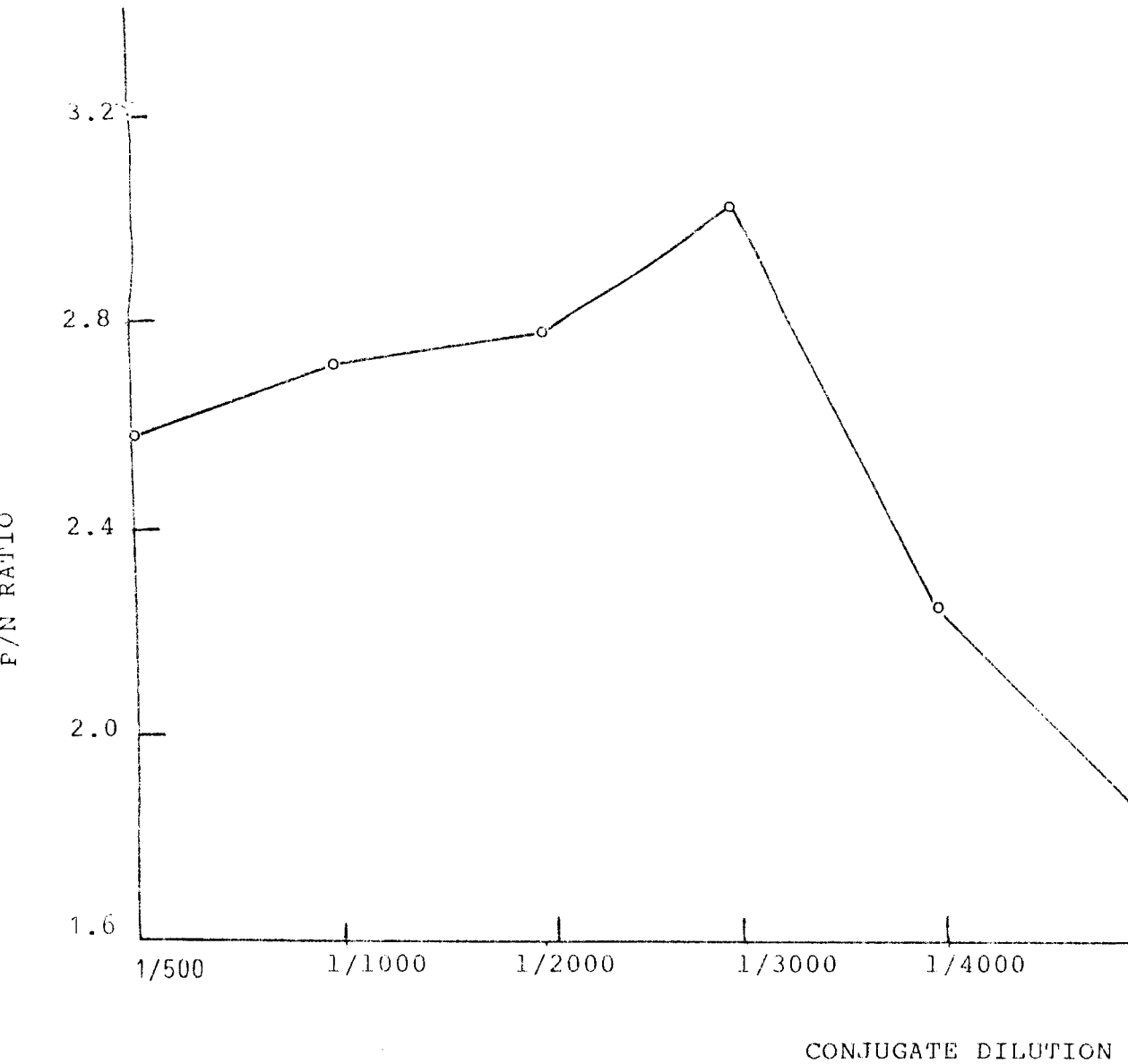

FIG.4.6 Standardization of conjugate dilution for S. bovis infection

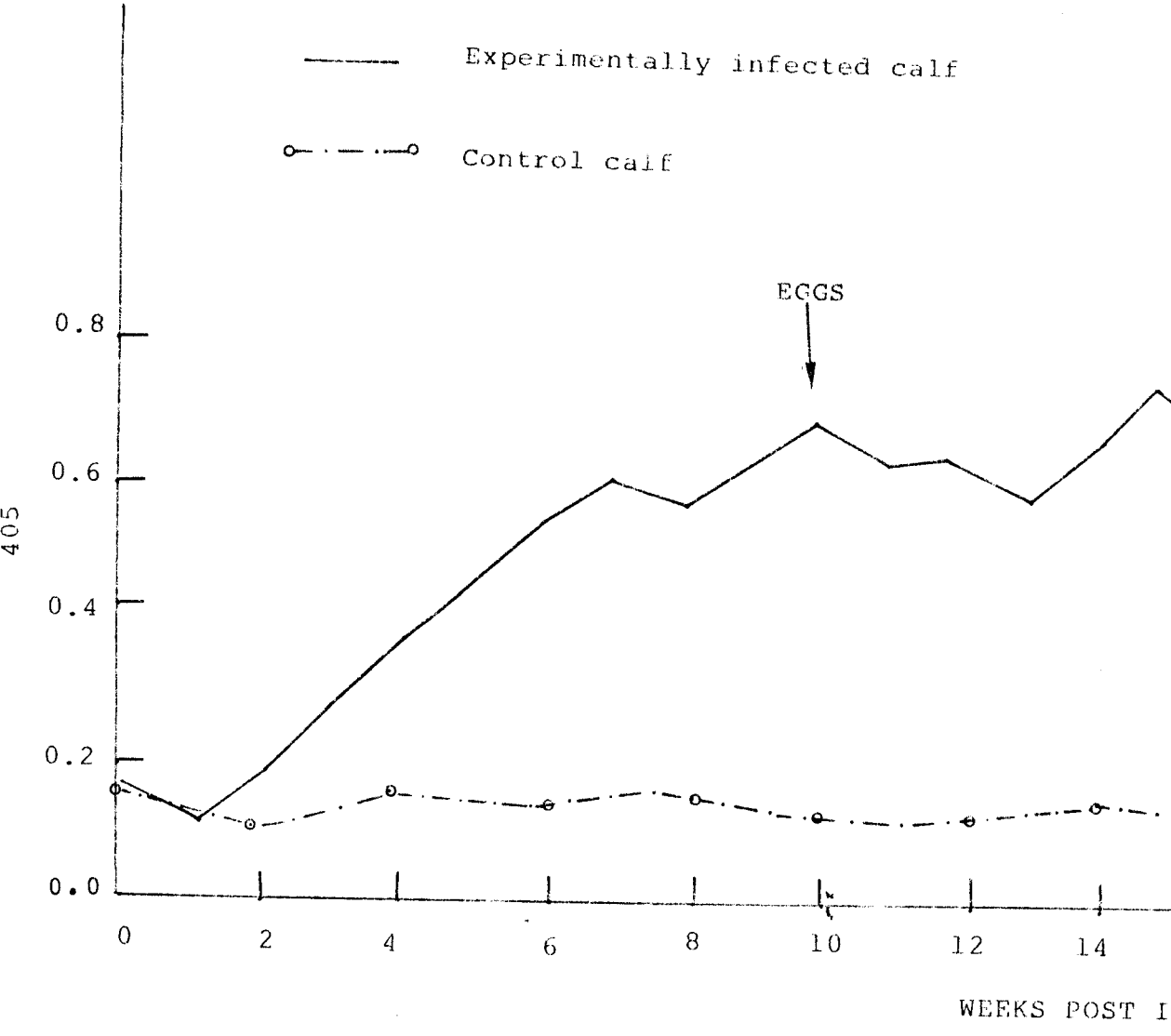

FIG. 4.7: ELISA OD measured at 405nm for sera of a calf inoculated orally with 400 F. hepatica metacercar

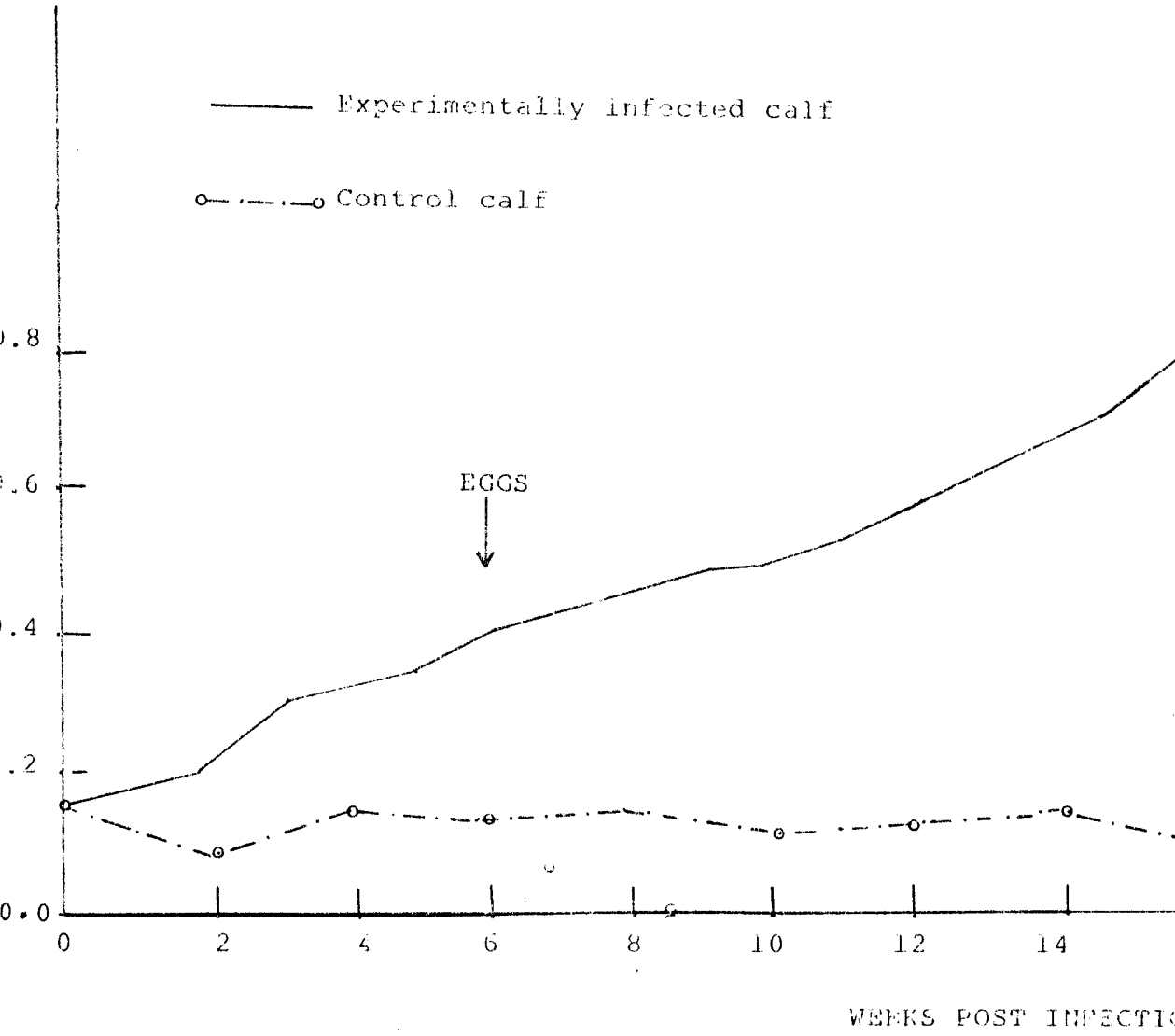

FIG. 4.8: ELISA O.D. measured at 405nm for sera of a calf infected with S. bovis cercariae.

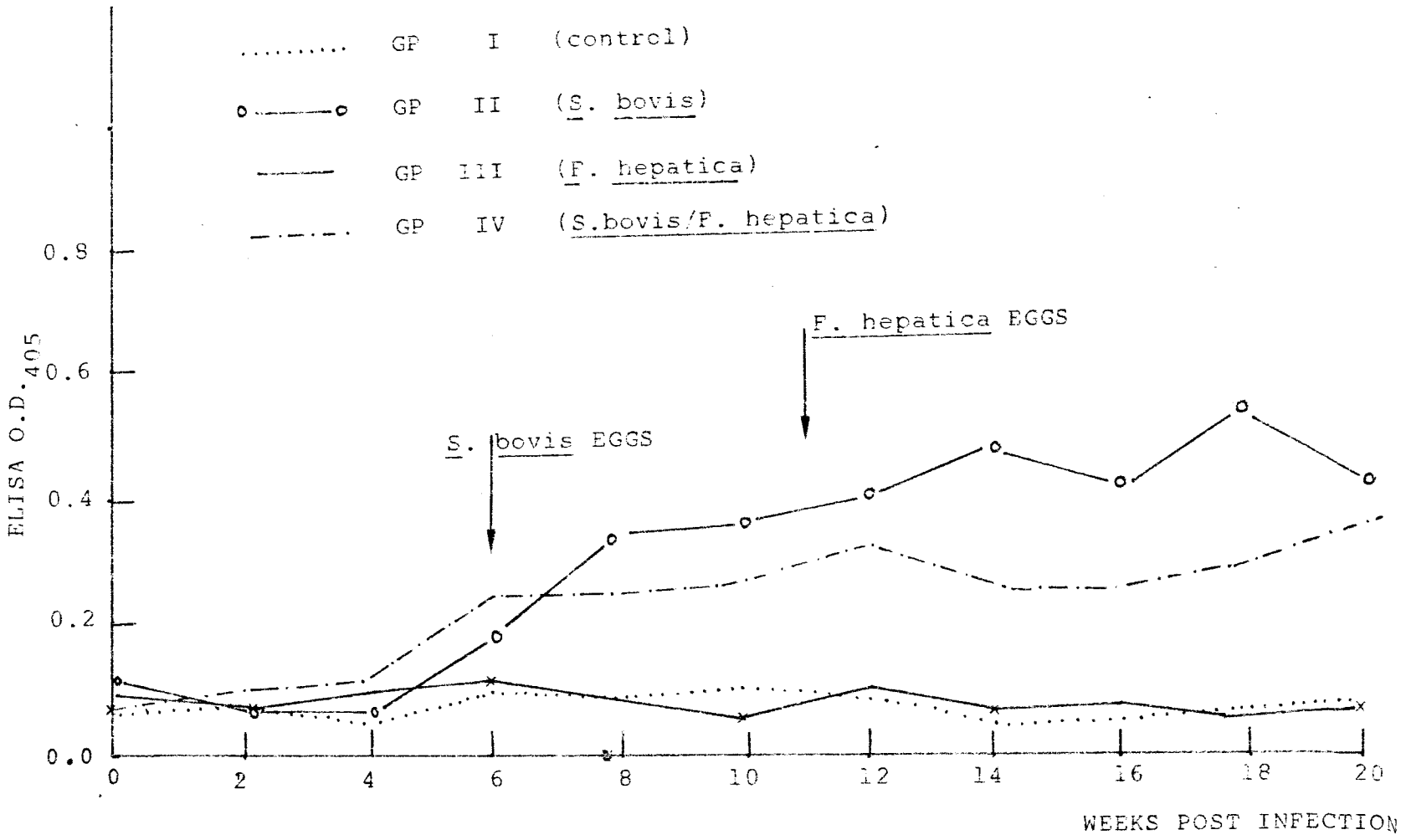

FIG. 4.9: ELISA $O.D_{405}$ of sera from calves experimentally infected with

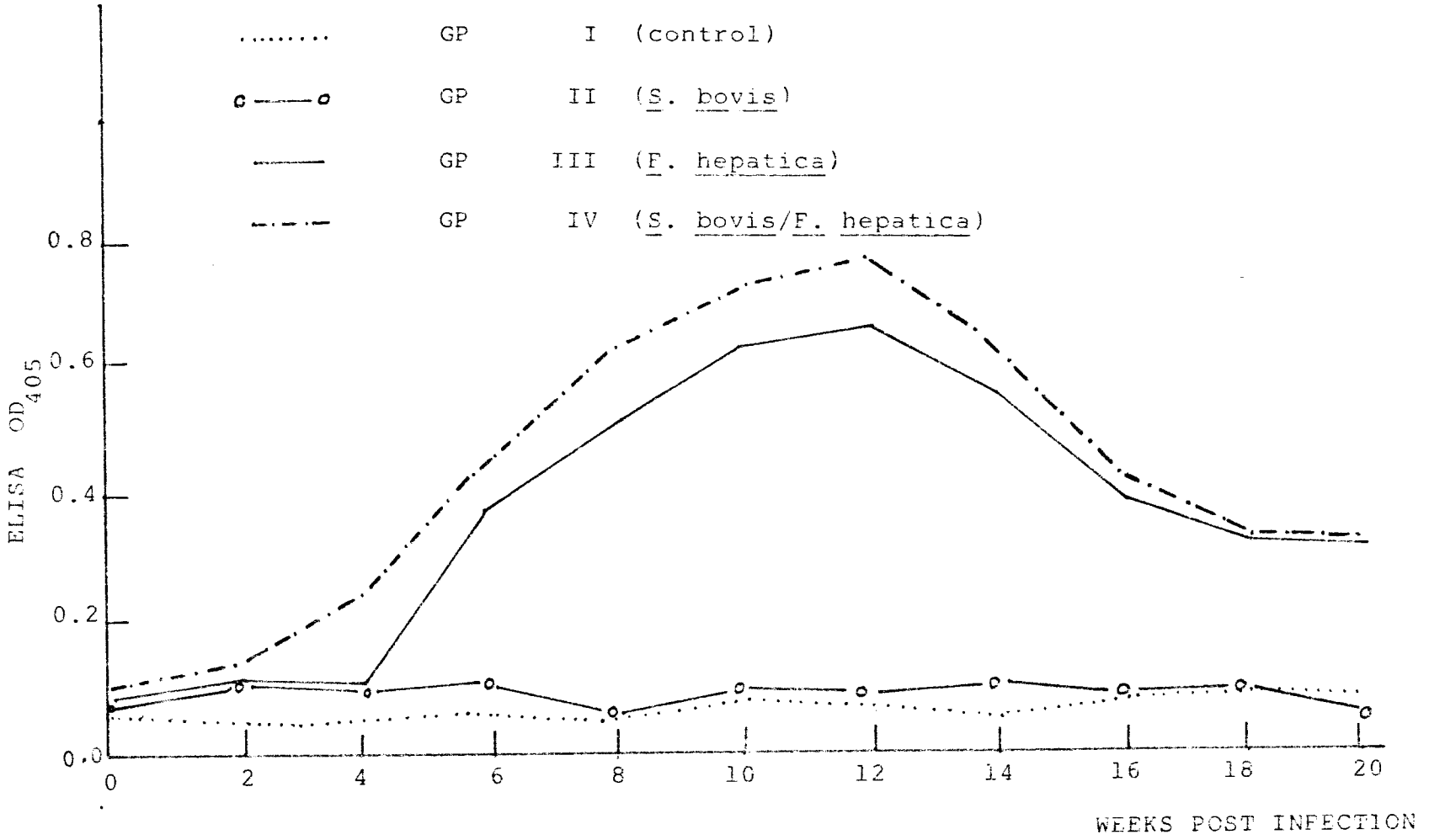

FIG. 4.10: ELISA $O.D_{405}$ of sera from calves experimentally infected with S. bovis and F. hepatica using FHA antigen.

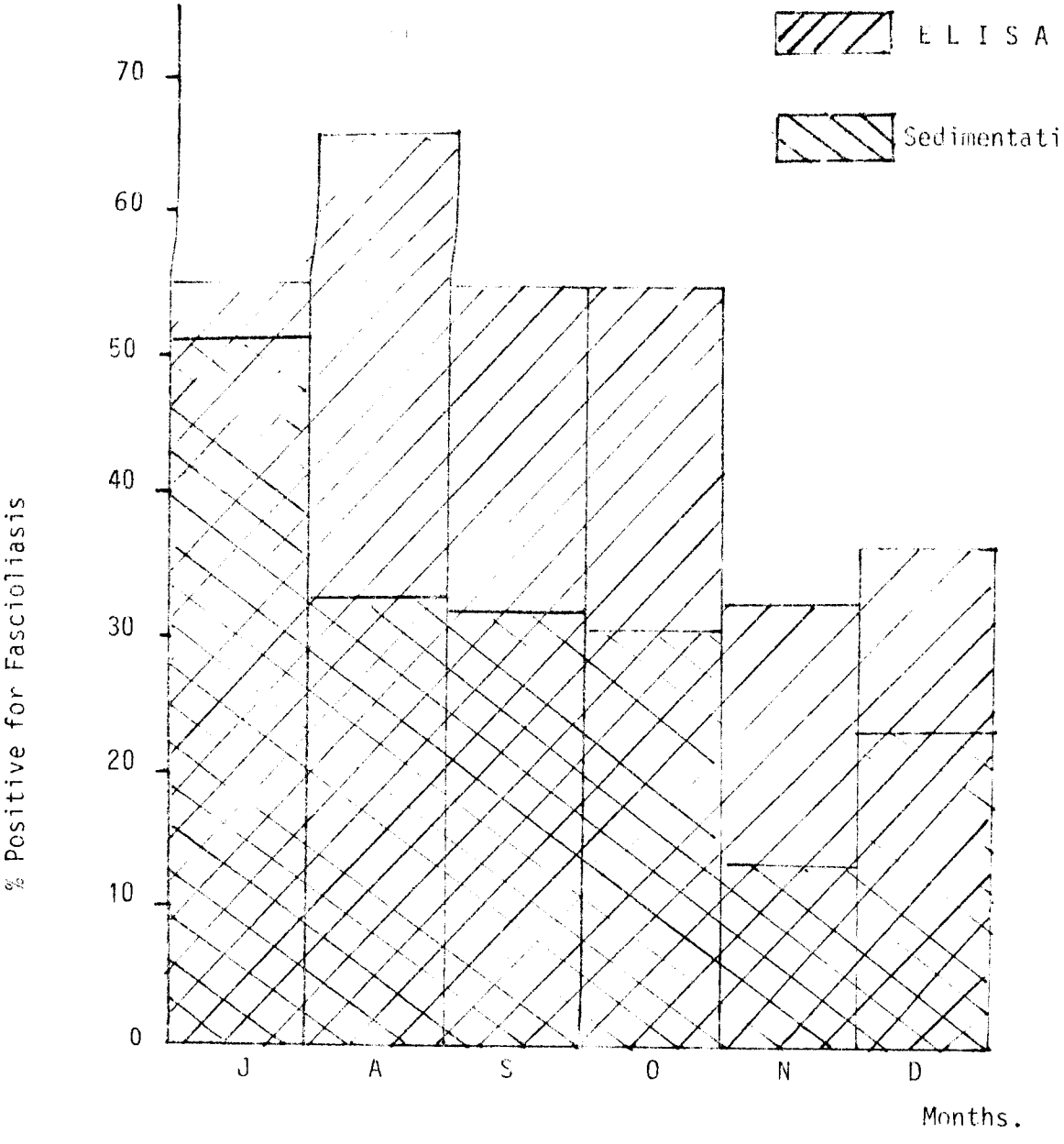

Fig. 5. Histogram showing proportion of sampled cattle over six months which were positive for fascioliasis by two techniques.

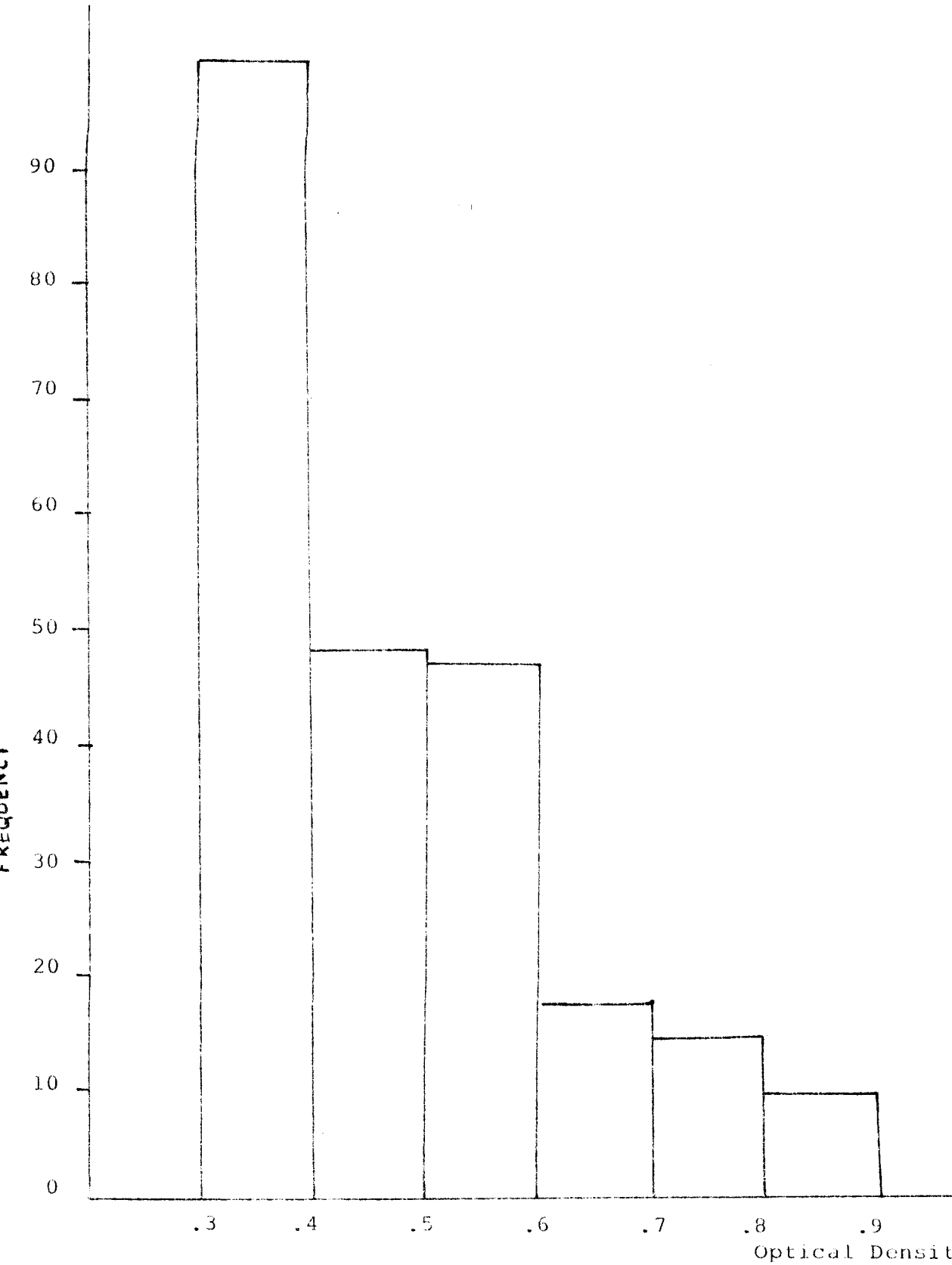

FIG. 6: FREQUENCY DISTRIBUTION OF THE OPTICAL DENSITY ($O.D._{405}$) VALUES FOR POSITIVE CASES

TABLE 2: RESULTS OF ELISA TEST AND SEDIMENTATION TECHNIQUE FOR DETECTION OF F gigantica INFECTION OVER SIX MONTHS

1983 MONTH	NUMBER EXAMINED	E L I S A		SEDIMENTATION	
		NO. POSITIVE	%	NO POSITIVE	%
JULY	70	39	56	36	51
AUGUST	84	55	65	27	32
SEPTEMBER	86	48	56	26	30
OCTOBER	88	47	53	29	36
NOVEMBER	117	37	32	16	14
DECEMBER	119	43	36	28	24
TOTALS	564	269	48	162	29

TABLE 3: RELATION BETWEEN ELISA O.D. VALUES AND THE AGE OF F. gigantica INFECTED ANIMALS

AGE (YEARS)	NO. EXAM.	ELISA		POSITIVE O.D. RANGE	PO M
		NO. POSITIVE	%		
1	48	13	27	0.432 - 0.867	0.
2	131	62	47	0.301 - 0.872	0.
3	88	27	31	0.302 - 0.756	0.
4	34	15	44	0.323 - 0.790	0.
5+	263	152	58	0.300 - 0.871	0.
TOTALS	564	269	48%		

TABLE 4: RESULTS OF THE ELISA TEST FOR *Fasciola sp.* AND *Schistosoma sp.* Summarised from Appendices 1 - 6.

	+ve Fasciola	-ve Fasciola	+ve Schistosoma	-ve Schist
TOTAL No. Examined	266	298	48	516
Mean ELISA O.D.	0.487	0.249	0.333	0.239
Range ELISA O.D.	0.300-0.932	0.106-0.299	0.300-0.459	0.107-

TABLE 5: RESULTS OF SAMPLES CONSIDERED AS POSITIVE FOR FASCIOLIASIS ON DIFFERENT DIAGNOSTIC TESTS,, BY MONTH.

SAMPLES POSITIVE FOR:-	J	A	S	O	N	D	TOTAL
Eggs + ELISA + Flukes	18	10	10	11	5	6	60
Eggs + Flukes	2	-	-	-	-	-	2
Flukes + ELISA	-	1	-	2	3	-	6
Eggs + ELISA	15	14	15	17	5	20	86
Eggs only	1	-	-	2	1	2	1
ELISA only	8	25	17	17	20	18	105
Flukes Only	-	-	-	1	-	-	1
Positive infection	14	44	35	38	23	35	189

TABLE 6: CORRELATION BETWEEN CATTLE FROM COMMERCIAL AND TRADITIONAL FARMS

	COMMERCIAL (FASCIOLA)						TRADITIONAL (FASCIOLA)				
Month	Total No. Examd	No. Examd	+ve Fasciola Eggs	+ve Elisa	+ve Flukes	+ve Any Test	No. Examd	Fasciola Eggs	+ve Elisa	+ve Flukes	+ve Any Test
July	70	31	10	14	8	14	39	26	25	19	27
August	84	68	17	23	11	44	16	5	9	0	10
September	86	68	21	32	8	35	18	6	12	3	14
October	88	64	26	38	10	38	24	2	8	4	10
November	117	69	11	23	10	23	48	5	14	0	14
December	119	98	22	34	1	35	21	6	5	2	8
TOTAL	564	398	107	164	48	189	166	50	73	28	83

TABLE 7: RESULTS OF SAMPLES FROM COMMERCIAL FARMS CONSIDERED AS POSITIVE FOR FASCIOLIASIS.

Cattle Origin	Total Exam.	Pos. Eggs.	Pos. ELISA	Pos. Flukes	Total Pos. Any Test
CE	267	81	124	24	119
CN	30	4	11	7	14
CW	88	21	27	17	54
CS	13	1	2	0	2
TOTAL	398	107	164	48	189

TABLE 8: RESULTS OF SAMPLES FROM TRADITIONAL FARMS CONSIDERED AS POSITIVE FOR FASCIOLIASIS

Cattle Origin	Total Exam.	Pos. Eggs	Pos. ELISA	Pos. Flukes	Total Pos. Any Test
TE	33	14	20	5	21
TN	33	7	15	5	18
TW	88	26	32	17	38
TS	12	3	6	1	6
TOTAL	166	50	73	28	83

CE = Commercial farms, east of Mazabuka
CN = Commercial farms, North of Mazabuka
CW = " " , West of Mazabuka
CS = " " , South of Mazabuka

TN = Traditional farms, East of Mazabuka
TN = Traditional farms, North of Mazabuka
TW = " " , West of Mazabuka
TS = " " , South of Mazabuka

NOTE: The above abbreviations are used in Appendices 1 -

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SECTION X

Appendices 1 - 6

Detailed results of the various tests for Fasciola and Schistosoma infections in cattle sampled for six months at the Mazabuka Abattoir, Zambia, during 1983.

For explanation of cattle origin, see Page 70.

POS. INF. = Sample positive for infection by any diagnostic method.

APPENDIX 1: MAZABUKA ABATTOIR SURVEY DATA FOR THE MONTH OF JULY 1983

			FASCIOLA				SCHISTOSOMA			
SAMPLE NO.	CATTLE ORIGIN	AGE YEARS	EGGS FAECES	ELISA O.D.	FLUKES LIVERS	POS. INF.	EGGS FAECES	ELISA O.D.	EGGS LIVER	PO IN
1.	TE	5	+	0.260	+	Pos	-	0.260	-	
2	TE	9	+	0.544 +	-	Pos	-	0.300+	-	
3	TE	5	-	0.279	-	Pos	-	0.299	-	
4	CN	1	+	0.534+	-	Pos	-	0.299	-	
5	CN	3	+	0.504+	-	Pos	-	0.314	-	
6	TE	5	+	0.326+	-	Pos	-	0.254	-	
7	TE	1	+	0.804 +	-	Pos	-	0.278	-	
8	TE	3	+	0.548 +	-	Pos	-	0.152	-	
9	CW	3	-	0.275	-		-	0.182	-	
10	CW	3	-	0.275	-		-	0.183	-	
11	CW	3	-	0.294	-		-	0.294	-	
12	TN	6	-	0.268	-		-	0.267	-	
13	TN	5	-	0.212	-	Pos	-	0.259	-	
14	CN	5	-	0.273	-		-	0.280	-	
15	TW	7	+	0.380+	-	Pos	-	0.212	-	
16	TW	9	+	0.267	-	Pos	-	0.259	-	
17	TN	2	-	0.271	-		-	0.169	-	
18	CW	5	-	0.314+	-	Pos	-	0.211	-	
19	TE	10	+	0.275	-		-	0.221	-	
20	CW	8	+	0.321+	-	Pos	-	0.214	-	
21TE	TE	2	+	0.319+	P	Pos	-	0.319+	-	
22	TE	5	-	0.322+	-	Pos	-	0.112	-	
23	TE	5	+	0.774 +	-	Pos	-	0.268	-	
24	TW	5	-	0.313 +	-	Pos	-	0.263	-	
25	CW	7	-	0.220	-	-	-	0.252	-	
26	TW	7	-	0.286	-		-	0.256	-	
27	TN	3	+	0.522 +	-	Pos	-	0.256	-	
28	TN	6	+	0.586 +	-	Pos	-	0.206	-	
29	TW	3	-	0.257	-		-	0.209	-	
30	CW	2	-	0.257	-		-	0.262	-	
31	CW	5	-	0.277	-		-	0.270	-	
32	CW	6	-	0.284	-		-	0.265	-	
33	CW	2	-	0.301 +	-	Pos	-	0.139	-	

			FASCICLA				SCHISTOSOMA		
SAMPLE NO.	CATTLE ORIGIN	AGE YEARS	EGGS FAECES	ELISA O.D.	FLUKES LIVERS	EGS. INF.	EGGS FAECES	ELISA O.D.	EGGS LIVER
34	TW	4	-	0.266	-			0.254	-
35	TW	8	+	0.361+	+	Pos	-	0.266	-
36	TE	5	+	0.314 +	+	Pos	-	0.314	-
37	TW	2	-	0.276	-		-	0.161	-
38	TW	6	-	0.317 +	-	Pos	-	0.274	-
39	TW	0	-	0.267	-		-	0.249	-
40	TW	5	+	0.459 +	+	Pos	-	0.262	-
41	CN	2	+	0.494 +	-	Pos	-	0.131	-
42	CN	2	-	0.168	-		-	0.113	-
43	CN	2	-	0.281	-		-	0.201	-
44	TN	2	+	0.510 +	-	Pos	-	0.202	-
45	TW	5	+	0.414 +	-	Pos	-	0.166	-
46	CE	7	-	0.268	-		-	0.218	-
47	TW	6	+	0.537 +	+	Pos	-	0.202	-
48	TW	6	+	0.379 +	+	Pos	-	0.192	-
49	TW	9	+	0.280	-		-	0.182	-
50	TW	4	-	0.284	-		-	0.217	-
51	CW	5	-	0.219	-		-	0.256	-
52	CW	6	-	0.171	-		-	0.171	-
53	CW	1	0	0.181	-		-	0.160	-
54	CW	6	-	0.171	-		-	0.182	-
55	CW	5	-	0.279	-		-	0.187	-
56	CW	5	-	0.642+	-	Pos	-	0.286	-
57	CW	9	+	0.543+	-	Pos	-	0.269	-
58	CW	2	+	0.507	+	Pos	-	0.107	-
59	CW	6	+	0.576 +	+	Pos	-	0.258	-
60	CW	7	+	0.573 +	+	Pos	-	0.176	-
61	CW	7	+	0.583 +	+	Pos	-	0.173	-
62	CW	5	+	0.543 +	+	Pos	-	0.298	-
63	TW	6	+	0.432 +	+	Pos	-	0.298	-
64	TW	8	+	0.444 +	+	Pos	-	0.191	-
65	TW	5	+	0.598 +	+	Pos	-	0.225	-
66	TW	9	+	0.552 +	+	Pos	-	0.298	-
67	TW	3	+	0.448 +	+	Pos	-	0.225	-
68	TW	2	+	0.366 +	+	Pos	-	0.231	-
69	CE	2		0.263	+		-	0.260	-
70	CE	7	-	0.390 +	-	Pos	-	0.280	-

APPENDIX 2: MAZABUKA ABATTOIR SURVEY DATA FOR THE MONTH OF AUG

			FASCIOLA				SCHISTOSOMA			
MPLE .	CATTLE ORIGIN	AGE YEARS	EGGS FAECES	ELISA O.D.	FLUKES LIVER	POS. INF.	EGGS FAECES	ELISA O.D.	EGGS LIVER	
	CE	3	-	0.352 +	-	Pos	-	0.196	-	
	CE	8	-	0.298	-		-	0.222	-	
	CN	5	+	0.585 +	-	Pos	-	0.213	-	
	CN	2½	-	0.276	-		-	0.256	-	
	CE	4	-	0.461 +	-	Pos	-	0.229	-	
	CE	10	-	0.360	-	Pos	-	0.260	-	
	CE	6	+	0.491 +	-	Pos	-	0.291	-	
	CE	8	+	0.365 +	-	Pos	-	0.222	-	
	CE	3	-	0.416 +	-	Pos	-	0.276	-	
	CE	6	+	0.390 +	-	Pos	-	0.290	-	
	PW	7	+	0.308 +	-	Pos	-	0.268	-	
	CW	5	-	0.372 +	-	Pos	-	0.299	-	
	CW	7	-	0.401 +	-	Pos	-	0.281	-	
	CW	4	-	0.223	-		-	0.257	-	
	CW	6	+	0.345 +	-	Pos	-	0.311 +	-	
	CS	1	-	0.274	-		-	0.200	-	
	TN	8	-	0.337 +	-	Pos	-	0.323 +	-	
	TN	6	-	0.326 +	-	Pos	-	0.224	-	
	CW	10	-	0.302 +	-	Pos	-	0.241	-	
	CW	7	-	0.225	-		-	0.253	-	
	CW	1	-	0.290	-		-	0.260	-	
	CE	6	-	0.346	-	Pos	-	0.273	-	
	CE	5	-	0.280	-		-	0.130	-	
	CW	10	-	0.266	-		-	0.172	-	
	CW	6	-	0.319 +	-	Pos	-	0.152	-	
	CW	7	-	0.336 +	-	Pos	-	0.165	-	
	CE	5	-	0.238	-		-	0.147	-	
	CW	5	-	0.309 +	-	Pos	-	0.287	-	
	CW	8	-	0.302 +	-	Pos	-	0.265	-	
00	CW	5	-	0.300 +	-	Pos	-	0.275	-	
01	CN	5	-	0.284	-		-	0.277	-	
02	CN	5	-	0.216	-		-	0.348 +	-	
03	CN	4	-	0.334 +	-	Pos	-	0.314 +	-	
04	TW	7	-	0.320 +	-	Pos	-	0.297	-	
05	CN	3	-	0.200	-		-	0.233	-	

			FASCIOLA				SCHISTOSOMA		
SAMPLE NO.	CATTLE ORIGIN	AGE YEARS	EGGS FAECES	ELISA O.D.	FLUKES LIVER	POS. INF.	EGGS FAECES	ELISA O.D.	EGGS LIVER
106	CE	3	-	0.218	-		-	0.221	-
107	PE	5	-	0.204	-		-	0.201	-
108	CW	10		0.270	-		-	0.257	-
109	CW	8	+	0.467 +	-	Pos	-	0.257	-
110	CW	9	+	0.484 +	-	Pos	-	0.289	-
111	CW	8	+	0.332 +	+	Pos	-	0.246	-
112	CW	10	-	0.336 +	-	Pos	-	0.246	-
113	CW	6	+	0.396 +	+	Pos	-	0.234	-
114	CW	8	+	0.482 +	-	Pos	-	0.243	-
115	TN	10	+	0.492 +	-	Pos	-	0.227	-
116	CW	4	-	0.298	-		-	0.200	-
117	CW	8	-	0.245	-		-	0.316 +	-
118	CW	8	+	0.322 +	+	Pos	-	0.270	-
119	CW	3	-	0.235	-		-	0.314 +	-
120	CE	6	-	0.322 +	-	Pos	-	0.334 +	-
121	CE	6	-	0.249	-		-	0.320 +	-
122	CE	8	-	0.192	-		-	0.236	-
123	CE	6	-	0.240	-		-	0.246	-
124	CE	5	-	0.165	-		-	0.236	-
125	CE	6	-	0.183	-		-	0.237	-
126	TE	1		0.211	-		-	0.256	-
127	TN	10	-	0.248	-		-	0.296	-
128	CE	8	-	0.301 +	-	Pos	-	0.253 +	-
129	CN	2	-	0.287	-		-	0.392 +	-
130	TS	10	-	0.261	-		-	0.271	-
131	TE	7	-	0.255	-		-	0.234	-
132	TE	7	+	0.475+	-	Pos	-	0.271	-
133	TE	10	-	0.710 +	-	Pos	-	0.323 +	-
134	TE	10	+	0.302 +	-	Pos	-	0.211	-
135	CW	5	+	0.393 +	-	Pos	-	0.173	-
136	CE	3	-	0.297	-		-	0.267	-
137	TE	5	+	0.361 +	-	Pos	-	0.210	-
138	CE	2	-	0.455 +	-	Pos	-	0.230	-
139	CW	6	-	0.304 +	-	Pos	-	0.265	-
140	TS	3	+	0.375 +	-	Pos	-	0.272	-
141	CE	4	-	0.275	-		-	0.299	-

			FASCIOLA				SCHISTOSOMA		
SAMPLE NO.	CATTLE ORIGIN	AGE YEARS	EGGS FAECES	ELISA O.D.	FLUKES LIVERS	POS. INF.	EGGS FAECES	ELISA O.D.	EGGS LIVER
142	CW	5	+	0.311 +	-	Pos	-	0.299	-
143	CS	6	+	0.448 +	-	Pos	-	0.591 +	-
144	TE	8	+	0.574 +	-	Pos	-	0.255	-
145	CE	2	-	0.341 +	-	Pos	-	0.284	-
146	CE	5	-	0.379 +	-	Pos	-	0.235	-
147	CE	6	-	0.465 +	-	Pos	-	0.264	-
148	CE	8	+	0.393 +	+	Pos	-	0.290	-
149	CW	7	+	0.841 +	-	Pos	-	0.262	-
150	CW	7	+	0.716 +	-	Pos	-	0.321 +	-
151	CW	2	-	0.723 +	+	Pos	-	0.254	-
152	CW	6	+	0.726 +	+	Pos	-	0.304 +	-
153	CW	6	+	0.871 +	+	Pos	-	0.209	-
154	CW	6	+	0.664 +	+	Pos	-	0.220	-

APPENDIX 3 : MAZABUKA ABATTOIR SURVEY DATA FOR THE MONTH OF SEPTEMBER 1

			FASCIOLA				SCHISTOSOMA		
SAMPLE NO.	CATTLE ORIGIN	AGE YEARS	EGGS FAECES	ELISA O.D.	FLUKES LIVER	POS. INF.	EGGS FAECES	ELISA O.D.	EGGS LIVER
155	CE	3	+	0.351 +	-	Pos	-	0.290	-
156	CE	10	+	0.392 +	-	Pos	-	0.185	-
157	TS	6	-	0.405 +	-	Pos	-	0.397 +	-
158	CW	5	+	0.465 +	-	Pos	-	0.254	-
159	CW	6	+	0.548 +	+	Pos	-	0.246	-
160	CW	4	+	0.790 +	+	Pos	-	0.393 +	-
161	CW	7	+	0.821 +	+	Pos	-	0.312 +	-
162	CW	9	+	0.519 +	+	Pos	-	0.270	-
163	CW	6	+	0.666 +	+	Pos	-	0.411 +	-
164	CW	8	+	0.654 +	?	Pos	-	0.278	-
165	CW	6	+	0.829 +	+	Pos	-	0.307 +	-
166	TS	2	+	0.601 +	+	Pos	-	0.233	-
167	TE	6	-	0.245	-		-	0.190	-
168	TN	6	+	0.333 +	+	Pos	-	0.317 +	-
169	TS	6	-	0.611 +	-	Pos	+	0,459 +	-
170	TN	10	-	0.310 +	-	Pos	-	0.203	-
171	CE	3	-	0.627 +	-	Pos	-	0.265	-
172	CE	7	-	0.345 +	-	Pos	-	0.252	-
173	CE	2	-	0.445 +	-	Pos	-	0.213	-
174	CE	7	+	0.593 +	-	Pos	-	0.234	-
175	TN	6	-	0.417 +	-	Pos	-	0.237	-
176	TN	1	+	0.632 +	-	Pos	-	0.368 +	-
177	CW	6	+	0.707 +	-	Pos	-	0.272	-
178	CW	8	+	0.316 +	-	Pos	-	0.260	-
179	CW	7	+	0.366 +	-	Pos	-	0.230	-
180	CW	10	+	0.665 +	-	Pos	-	0.259	-
181	CW	8	+	0.571 +	-	Pos	-	0.246	-
182	CW	4	+	0.369 +	-	Pos	-	0.237	-
183	TS	4	-	0.323 +	-	Pos	-	0.275	-
184	CN	10	-	0.376 +	-	Pos	-	0.278	-
185	CN	7	-	0.290	-		-	0.286	-
186	CN	8	-	0.284	-		-	0.227	-
187	CN	9	-	0.284	-		-	0.254	-
188	CN	10	-	0.373 +	-	Pos	-	0.234	-

			FASCIOLA				SCHISTOSOMA		
SAMPLE NO.	CATTLE ORIGIN	AGE YEARS	EGGS FAECES	ELISA O.D.	FLUKES LIVER	POS. INF.	EGGS FAECES	ELISA O.D.	EGGS LIVE
189	CN	8	-	0.286	-		-	0.279	-
190	TW	7	+	0.418 +	-	Pos	-	0.217	-
191	CE	5	+	0.477 +	-	Pos	-	0.112	-
192	CE	5	-	0.403 +	-	Pos	-	0.261	-
193	CE	2	+	0.360 +	-	Pos	-	0.256	-
194	CE	10	-	0.232	-		-	0.197	-
195	CE	2	-	0.197	-		-	0.232	-
196	CE	3	-	0.279	-		-	0.240	-
197	TW	2	-	0.288	-		-	0.189	-
198	TW	1	+	0.433 +	-	Pos	-	0.198	-
199	TW	3	-	0.244	-		-	0.235	-
200	CW	3	-	0.189	-		-	0.177	-
201	CW	3	-	0.156	-		-	0.230	-
202	CW	3	-	0.282	-		-	0.262	-
203	TW	7	-	0.190	-		-	0.151	-
204	TS	10	+	0.329 +	-	Pos	-	0.168	-
205	CS	2	-	0.269	-	-	-	0.168	-
206	CS	6	-	0.267	-		-	0.285	-
207	CS	3	-	0.203	-		-	0.281	-
208	CS	7	-	0.199	-	-	-	0.214	-
209	CS	8	-	0.224	-		-	0.198	-
210	CS	5	-	0.240	-		-	0.173	-
211	CS	10	-	0.242	-		-	0.361	-
212	CS	7	-	0.332 +	-	Pos	-	0.151	-
213	CS	4	-	0.198	-		-	0.248	-
214	CS	5	-	0.292	-		-	0.214	-
215	CS	8	-	0.286	-		-	0.141	-
216	CS	9	-	0.210	-		-	0.190	-
217	CE	2	-	0.223	-		-	0.192	-
218	CE	3	-	0.236	-		-	0.207	-
219	CE	2	-	0.217	-		-	0.172	-
220	CE	8	-	0.212	-		-	0.279	-
221	CE	6	-	0.161	-		-	0.249	-
222	CE	3	-	0.256	-		-	0.217	-
223	CE	3	-	0.136	-		-	0.275	-
224	CE	5	-	0.402+	-		-	0.144	-

			FASCIOLA				SCHISTOSOMA		
SAMPLE NO.	CATTLE ORIGIN	AGE YEARS	EGGS FAECES	ELISA O.D.	FLUKES LIVERS	POS. INF.	EGGS FAECES	ELISA O.D.	EGGS LIVER
225	CE	6	-	0.224	-		-	0.181	-
226	CE	7	-	0.213	-		-	0.204	-
227	CE	4	-	0.201	-		-	0.250	-
228	CE	10	-	0.306 +	-	Pos	-	0.292	-
229	CE	3	-	0.198	-		-	0.184	-
230	CE	10	-	0.193	-		-	0.199	-
231	CE	4	-	0.334 +	-	Pos	-	0.277	-
232	CE	5	-	0.470 +	-	Pos	-	0.282	-
233	CE	9	-	0.658 +	-	Pos	-	0.229	-
234	CE	10	-	0.529 +	-	Pos	-	0.325 +	-
235	CE	1	-	0.569 +	-	Pos	-	0.297	-
236	CE	1	-	0.867 +	-	Pos	-	0.234	-
237	TW	5	-	0.350 +	-	Pos	-	0.209	-
238	TE	10	-	0.239	-		-	0.211	-
239	CE	10	-	0.518 +	+	Pos	-	0.268	-
240	TN	3	+	0,592 +	+	Pos	-	0.176	-

APPENDIX 4 : MAZABUKA ABATTOIR SURVEY DATA FOR THE MONTH OF OCTOBER 1

			FASCIOLA				SCHISTOSOM		
SAMPLE NO.	CATTLE ORIGIN	AGE YEARS	EGGS FAECES	ELISA O.D.	FLUKES LIVER	POS. INF.	EGGS FAECES	ELISA O.D.	EGGS LIVE
241	CE	3	+	0.599 +	+	Pos	-	0.307 +	-
242	CE	2	+	0.337 +	+	Pos	-	0.216	-
243	CE	2	+	0.752 +	+	Pos	-	0.308 +	-
244	CE	3	-	0.498 +	-	Pos	-	0.279	-
245	CE	3	-	0.273	-		-	0.346 +	-
246	CE	8	-	0.932 +	-	Pos	-	0.398 +	-
247	CE	7	+	0.259	-	Pos	-	0.179	-
248	TE	1	-	0.517 +	+	Pos	-	0.272	-
249	CE	7	+	0.707 +	+	Pos	-	0.267	-
250	CE	6	-	0.731 +	+	Pos	-	0.360 +	-
251	CE	6	+	0.654 +	+	Pos	-	0.228	-
252	CE	5	+	0.508 +	+	Pos	-	0.225	-
253	CE	1	+	0.559 +	-	Pos	-	0.285	-
254	CE	5	+	0.535 +	+	Pos	-	0.269	-
255	TN	3	-	0.222	-		-	0.202	-
256	TN	2	-	0.225	-		-	0.183	-
257	TN	2	-	0.124	-		-	0.199	-
258	TN	2	-	0.819 +	-	Pos	-	0.324 +	-
259	CE	3	-	0.230	-		-	0.221	-
260	CE	4	+	0.378 +	-	Pos	-	0.265	-
261	CE	3	-	0.228	-		-	0.298	-
262	CE	3	-	0.238	-		-	0.197	-
263	CE	3	-	0.282	-		-	0.283	-
264	CE	2	-	0.106	-		-	0.227	-
265	CE	2	-	0.221	-		-	0.232	-
266	CE	2	-	0.270	-		-	0.161	-
267	TS	4	-	0.239	-		-	0.216	-
268	TE	7	-	0.230	-		-	0.253	-
269	CE	9	-	0.239	-		-	0.234	-
270	CE	3	+	0.549 +	-	Pos	-	0.205	-
271	CE	3	+	0.304 +	-	Pos	-	0.290	-
272	CE	2	+	0.354 +	+	Pos	-	0.276	-
273	CE	4	+	0.379 +	-	Pos	-	0.287	-
274	CE	2	+	0.387 +	+	Pos	-	0.255	-
275	CE	2	+	0.699 +	-	Pos	-	0.236	-

			FASCIOLA				SCHISTOSOMA		
SAMPLE NO.	CATTLE ORIGIN	AGE YEARS	EGGS FAECES	ELISA O.D.	FLUKES LIVER	POS. INF.	EGGS FAECES	ELISA O.D.	EGGS LIVER
312	TW	5	-	0.240	-		-	0.234	-
313	TW	3	-	0.499 +	+	Pos	-	0.275	-
314	TW	6	-	0.299	+		-	0.295	-
315	TW	5	-	0.446 +	-	Pos	-	0.213	-
316	TW	4	-	0.499 +	-	Pos	-	0.241	-
317	TW	4	-	0.237	-		-	0.223	-
318	CE	7	-	0.215	-		-	0.236	-
319	CE	6		0.358 +	-	Pos	-	0.199	-
320	CE	6	+	0.495 +	-	Pos	-	0.248	-
321	CE	5	+	0.669 +	-	Pos	-	0.263	-
322	TW	10	-	0.268	-		-	0.208	-
323	TN	8	-	0.330 +	-	Pos	-	0.256	-
324	CE	3	-	0.368 +	-	Pos	-	0.262	-
325	CE	2	-	0.318 +	-	Pos	-	0.230	-
326	CE	2	-	0.394 +	-	Pos	-	0.252	-
327	CE	2	+	0.340 +	-	Pos	-	0.251	-
328	CE	2	-	0.268	-		-	0.262	-

APPENDIX 5: MAZABUKA ABATTOIR SURVEY DATA FOR MONTH OF NOVEMBER 1983

			FASCIOLA				SCHISTOSOMA		
SAMPLE NO.	CATTLE ORIGIN	AGE YEARS	EGGS FAECES	ELISA O.D.	FLUKES LIVER	POS. INF.	EGGS FAECES	ELISA O.D.	EGGS LIVER
329	CE	3	+	0.280	-		-	0.231	-
330	CE	2	-	0.270	-		-	0.231	-
331	CE	2	-	0.261	-		-	0.251	-
332	CE	3	-	0.296	-		-	0.253	-
333	CE	2	-	0.258	-		-	0.276	-
334	CN	7	+	0.422 +	-	Pos	-	0.298	-
335	TE	7	-	0.336 +	-	Pos	-	0.235	-
336	TE	3	-	0.423 +	-	Pos	-	0.266	-
337	TE	2	-	0.361 +	-	Pos	-	0.252	-
338	TW	10	+	0.469 +	-	Pos	-	0.257	-
339	TW	9	+	0.470 +	-	Pos	-	0.297	-
340	TN	8	+	0.467 +	-	Pos	-	0.269	-
341	TN	1	-	0.370 +	-	Pos	-	0.251	-
342	CE	2	-	0.282	-		-	0.195	-
343	CE	2	-	0.375 +	-	Pos	-	0.275	-
344	CE	2	-	0.350 +	-	Pos	-	0.230	-
345	CE	3	-	0.295	-		-	0.203	-
346	CE	8	-	0.502 +	-	Pos	-	0.207	-
347	TW	10	-	0.539 +	-	Pos	-	0.289	-
348	TE	2	-	0.601 +	-	Pos	-	0.285	-
349	CE	2	+	0.385 +	-	Pos	-	0.205	-
350	CE	2	-	0.535 +	-	Pos	-	0.222	-
351	CE	2	+	0.872 +	-	Pos	-	0.131	-
352	CE	2	-	0.408 +	-	Pos	-	0.133	-
353	CE	2	+	0.428 +	-	Pos	-	0.251	-
354	CE	2	+	0.581 +	-	Pos	-	0.238	-
355	CE	3	-	0.195	-		-	0.202	-
356	TN	3	-	0.199	-		-	0.302	-
357	TN	3	-	0.182	-		-	0.154	-
358	CE	3	+	0.238	-		-	0.127	-
359	CE	3	+	0.582 +	-	Pos	-	0.238	-
360	CE	3	-	0.202	-		-	0.202	-
361	CE	3	-	0.190	-		-	0.302 +	-
362	CE	2	-	0.202	-		-	0.157	-

			FASCIOLA				SCHISTOSOMA		
SAMPLE NO.	CATTLE ORIGIN	AGE YEARS	EGGS FAECES	ELISA O.D.	FLUKES LIVER	POS. INF.	EGGS FAECES	ELISA O.D.	EGGS LIVE
363	CE	2	-	0.256	-		-	0.127	-
364	CE	3	-	0.269	-		-	0.126	-
365	CE	2	-	0.774 +	-	Pos	-	0.238	-
366	CE	2	-	0.265	-		-	0.259	-
367	TE	9	-	0.266	-		-	0.139	-
368	CE	2	-	0.280	-		-	0.246	-
369	CE	2	-	0.286	-		-	0.251	-
370	CE	2	-	0.444 +	-	Pos	-	0.226	-
371	CE	2	+	0.284	-		-	0.237	-
372	CE	2	-	0.291	-		-	0.237	-
373	CE	3	-	0.290	-		-	0.251	-
374	TW	9	-	0.179	-		-	0.244	-
375	TW	8	-	0.187	-		-	0.214	-
376	TW	6	-	0.225	-		-	0.261	-
377	TW	8	+	0.695 +	-	Pos	-	0.256	-
378	TW	3	-	0.230	-		-	0.286	-
379	TW	10	-	0.228	-		-	0.264	-
380	TW	8	-	0.204	-		-	0.164	-
381	TW	2	-	0.203	-		-	0.237	-
382	TW	2	-	0.318 +	-	Pos	-	0.244	-
383	TW	6	-	0.208	-		-	0.254	-
384	CN	1	-	0.171	-		-	0.158	-
385	TW	6	-	0.311 +	-	Pos	-	0.158	-
386	TW	1	-	0.190	-		-	0.157	-
387	TW	2	-	0.177	-		-	0.254	-
388	TW	2	-	0.243	-		-	0.254	-
389	CE	7	+	0.746 +	-	Pos	-	0.265	-
390	CE	2	-	0.590 +	-	Pos	-	0.267	-
391	CE	2	-	0.176	-		-	0.221	-
392	CE	2	-	0.232	+		-	0.217	-
393	CE	3	+	0.618 +	+	Pos	-	0.200	-
394	CE	4	+	0.669 +	-	Pos	-	0.187	-
395	TW	2	+	0.357 +	-	Pos	-	0.153	-
396	TW	2	-	0.324 +	-	Pos	-	0.266	-
397	TW	2	-	0.286	-		-	0.266	-
398	TW	2	-	0.295	-		-	0.209	-

			FASCIOLA				SCHISTOSOMA		
SAMPLE NO.	CATTLE ORIGIN	AGE YEARS	EGGS FAECES	ELISA O.D.	FLUKES LIVER	POS. INF.	EGGS FAECES	ELISA O.D.	EGGS LIVER
399	TW	3	-	0.294	-		-	0.258	-
400	TW	3	-	0.165			-	0.277	-
401	TW	2	-	0.225	-		-	0.175	-
402	TW	10	-	0.285	-		-	0.318 +	-
403	TW	3	-	0.273	-		-	0.282	-
403	TW	3	-	0.174	-		-	0.269	-
405	TW	10	-	0.159	-		-	0.249	-
406	TW	7	-	0.158	-		-	0.178	-
407	TS	8	-	0.250	-		-	0.179	-
408	CE	2	-	0.282	-		-	0.249	-
409	CE	2	-	0.266	-		-	0.254	-
410	CE	2	-	0.234	-		-	0.207	-
411	CE	2	-	0.278	-		-	0.228	+
412	CE	6	-	0.259	-		-	0.253	-
413	CE	3	-	0.240	-		-	0.203	-
414	CN	2	-	0.251	-		-	0.209	-
415	CN	2	-	0.276	-		-	0.276	-
416	CN	2	-	0.336 +	-	Pos	-	0.176	-
417	CN	2	-	0.201	-		-	0.213	-
418	CE	2	-	0.297	-		-	0.254	-
419	CE	6	-	0.263	-		-	0.207	-
420	CE	2	-	0.177	-		-	0.225	-
421	CE	5	-	0.245	-		-	0.243	-
422	CE	7	-	0.204	-		-	0.257	-
423	CE	2	-	0.571 +	+	Pos	-	0.235	-
424	CE	2	-	0.310 +	-		-	0.237	-
425	CE	2	-	0.470 +	-	Pos	-	0.250	-
426	CE	2	-	0.249	-		-	0.215	-
427	CE	2	-	0.223	-		-	0.211	-
428	CE	3	-	0.263	-		-	0.244	-
429	CE	8	-	0.316 +	-	Pos	-	0.253	+
430	CE	10	-	0.232	-		-	0.236	-
431	TW	2	-	0.237	-		-	0.295	-
432	TW	2	-	0.214	-		-	0.211	-
433	TW	3	-	0.232	-		-	0.333 +	-
434	TW	3	-	0.193	-		-	0.232	-

			FASCIOLA				SCHISTOSOMA		
SAMPLE NO.	CATTLE ORIGIN	AGE YEARS	EGGS FAECES	ELISA O.D.	FLUKES LIVER	POS. INF.	EGGS FAECES	ELISA O.D.	EGGS LIVER
435	TW	2	-	0.244	-		-	0.246	-
436	TW	3	-	0.223	-		-	0.222	-
437	TW	3	-	0.236	-		-	0.241	-
438	TW	3	-	0.253	-		-	0.241	-
439	TW	3	-	0.244	-		-	0.254	-
440	CE	8	-	0.214	-		-	0.199	-
441	CE	2	-	0.208	-		-	0.242	-
442	CE	4	-	0.229	-		-	0.213	-
443	CE	2	-	0.264	-		-	0.251	-
444	CE	4	-	0.282	-		-	0.269	-
445	CE	8	-	0.432	+	+	-	0.239	-

APPENDIX 6: MAZABUKA ABATTOIR SURVEY DATA FOR MONTH OF DECEMBER 198

			FASCIOLA				SCHISTOSO		
SAMPLE NO.	CATTLE ORIGIN	AGE YEARS	EGGS FAECES	ELISA O.D.	FLUKES LIVER	POS. INF.	EGGS FAECES	ELISA O.D.	EGGS LIVE
446	CE	10	-	0.218	-		-	0.221	-
447	CE	8	-	0.257	-		-	0.207	-
448	CE	10	-	0.231	-		-	0.233	-
449	CE	2	+	0.235	-		-	0.200	-
450	CE	4	+	0.369 +	+	Pos	-	0.285	-
451	CE	2	+	0.310 +	-	Pos	-	0.234	-
452	CE	2	+	0.409 +	-	Pos	-	0.177	-
453	CE	3	+	0.580 +	-	Pos	-	0.254	-
454	CE	4	+	0.455 +	-	Pos	-	0.235	-
455	TN	10	+	0.604 +	-	Pos	-	0.217	-
456	CE	1	-	0.269	-		-	0.237	-
457	CE	4	-	0.229	-		-	0.265	-
458	CE	4	+	0.637 +	-	Pos	-	0.237	-
459	CE	4	-	0.290	-		-	0.222	-
460	CE	3	-	0.258	-		-	0.195	-
461	CE	5	-	0.256	-		-	0.207	-
462	CE	5	-	0.345 +	-	Pos	-	0.209	-
463	CE	3	-	0.235	-		-	0.206	-
464	CE	3	-	0.199	-		-	0.179	-
465	CE	4	-	0.181	-		-	0.269	-
466	CE	4	-	0.305 +	-		-	0.260	-
467	CE	2	-	0.194	-		-	0.227	-
468	CE	3	-	0.216	-		-	0.190	-
469	CE	3	-	0.244	-		-	0.288	-
470	CE	3	-	0.216	-		-	0.207	-
471	CE	3	-	0.246	-		-	0.190	-
472	CE	3	-	0.219	-		-	0.284	-
473	CE	3	-	0.256	-		-	0.270	-
474	CE	3	-	0.209	-		-	0.291	-
475	CE	3	-	0.291	-		-	0.245	-
476	CE	1	-	0.202	-		-	0.216	-
477	CE	1	-	0.204	-		-	0.254	-
478	CS	3	-	0.260	-		-	0.208	-
479	TN	1	-	0.234	-		-	0.208	-

			FASCIOLA				SCHISTOSOMA		
SAMPLE NO.	CATTLE ORIGIN	AGE YEARS	EGGS FAECES	ELISA O.D.	FLUKES LIVER	POS. INF.	EGGS FAECES	ELISA O.D.	EGG LIV
480	TN	6	-	0.310 +	-	Pos	-	0.245	-
481	CE	2	+	0.364 +	-	Pos	-	0.230	-
482	TW	5	+	0.535 +	-	Pos	-	0.260	-
483	TN	6	-	0.237	-		+	0.445 +	+
484	CE	2	+	0.768 +	-	Pos	-	0.283	-
485	CE	2	+	0.337 +	-	Pos	-	0.284	-
486	CE	5	-	0.306 +	-	Pos	-	0.252	-
487	CE	2	-	0.332 +	-	Pos	-	0.222	-
488	CE	2	-	0.352 +	-	Pos	-	0.274	-
489	CE	8	-	0.205	-		-	0.257	-
490	CN	10	-	0.260	-		-	0.217	-
491	CN	7	-	0.332 +	-	Pos	-	0.253	-
492	CN	10	-	0.280	-		-	00.255	-
493	CN	10	-	0.279	-		-	0.257	-
494	TN	2	-	0.278	-		-	0.247	-
495	TW	6	+	0.348 +	-		-	0.257	-
496	TW	9	-	0.250	-		-	0.255	-
497	TW	2	+	0.522 +	+	Pos	-	0.267	-
498	CE	2	+	0.512 +	-	Pos	-	0.268	-
499	CE	2	+	0.687 +	+	Pos	-	0.249	-
500	CE	2	+	0.513 +	-	Pos	-	0.262	-
501	CE	2	+	0.513 +	-	Pos	-	0.266	-
502	CE	2	-	0.255	-		-	0.295	-
503	CE	2	-	0.260	-		-	0.228	-
504	CE	2	-	0.244	-		-	0.211	-
505	CE	2	-	0.448 +	-	Pos	-	0.231	-
506	PE	4	-	0.244	-		-	0.238	-
507	PN	4	-	0.308 +	-	pos	-	0.217	-
508	CE	1	-	0.240	-		-	0.283	-
509	CE	8	+	0.321 +	+	Pos	-	0.249	-
510	CE	2	+	0.359 +	-	Pos	-	0.209	-
511	CE	2	+	0.486 +	-	Pos	-	0.281	-
512	CE	2	+	0.443 +	-	Pos	-	0.222	-
513	CE	2	+	0.309 +	-	Pos	-	0.198	-
514	CE	2	-	0.289	-		-	0.199	-

			FASCIOLA				SCHISTOSOMA		
SAMPLE NO.	CATTLE ORIGIN	AGE YEARS	EGGS FAECES	ELISA O.D.	FLUKES LIVER	POS INF.	EGGS FAECES	ELISA O.D.	EGG FÆ
515	CE	2	-	0.245	-		-	0.236	-
516	CE	7	-	0.191	-		-	0.248	-
517	CE	6	-	0.269	-		-	0.213	-
518	CN	2	0	0.324 +	-	Pos	-	0.207	-
519	CE	3	+	0.756 +	-	Pos	-	0.210	-
520	CE	1	+	0.262	-		-	0.209	-
521	CN	9	-	0.239	-		-	0.198	-
522	TW	7	-	0.302 +	-	Pos	-	0.218	-
523	TW	2	+	0.380 +	-	Pos	-	0.236	-
524	TW	2	+	0.255	-		-	0.195	-
525	CE	2	-	0.266	-		-	0.216	-
526	CE	2	-	0.185	-		-	0.227	-
527	CE	2	-	0.186	-		-	0.208	-
528	CE	2	-	0.287	-		-	0.240	-
529	CE	3	-	0.297	-		-	0.232	-
530	CW	4	-	0.194	-		-	0.206	-
531	CW	3	-	0.295	-		-	0.224	-
532	CW	3	-	0.185	-		-	0.210	-
533	CW	3	-	0.179	-		-	0.208	-
534	CW	8	-	0.208	-		-	0.223	-
535	CE	10	-	0.181	-		-	0.209	-
536	TS	4	-	0.200	-		-	0.198	-
537	TS	3	-	0.261	-		-	0.300 +	-
538	TN	6	-	0.205	-		-	0.249	-
539	CE	2	-	0.198	-		-	0.209	-
540	CE	1	-	0.277	-		-	0.200	-
541	CE	1	-	0.188	-		-	0.253	-
542	CE	1	+	0.395 +	-	Pos	-	0.241	-
543	CE	1	-	0.221	-		-	0.227	-
544	CE	1	-	0.296	-		-	0.272	-
545	CE	1	-	0.286	-		-	0.280	-
546	TN	6	-	0.241	-		-	0.248	-
547	CE	3	-	0.240	-		-	0.187	-
548	TN	8	-	0.240	-		-	0.247	-
549	TN	10	-	0.258	-		-	0.171	-
550	CE	8	-	0.303 +	-	Pos	-	0.166	-
551	CE	9	-	0.260	-		-	0.268	-
552	CW	2	-	0.357 +	-	Pos	-	0.305 +	-

			FASCIOLA				SCHISTOSOMA		
SAMPLE NO.	CATTLE ORIGIN	AGE YEARS	EGGS FAECES	ELISA O.D.	FLUKES LIVER	POS. INF.	EGGS FAECES	ELISA O.D.	EGGS LIV
553	CW	1	-	0.309 +	-	Pos	-	0.173	-
554	CW	1	-	0.296	-		-	0.273	-
555	CW	2	-	0.309 +	-	Pos	-	0.207	-
556	CE	1	-	0.306 +	-	Pos	-	0.262	-
557	CE	1	-	0.259	-		-	0.270	-
558	CE	1	-	0.212	-		-	0.222	-
559	CE	1	-	0.276	-		-	0.261	-
560	CE	1	-	0.264	-		-	0.273	-
561	CE	1	-	0.288	-		-	0.247	-
562	TW	8	-	0.337 +	-	Pos	-	0.210	-
563	TS	10	-	0.257	-		-	0.168	-
564	CE	3	-	0.307 +	-	Pos	-	0.270	-