SEROLOGICAL AND PARASITOLOGICAL SURVEY OF TOXOPLASMOSIS IN FOOD ANIMALS (CATTLE, SHEEP, GOATS AND SWINE) IN KANO AND KADUNA STATES OF NIGERIA

By

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I pass with relief from the tossing sea of Cause and Theory to the firm ground of Result and Fact.

- Sir Wiston S. Churchill

DEDICATED TO MY LOVING WIFE, ADEMIJU

AND

OUR ADORING SON, OLATUNBOSUN JUNIOR

DECLARATION

I hereby declare the originality of this piece of work which was carried out by me in the laboratory of the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria, under the supervision of Drs. E. D. Belino, D. S. Adegboye and Prof. A. A. Ilemobade. Help received in the execution of the project is duly acknowledged.

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THESIS APPROVAL

This thesis by Andrew Olatunbosun Aganga meets the regulations governing the degree of Master of Science of Ahmadu Bello University and is approved for its contribution to scientific knowledge and literary presentation.

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SUMMARY

A serological and parasitological survey of toxoplasmosis was conducted on some food animals (cattle sheep, goats and swine) in Kano and Kaduna States of Nigeria to determine prevalence of T. gondii infection.

A combination of Indirect Haemagglutination (IHA) and Indirect Fluorescent Antibody (IFA) tests were the serological methods used for cattle, goats and sheep serum samples while the IHA and the Sabin-Feldman (SF) dye tests were used for the swine serum samples.

Attempts were made to isolate T. gondii from seropositive animals by the mouse inoculation technique,

A total of 762 animals were sampled comprising 200 each of cattle, goats, sheep and 162 swine. Serum samples were collected prior to slaughter while tissue samples of the same animals were collected after slaughter at various abattoirs in these two States. Results show that 3, 4,5, 9 and 6.8 per cent respectively of cattle, goats, sheep and swine were positive by the Indirect Haemagglutination te3t while 3, A, 9.5 per cent of cattle, goats and sheep were

positive by the Indirect Fluorescent Antibody Test and 8 per cent of swine by the Sabin-Feldman (SF) dye test.

There was no statistical significant difference in the results based on age, sex, breed and geographical location P<0.0.05(chi square). Attempt to isolate Toxoplasma gondii from the tissues of the serologically positive animals was unsuccessful.

It is concluded, based on the present study, that meat of commonly slaughtered animals in Northern Nigeria present minimal public health hazard as far as T. gondii is concerned.

CHAPTER I

INTRODUCTION

Toxoplasmosis is a world-wide zoonotic disease affecting all warm-blooded animals including man. It is caused by the protozoan parasite, Toxoplasma gondii.

The organism was first described by Nicolle and Manceaux in 1908 in the North African rodent Ctenodactylus gondii. Among domestic animals, the first case of toxoplasmosis was reported in the dog by Mello (1910) in Turin, Italy. Wolf and Cowen (1937) described the first congenital case in humans, while Olafson and Monlux (1942) were the first to report the disease in sheep and cats. Farrell et al. (1952) in Ohio came across the first cases of toxoplasmosis in swine. They found the disease in eight pigs from a farm where an undiagnosed disease had recurred for many years. Reports of toxoplasmosis in cattle were described by Marotel and Pierron (1943) and later by Sanger et al. (1953) who found Toxoplasma in four herds of cattle in Ohio. Hartley, Jebson and McFarlane (1954) demonstrated that Toxoplasma was the agent producing abortion in a large number of ewes in

Australia. In 1970, several workers independently made a significant advance to our knowledge of the epizootiology of toxoplasmosis (Frenkel, Dubey and Miller, 1970; Weiland and Kuhn, 1970; Overdulve, 1970; Sheffield and Melton, 1970; Hutchison, Dunachie, Siim and Work, 1970; Witte and Piekarski, 1970; Walton and Werner, 1970). These workers demonstrated that Toxoplasma gondii is a coccidian parasite with development occuring in the epithelium of feline animals, being the final host.

In Nigeria, reports indicate that toxoplasmosis may be widespread in human population. Jelliffe, (1951) serologically diagnosed a congenital form of the disease in a 3-year-old Yoruba girl in Ibadan. Olurin, Fleck and Osuntokun (1972) reported a reaction rate to the SF dye test amongst Nigerian patients with chorioretinitis of 95.4% but also of 72.2% amongst control patients who did not suffer from uveitis. They concluded that many cases of focal chorioretinitis were probably toxoplasmic but that the dye test was of limited value in diagnosis in patients over 40 years. Emejuaiwe, Njoku-Obi, Talwar and Kodilinye (1973) observed an increasing incidence of human Toxoplasma

infection in the Eastern part of Nigeria. Overdulve (cited by Ogunrinade, 1977) detected antibodies at a titre of 1/160 by the fluorescent tagged antibody technique in 29 of 144 patients at Ahmadu Bello Teaching Hospital and Guiness Eye Hospital in Northern Nigeria. In his survey, Overdulve found out that the 29 positive cases were Christians while no Muslim carried antibodies to Toxoplasma infection. He therefore concluded that the consumption of infected pork or pork products may be the source of infection to these patients since Muslims don't eat pork because of their religious doctrines.

The first indication of toxoplasmosis in domestic animals in Nigeria was by Ogunrinade (1977). In an investigation into the prevalence of Toxoplasma antibodies in dogs in Ibadan, 40 randomly selected sera were dye-tested. About 58% were positive at titres of 1/16 and above. Local breed of dogs had a higher proportion of positive reactors (12 out of 15) than exotic breeds or crosses. It was the opinion of Ogunrinade that this may be due to differences in management and habits. Young dogs below 10 months of age had higher titres than other positive reactors.

In an outbreak of a neurological disease in a buffalo ranch in Bendel State of Nigeria, eleven out of about 200 animals died within three weeks. Toxoplasma cysts and trophozoites were found in the brain of a buffalo. Falade (1978) serologically tested 751 sera from female goats collected from Kano abattoir. Using the latex agglutination test, he found 23 (0.06%) of 751 sera examined to be positive at titres ranging from 1/16 to 1/256. Isolation attempts were not carried out in this survey.

In view of these reports and since it has been suggested that human beings may acquire toxoplasmosis from meat consumption (Siim, 1956; Weinman and Chandler, 1956), especially if the meat is undercooked (Jacobs et al., 1960a) the objectives of this study were:

(i) to carry out prevalence studies on food animals (cattle, sheep, goats and swine) in Nigeria using the Indirect Haemagglutination (IHA) and the Indirect Fluorescent Antibody (IFA) tests and

(ii) to attempt isolation of the organism in the tissues of these food animals by mice inoculation method.

CHAPTER II

LITERATURE REVIEW

Historical Background

In 1908, Charles Nicolle and L. Manceaux showed that a fatal disease contracted by some cricetine rodents (gondis) maintained at the Pasteur Institute in Tunis was caused by a new organism which they named Toxoplasma (Frenkel, 1970). Through the 1920s, toxoplasmosis remained an obscure disease of laboratory animals studied at the Pasteur Institute in Paris (Levaditi et al., 1929) and the Rockefeller Institute in New York (Sabin, 1939). It was not until between 1937 and 1940 that it was identified as the cause of congenital meningo-encephalitis by Abner Wolf and David Cowen at Columbia University in New York (Wolf and Cowen, 1937).

Since then, the amount of literature on toxoplasmosis in man and animals has been phenomenal, a reflection, no doubt, of the biological and public health significance of the causative agent, Toxoplasma gondii. Between 1963 and 1970, many reviews have been written on toxoplasmosis and these have in turn

stimulated increased efforts at delineating the cause of the disease. Notable among these reviews are those of Eyles (1956), Jacobs (1956, 1957, 1961, 1962, and 1963), Siim (1956), Goldman, Carver and Solzer (1958), Galuzo and Zasukhin (1963) in Russian, Frenkel (1967) and Kozar (1970).

Life Cycle

The life-cycle of *Toxoplasma* has been studied by Frenkel, Dubey and Miller (1970a), Hutchison *et al.* (1970), Sheffield and Melton (1970) and Overdulve (1970). *T. gondii* has been found to have a cyst stage in the faeces of cats which is indistinguishable from the oocyst of *Isospora bigemina*. The life-cycle was found to be completed in the domestic cat and in some other members of the cat family only (Frenkel, Dubey and Miller, 1970a). They found that cats can be infected by feeding on mice which have been infected with the vegetative stages of *T. gondii* with subsequent production of oocysts in the epithelial cells of the ileum by the union of male and female gametes, as in *Eimeria* and *Isospora*. According to these workers the oocysts pass out in the faeces, sporulate in 2 to 3 days

at 24°C, 5 to 8 days at 15°C and 14 to 21 days at 11°C. These oocysts when ingested infect various animals in which they develop parenterally. The prepatent period in cats is 3 to 5 days after being fed parenteral cysts, 7 to 10 days after being fed trophozoites, and 20 to 24 days after being fed faecal oocysts (Frenekl, Dubey and Miller, 1970; Frenkel, Dubey, and Miller 1970a).

Disease Patterns In Animals and Man

The strains of T. gondii recovered from different species vary in pathogenicity but there is only one recognised serotype (Quinn and McCraw, 1972). Exposure to the organism and infections by it are common, but clinically apparent disease is uncommon. The clinical signs of toxoplasmosis are reported to vary considerably with the species affected and very likely with the strain of organism involved (Quinn and McCraw, 1972).

Dogs

It has been observed that while adult dogs do not show characteristic clinical signs, young dogs under one

year old tend to develop the acute form of toxoplasmosis characterized by weakness, depression, cough, tremors and incoordination (Cole, Sanger, Farrell and Kornder, 1954). In a number of cases studied by Campbell, Martin and Gordon, (1955), none had signs which could be regarded as specific for Toxoplasma infection. These authors, however, noted an association between T. gondii and clinical evidence of distemper. This may be due to the fact that younger dogs are more prone to these diseases.

Cats

Toxoplasmosis manifests itself in two disease syndromes in the cat: an acute or subacute generalised form in young animals which is characterised by respiratory signs, jaundice and lymphadenopathy, and a chronic localized disease in older animals usually with wasting and intestinal signs (Jubb and Kennedy, 1970). Frenkel and Dubey (1972) showed that new born kittens developed enteritis, hepatitis, pneumonitis and encephalitis and were moribund by the ninth day after experimental infection. Kittens infected at 2 weeks of age or older were also similarly affected but often

survived, whereas adult cats remained asymptomatic.

Sheep

Abortion or death of newborn lambs throughout the world has been associated with toxoplasmosis (Hartley and Marshall, 1957; Beverly and Watson, 1959 and 1971; Jacobs and Hartley, 1964). Respiratory and nervous signs are features of the disease in ewes and lambs from which Toxoplasma has been recovered (Cole et al. 1954). A history of several years of abortion may be associated with the diagnosis of toxoplasmosis on a farm (Beverley and Watson, 1971a). Hartley et al. (1954) and Hartley and Marshall (1957) have detailed the clinical aspects of Toxoplasma abortion in sheep. Death of the foetus may occur antepartum, at the end of an apparently normal parturition or within a few hours of birth. Mummification, mild to marked uterine decomposition, subcutaneous oedema of the foetus and excess of fluid in the body cavities may be seen. However, these changes are not specific for toxoplasmosis but are generally indicative of abortion and uterine infection. Essentially there is no diagnostic lesion which can be specifically attributed to

Toxoplasma infection (Soulsby, 1971). There appears to be agreement that congenital transmission occurs only in ewes infected during pregnancy (Beverley and Watson, 1971). Moreover, early infection of pregnant ewes frequently results in death and resorption of the foetus, whereas infection at a later time, for instance, at 90 days pregnancy, is followed by congenital infection with abortion less often resulting (Quinn and McCraw, 1972).

Swine

Most of the reported outbreaks of toxoplasmosis in swine are associated with the newborn. Moller *et al.* (1970) studied *Toxoplasma* in experimentally infected pregnant sows. In six sows infected in the third month of gestation, the disease varied from mild to severe depending upon the strain of *Toxoplasma* used. The severe form was characterized by fever, anorexia and dyspnoea. In another experimental *Toxoplasma* infection of pigs using the oocysts of *Isospora bigemina* of feline origin, Sasaki *et al.* (1974) observed that some of the pigs manifested an increase in body temperature, loss of vitality and appetite, discharge from the eyes,

coughing, accelerated respiration and incoordination of the hindquarters 4 days after the inoculation. Their faeces were watery and contained mucus. From the 7th day of infection onwards, these pigs were observed to have cyanosis at the apices of the ears and noses, and in the lower part of abdomen.

Cattle

Reports concerning toxoplasmosis in cattle are scarce. Sanger et al. (1953) recorded an outbreak of a disease they suspected to be toxoplasmosis in a herd of Brown Swiss cows in which forty-five out of seventy-eight calves born over a period of a year died at various ages. The clinical signs included dyspnoea, coughing, sneezing, a nasal discharge, trembling and shaking of the head. An elevated temperature was noted in several cases, but in others the temperature appeared normal. At times, death was sudden without any previous evidence of illness; in other cases the disease lasted for several months. These signs however, have not been known to be associated with Toxoplasma infection and hence other disease conditions need be considered.

Buffalo

Neurologic disorders and deaths have been reported in the buffalo (Ikede, et al., 1978). 11 out of about 200 animals died within three weeks. Toxoplasma cysts and trophozoites were found in the brain of a buffalo; also appreciable levels of antibodies were found in the sera of animals examined.

Goats

Toxoplasmosis in goats has not been adequately documented. Feldman and Miller (1956) found serological evidence of infection in two goat populations in Central New York. In a survey conducted by Falade (1978) he detected antibodies to T. gondii in the sera of 27 out of 848 goats in Nigeria, but made no attempt at isolation.

Man

Congenital transmission in man can be suspected when the following clinical signs are manifested: retinochoroiditis, hydrocephaly or microcephaly, psychomotor retardation and cerebral calcification (Feldman, 1968). Congenital infection in human infants is usually the result of asymptomatic infections of their

mothers (Miller, Aronson and Remington, 1969). Majority of infants born to women who had experienced infection during pregnancy are born without visible evidence of defects. Some of these infants, however, do develop active disease a few days to months after birth. This may express itself as hydrocephaly, chorioretinitis, blindness, mental retardation, epilepsy or deafness. In those infants with severe disease present at birth the mortality is about 7-16 per cent. 90 per cent of the survivors are permanently mentally retarded or have marked visual impairment (Miller et al., 1969). In the adult, T. gondii may cause lymphadenopathy. It is also an opportunist as it may cause a lethal infection in the immunologically compromised host.

Pathological changes

The extent of reaction to the presence of the parasite varies according to the organ involved and the age of the host. Often the only response observed in tissues is necrosis and this is especially true in acute cases (Quinn and McCraw, 1972). According to Smith, Jones and Hunt (1972), infection with Toxoplasma is

believed to result first in parasitaemia, then in localization and multiplication of the organisms in various tissues. This localization may be followed by active lesions in the affected tissue or by encystment of the Toxoplasma, in which form they remain viable for a long time. This infection is most likely to occur in the brain and other tissues where living organisms have been found several years following infection. Toxoplasma has been found in the liver, spleen and lymph nodes of experimentally infected mice. Ito et al. (1967) observed the following characteristic changes in the infected mice: activation of the reticulo-endothelial system, necrosis of host cells and circumscribed circulatory disturbances. In the dog and cat, the lungs and liver are usually affected and in the latter, focal necrosis may be obvious. The spleen and lymph nodes may be enlarged. Hirth and Nielsen (1969) reported that the most consistent pathological finding in feline toxoplasmosis was interstitial pneumonia accompanied by fibrinous exudate, focal necrosis, proliferation of alveolar lining cells and the presence of large number of alveolar macrophages. Lesions most often seen in the brain were areas of focal gliosis and perivascular cuffing.

In other domestic animals, lesions are less characteristic. The disease in swine shares common features with that in dogs such as pneumonitis, enteritis and focal hepatitis. Young dogs are more susceptible than adults (Levine, 1961). In an experimental infection of 8 pigs and 6 pregnant sows with cysts and trophozoites of T. gondii respectively, Moller et al. (1970) observed gross lesions in the lungs and uterus. Hyperaemia and oedema were the most prominent features of the gross pneumonic lesions; the lungs of one sow contained numerous miliary nodules with necrotic centres. Mummified foetuses were found in one uterus. Microscopically, acute focal necrosis was seen in the lungs, liver, pancreas, adrenal and thyroid glands and some lymph nodes. In still-born piglets, degenerative changes were recorded in the central nervous system, heart, lung and liver.

In ruminants, the skeletal muscles appear to be a favoured site of infection and proliferation by the parasite (Jacobs and Hartley, 1964). Cattle and sheep with acute disease may exhibit respiratory and central nervous system involvement (Levine, 1961; Fulton, 1967). In pregnant animals, the placenta is a common site of

proliferation and small necrotic foci in the foetal cotyledons or maternal caruncles are frequently observed. The characteristic lesions of the placenta in ovine abortion due to toxoplasmosis is a focal inflammation of the cotyledonary parts of the placenta (Beverley et al., 1971). These workers also recorded pathological changes in the lymph nodes, liver, hear and brain of ovine foetuses.

Transmission

Toxoplasma is in many respects a very unique protozoan parasite. It is a remarkably ubiquitous organism which has been isolated from nearly every organ and tissue, although, depending on the host, some organs are more parasitized than others (Overdulve, 1970). Furthermore, T. gondii is one of the most successful parasite of which so many possible ways of transmission are known, although all the experimentally proved mechanisms of transmission such as through the consumption of infected meat and meat products as well as the infectivity of oocysts shed by the cat and cat family, cannot sufficiently explain the high rates of Toxoplasma infection in such groups as, for instance,

strict vegetarians with a prevalence rate ranging between 84%-100% amongst the adult population tested, and herbivores (Overdulve, 1970, Wallace, 1976).

Contaminated faeces

It is highly likely that the cat, as the shedder of oocyts, and the invertebrates, as transport hosts play important roles in the spread of T. gondii among herbivorous and omnivorous animals. It has been observed that almost all cats without Toxoplasma antibody became infected after eating cysts, and they excrete oocysts for 1 to 2 weeks (Dubey, Miller and Frenkel, 1970). Recently infected cats generally do not excrete Toxoplasma again following a second feeding (Frenkel, Dubey and Miller, 1969; Dubey, Miller and Frenkel, 1970; Whitte and Piekarski, 1970). However, about half of the cats with naturally acquired antibodies to Toxoplasma excrete oocysts after cyst feeding (Dubey, Miller and Frenkel, 1970). This indicates that immunity is not absolute and that a cat may shed oocysts and be infectious several times in a lifetime (Dubey, Miller and Frenkel, 1970). However, without the cat or its relatives, T. gondii infections would undoubtedly persit in nature in cannibalistic rodents and in swine (Jacobs,

1974). Dobos-Kovacs, Pellerdy and Balsao (1974) threw more light on the transmission of toxoplasmosis when they reported the first Toxoplasma infection in captive Derby Kangaroos at the Budapest Zoo. Three of the animals died of the infection, the origin of which they traced to oocysts shed along with cat faeces. Investigations disclosed that the kangaroos became infected by the ingestion of fodder contaminated with cat faeces. Dry cat faeces found in the preparatory room, and the fresh faeces of a cat living there, both contained Toxoplasma oocysts, which caused fatal disease on experimental transmission to albino mice.

Beverley et al. (1975) in their studies on the spread of Toxoplasma gondii to sheep, found no oocysts in the faeces of sheep, lambs, crows or pigeons infected orally with sporulated oocysts, or of sheep and lambs inoculated subcutaneously with the tissue cysts. All pregnant ewes given oocysts aborted about 28 days after infection. All those given tissue cysts aborted in about 34 days after infection. All the inoculated crows and pigeons became infected and developed histological lesions.

Conducting epidemiological studies in flocks of

sheep with reproductive loss resulting from toxoplasmosis, Waldeland(1977) diagnosed toxoplasmosis in 15 out of 51 flocks using the SF dye test. Though they suspected that transmission was via silage consumption on one farm, cats were incriminated to be responsible as they were found to be common in the district.

Sengbusch and Sengbusch (1976) however, refused to share the view of the role of cats in the transmission of toxoplasmosis. In a prevalence study carried out, they questioned veterinarians, animal hospital workers, and a selected population of non-contact subjects about rural or urban residency, pets owned, contact with cats and whether their cats used a litter box in the house. Serum samples were collected for serology by the complement-fixation and indirect haemagglutination tests. None of the 60 non-contact individuals was positive and only 18 per cent of the 60 contact subjects had detectable antibodies. Because only one veterinarian was seropositive, they proposed that exposure to cats per se did not supply sufficient evidence upon which to expect a risk of Toxoplasma infection. They therefore suggested that other factors such as direct or indirect association with cats, duration of exposure and the infectivity of the cats should also be considered.

Consumption of infected meat

The findings of Jacobs et al. (1960a) and the data of Desmonts et al. (1965) have suggested that in certain human populations, such as in France, the cyst stage consumed in raw or undercooked tissues of domestic animals is of primary importance in causing human Toxoplasma infection and disease. Two epidemics of human toxoplasmosis were attributed to meals of undercooked beef in the United States of America (Kean, Kimball and Christenson, 1969; Lord et al., 1975). In the 1969 epidemic 5 college students became ill after eating undercooked hamburgers while in the 1975 epidemic 4 out of 19 adults developed clinical toxoplasmosis apparently after feeding on a meat dish made from raw beef in a Syrian restaurant. Dubey and Streitel (1976) are of the view that most Toxoplasma infections in man are acquired postnatally, either by ingesting infected meat or by ingesting oocysts in feline faeces.

Boch (1967) however, rules out raw beef and veal as a possible source of human toxoplasmosis after being unable to isolate T. gondii from the flesh of these animals.

Role of arthropods and coprophages

The role of various arthropods and coprophages in the natural spread of toxoplasmosis, while it is attractive, has apparently been doubted by Quinn and McCraw (1972). Wallace (1973) has however, presented evidence that coprophagous invertebrates such as cockroaches, flies, snails and slugs may serve as transport hosts after ingesting oocysts in cat faeces if these animals are eaten by small mammals and birds. Wallace (1971, 1972) had earlier on demonstrated the experimental transmission of the parasite by filth-flies and cockroaches. He has also pointed out that some epidemiologic information indicates the possibility of the transmission of T. gondii to human food via coprophagous insects.

Congenital transmission in both animals and humans has been studied extensively by various workers. It has been found that the dam may have latent infection that becomes activated during pregnancy or much more likely, may acquire infection during pregnancy (Theis, 1977). In general, continued vertical transmission from dam to offspring for several generations has not been proven to occur. In humans, the majority of infants born

to women that experienced infection during pregnancy are born without visible evidence of defects. Presently there is apparently no way to predict the outcome of the latently infected infant (Theis, 1977, unpublished).

Venereally

Venereal mode of transmission has been documented by Spence et al. (1978) in the sheep. These workers investigated the possible role of the ram in the transmission of the disease. Two rams were inoculated subcutaneously with an ovine strain of T. gondii vegetative cysts. Specimens of semen were taken twice weekly for one month and inoculated into mice. T. gondii was found in the semen of one ram on the 20th day and in the other on the 20th and 25th days after infection. The experiment was repeated later and they showed that T. gondii was present in the semen of one ram from the 7th to the 32nd days after infection and in the other from the 14th to the 32nd days only. T. gondii was also recovered from several tissues of both. At the same time, two ewes were infected by vaginal inoculation of vegetative cysts of T. gondii and the parasite was subsequently recovered from the heart, skeletal muscle and liver of both sheep. These workers therefore concluded

that transmission at coitus in sheep is possible.

Human to human

There are exceptional cases of human to human transmission of the disease from organ transplantation (Reynolds, Walls and Pfeiffer, 1966) and blood transfusion (Roth et al., 1971; Siegel, Lunde and Gelderman, 1971). Jacobs (1970) reported an interesting observation of five experienced researchers who were involved in studies on transmission of toxoplasmosis. These workers had handled the trophozoite and cyst forms of T. gondii for several years without acquiring infection, but they became serologically positive after working with the oocysts.

Survival of the organism

Jacobs, Remington and Melton (1960) found that the cysts of T. gondii are not able to survive freezing and drying, but they survived as long as 8 days at 4°C. Proliferative forms were destroyed within a few minutes by artificial gastric juice, but the cysts remained infective in tissue up to three hours, while trophozoites liberated by peptic juice from isolated cysts survived

up to two hours. Trypsin destroyed the cyst wall immediately but the liberated trophozoites survived at least six hours; proliferative forms remained viable for at least three but less than six hours. Thus, parasites encysted in tissues could survive the normal digestive period in the stomach and should remain viable for even a longer period in the duodenum.

Supporting the observations made by Jacobs et al. (1960), Raisanen and Saari (1976) investigated environmental transmission of trophozoites. They subjected Toxoplasma trophozoites to different changes in osmotic pressure using ascitic fluid in different dilutions. Their results showed that trophozoites remained infectious for 5 to 29 days in different osmotic concentrations. They therefore suggested that trophozoites, being resistant to changes in osmotic pressure, may survive outside the body long enough to transmit the disease.

Diagnosis

Toxoplasmosis may not present a readily recognizable clinical form and on other occasions, it may occur in association with other infectious diseases which may mask

the clinical signs of the disease. Diagnosis may be made by:-

(a) isolation of the organism.

(b) microscopic examination of tissue sections or smears and

(c) serologic procedures.

Isolation: The most conclusive method of diagnosis is isolation of the parasite. Experimental animals such as mice, cell culture, and embryonated eggs are suitable for isolation purposes. Blood, cerebrospinal fluid, biopsy or autopsy material may also be used for isolation procedures (Quinn and McCraw, 1972; Krongstad, Juranek and Walls, 1972; Fleck, 1979).

Taking into consideration partinent aspects of previous studies on the resistance of cysts of Toxoplasma gondii to digestive juices (Jacobs and Melton, 1957) and on the distribution of the parasites in the striated muscle of rat with chronic infections (Jacobs and Melton, 1957a), Jacobs et al. (1960a) carried out a survey of meat samples from swine, cattle and sheep for the presence of encysted Toxoplasma, by the digestion method which they found adequate.

Various workers have since adopted the method of isolation with or without slight modifications.

Work (1967) used this method in its entire form while attempting Toxoplasma isolation from the flesh of sheep, swine and cattle in Denmark. Foster, Forrest and Blance (1969) modified the technique by digesting for only 45 minutes instead of the 2 hours by Jacobs et al. while attempting to isolate Toxoplasma gondii from naturally infected chickens. The results obtained indicated that isolation was also possible even at the shorter digestion period.

Although parasite isolation is known to be the most conclusive means of diagnosis, various workers have reported difficulty in isolating Toxoplasma even in serologically positive animals. Reasons for such failure were however, not given. Boch (1967) and co-workers in Berlin failed to isolate the parasite in samples from 500 diaphragm, 108 heart, 100 brains and 149 eyes of 500 cattle that had shown a 60% reaction rate to the Sabin-Feldman (SF) dye test. Boch (1967) has however, isolated Toxoplasma from pork. T. gondii has not been isolated from French meat and it is difficult to isolate T. gondii from cattle, and retail sausage meat in Britain, although in other parts of the world, e.g. Germany, it has been isolated from mutton (Fleck, 1979).

Toxoplasma can be cultivated readily in chicken embryos and tissue culture. It was first cultivated in both systems by Levaditi *et al.* (1929). Cook and Jacobs (1958) cultivated it in a wide variety of mammalian and avian cells including human, monkey, mouse rabbit, guinea pig, rat, ox and chick normal tissues, and in human and mouse cancer cells. This method is however, not a routine diagnostic method.

Microscopic examination of tissue sections:

Frenkel (1956) and Smith, Jones and Hunt (1972) reviewed the histopathology of toxoplasmosis. In the brain, *Toxoplasma* multiplies in the neurons and other cells and may cause cellular and interstitial necrosis. Sometimes, infarction necrosis causes extensive lesions. Whenever aqueductal obstruction and internal hydrocephalus are present, periventricular vasculitis and necrosis are generally observed; these constitute lesions unique for toxoplasmosis.

Demonstration of the parasite may be achieved in sections routinely stained with hemotoxylin and eosin (H&E). The cyst form is by far the most common form observed, although in acute forms of the disease, trophozoites may be seen also. Cysts may be sparsely

distributed and careful examination may be necessary to locate them in sections. It is often observed that cysts are found in areas away from inflammatory sites or necrotic foci (Quinn and McCraw, 1972).

Fluorescein labelled antibodies can also be used to stain frozen and specially fixed paraffin embedded sections in order to detect the organism (Carrer and Goldman, 1959).

Serology

Several serological tests are available for detecting toxoplasmosis. These include the Complement Fixation Test (CFT), Indirect Hemagglutination Test (IHA), Sabin-Feldman dye test (SF) and the Indirect Fluorescent Antibody Test (IFA).

The Dye Test:

The dye test, also known as the cytoplasma modifying antibody test, was developed by Sabin and Feldman in 1948. Since then, various research centres have adopted this test with different types of modifications (Remington, 1970). The dye test is still one of the most specific and sensitive system for measuring antibodies against this parasite (Feldman, 1968).

The test works on the principle that when Toxoplasma organisms (usually the RH strain) are incubated at 37°C in sera containing antibody against T. gondii and then placed in contact with an alkaline solution of methylene blue, the cytoplasm of the organism fails to stain while the nuclear endosomes stain blue. The cytoplasm and nucleus of Toxoplasma incubated in non-immune sera both pick up the dye. This is so because the immune sera affects the concentration of RNA in the cytoplasm, while the DNA in the nucleus is unaffected. Thus, with immune sera, the nucleic acid is unavailable to take up the stain in the cytoplasm while the nucleus can be stained (Theis, 1977). This test requires the inclusion of complement usually called the Accessory Factor. This is fresh normal human serum containing no Toxoplasma antibody and stored at -20°C to preserve complement activity. The serum must be tested to ascertain that it has the necessary properties required in the test. A live supply of the RH strain of Toxoplasma gondii must be used. This requires recovery from mice no more than 4 days after serial passage. It is important that a fresh alkaline solution of methylene blue be used for

each run (Theis, 1977). In reading the test, the diluted sera of the suspect animal preventing 50% of the organisms from picking up the stain in their cytoplasm represents the highest positive titre; 90% of the control Toxoplasma must have stained in both the cytoplasm and nucleus.

The SF test antibodies are usually elevated by the end of the second week of infection. The level at which they remain or fluctuate and the length of time they remain detectable are not uniform for all species nor do all individuals maintain a high titre indefinitely. In congenital cases particularly, the titre may be down to 1:4 within one or two years, even though there may be organisms still recoverable from the host (Eichenwald, 1956).

SF titres in cats can be as high as 1:4096 and yet the animal may remain clinically asymptomatic. However, some cats with SF titres of 1:256 or above can almost always be shown to have parasites present by mouse inoculation. If however, the SF test titre is 1:64 or lower, only in about 50% of the cats can the parasite be isolated (Fish, 1961). In cattle and swine, however, the SF titres are usually low even when organisms are present; titres obtained are rarely above 1:250 in these animals.

The complication of this test plus the expense inherent in maintaining the many factors necessary for running the test have restricted its use to only a few laboratories.

The Complement Fixation Test (C.F.):

This test was developed for toxoplasmosis by Sabin (1949). It is thought to be highly specific but is much less sensitive than the SF test. Titres are much lower when determined by this test, and they develop more slowly after infection and decline much more rapidly (Frenkel, 1970). A positive CF titre of 1:32 or above is considered to indicate relatively recent infection. The antigen for this test may be prepared from chicken embryos, mouse brain, peritoneal exudate or cell cultures of T. gondii. Eichenwald (1956) preferred chorioallantoic membrane or cell culture because peritoneal exudate has a strong anti-complementary activity. An advantage of this test is that live organisms are not required as antigens.

The Indirect Haemagglutination Test (IHA):

Several haemagglutination tests have been used, and these offer several advantages. They are economical,

rapid, relatively simple to perform and employ a non-viable antigen. The tests come in kits from various manufacturers. The ToxHA(R) test kit provides a simple, sensitive and standardized test system for the rapid detection and titration of antibodies to Toxoplasma gondii soluble extracts. The method, which is often referred to as the indirect or passive haemagglutination test for toxoplasmosis, is based on the work of Jacobs and Lunde (1957) and subsequently those of Jennis (1966) and Karim and Ludlam (1975). ToxHA(R) test utilizes extracts from purified trophozoites of Toxoplasma gondii RH strain to sensitize aldehyde-treated avian red blood cells. The test uses a suspension of formalin treated, tanned, turkey erythrocytes coated with a soluble sonicate of T. gondii and freeze-dried. In the presence of homologous antibodies, the sensitized cells will agglutinate to give characteristic patterns which can be read after a few hours in a plate test. Non-specific reactions may be detected, and where necessary, absorbed by the use of unsensitized control cells.

ToxHA(R) test detects and measures antibodies reacting principally with the soluble somatic antigens of

T. gondii which develop slowly in the late stage in the infection. These late-developing antibodies which are revealed by ToxHA(R) test is of particular value for detecting a diagnostic increase in antibody titres over the course of several months. Since the ToxHA(R) test system is based on the use of sensitized avian erythrocytes rather than the more commonly used mammalian cells, there is a lower incidence of agglutination due to heterophil antibody and therefore less need for serum adsorption.

The ToxHA(R) test is, however, limited in its application as it is sensitive principally to antibodies directed against the somatic antigens in contrast to the Fluorescent-Antibody Test (FA), SF and Direct Agglutination tests which all detect cuticular antibodies. Discrepancies will therefore exist between results by the different test procedures during the early stages of infection when little or no somatic antibodies may be found despite the presence of high levels of cuticular antibodies. A negative ToxHA(R) test results in the presence of clinical infection cannot be regarded as conclusive and either the haemagglutination test should be repeated after a few

weeks or the serum should be re-tested by the FA procedure (Anon, 1977).

The Fluorescent Antibody (FA) Test:

The fluorescent antibody test, introduced by Coons, Creech and Jones (1941) employs immune serum globulin labelled with fluorescent dye test to locate the corresponding antigen. It has been used for the visualization and identification of bacterial, viral, protozoal, helmintic, fungal and animal tissue antigens, and in modified form, for identifying the localization of serum antibodies in tissues, and also for the detection and characterization of serum antibodies.

Serological studies with fluorescein labelled Toxoplasma antibodies have been carried out by Goldman, Carver and Sulzer (1957). Further studies on the FA test were carried out by Goldman, Gordon and Carver (1962) and Capponi (1966). Two FA procedures have been developed: a fluorescence inhibition test, and an IFA test (Goldman, 1957; Kelen, Ayllon-Leindi and Labzoffshy, 1962). These tests offer a number of advantages. They are sensitive and do not require live antigen or accessory factor. Moreover the reagents used lend

themselves to preparation and standardization, and they keep indefinitely. In one comparative study of the SF test and the IFA test using 1,000 serum samples, there was over 95% agreement between the two tests (Walton, Benchoff and Brooks, 1966). Other investigators support this finding (Fletcher, 1965). It would appear, therefore, that the IFA test approaches the SF test in specificity and sensitivity.

Fluorescein - labelled anti-species immunoglobulins are intended for the qualitative detection of the major immunoglobulin classes of the appropriate species antibodies by FA procedures (Goldman, 1968; Nairn, 1969). The labelled reagent is also used to reveal the presence of infection by detecting specific antibodies in animal sera. For the detection of past or present parasitic infections, serum samples may be tested by the IFA technique at dilutions of 1:10-1:10,000, using polyvalent reagents. Suitable microscopic preparations (the substrates) are first treated with unlabelled test sera. Following a period of incubation, excess serum is removed by washing in Phosphate buffered saline (PBS). The appropriate fluorescent anti-species immunoglobulins are then applied.

Following further incubation and washing, the specimens are mounted and viewed in the fluorescent microscope. Positive reactions are shown by fluorescence of the parasites varying from an intense overall apple-green to an outline of the body walls of the Toxoplasma organisms. Capping of one end of the organisms with fluorescence is an unspecific reaction of some sera which reactions are usually diluted out above 1:64.

Methods of Control

Preventive measures

Human beings contract toxoplasmosis primarily by handling and ingestion of raw or improperly cooked meat, and probably also by ingestion of T. gondii oocysts. Any activities occupational or recreational, that bring people into contract directly with these sources are potential risks (Frenkel and Dubey, 1972a).

All meat should be thoroughly cooked to at least 66°C (100°F) before eating and hands should be washed with soap and water after handling meat to remove organisms contaminating the skin. Freezing meat at -14°C (5°F) for 24 hours may render the organisms nonviable (Frenkel and Dubey, 1972a).

Cat faecal oocysts have been implicated as a source of infection in laboratory workers (Miller, Frenkel and Dubey, 1972) and have been used to infect experimental animals (Work, 1971; Miller et al., 1972). There is also evidence of the isolation of these oocysts

from faeces of naturally infected cats (Wallace, 1971a), and from sand (Fleck et al., 1972), and soil (Ruiz, Frenkel and Cerdas, 1973). Transmission through cat faecal oocysts to man and animals is therefore a possibility.

Cat faecal oocysts of T. gondii do not become infective for 1 to 5 days or longer after being shed (Dubey et al., 1970). They can survive in moist soil for months. Because T. gondii oocysts are quite resistant to acids, alkalis and to common laboratory and hospital detergents, it is imperative that fresh faeces be disposed of daily (Dubey et al., 1970), whether in the home, hospital, kennels or in Zoos that keep felidae.

Serological surveillance will not indicate whether cats are shedding T. gondii oocysts; however, cats with antibodies are safer pets, since after re-infection they shed oocysts only briefly and in reduced numbers or not at all (Frenkel and Dubey, 1972a).

A number of preventive measures have been put forward from the Centre for Disease Control (CDC) in Georgia, USA, Anon (1971), while Work (1971) and Frenkel and Dubey, (1972a) have also added to the list.

These measures include the dumping of cat faeces daily followed by hand washing; prevention of pregnant women from eating raw meat or dumping cat faeces; conduction of serological tests on pregnant women with potential exposure to cat faeces; avoidance of the introduction of newly acquired cats to the household of a pregnant woman because prior diet and parasite history are not always known; wearing of gloves while working in sand or soil potentially contaminated by cat faeces or washing of hands afterwards and finally, the addition of boiling water or application of dry heat (over 66^{o}C) to faeces to kill faecal oocysts.

Chemotherapy

Sulphonamides are active against Toxoplasma gondii and among them, sulphadiazine and sulfamerazine are most useful (Frenkel, 1971). These drugs are believed to react in the same way as against other micro-organisms i.e. by competing with p-aminobenzoic acid (PABA). Their effect is inhibited by PABA and folic acid (Fulton, 1967). The antimalarial drug, pyrimethamine (Daraprim)$^{(R)}$, an antifolic agent, is also active against T. gondii. Sulphadiazine acts synergistically against Toxoplasma, and it is accepted

as the treatment of choice in human and experimental animal infections (Fulton, 1967 and Feldman, 1968). Chemotherapy is generally used to suppress proliferation of the parasite until immunity is acquired; consequently, treatment is given for some weeks. It is doubtful whether cyst forms are affected by such treatment, although trophozoites are susceptible (Feldman, 1968).

Thrombocytopenia and leukopenia occasionally result from such chemotherapy and can be counteracted by administration of folinic acid and yeast, which mammals can utilize but Toxoplasma cannot (Feldman, 1968 and Frenkel, 1971). Sulphones, tetracyclines and spiramycin do inhibit Toxoplasma to some degree, but they are much less effective than treatment with sulphadiazine and pyrimethamine (Christie, 1969 and Frenkel, 1971).

Immunity

Natural resistance

A number of serum factors are capable of reacting with trophozoites in vitro, thereby producing deleterious effects on the organisms. Antibody and

"accessory factor" - a heat-labile component present in normal human serum cooperate in this way. The cyst form of Toxoplasma is not affected by these factors. Apart from man, most other animals and birds possess what has been termed a "Toxoplasma-hostile factor", a substance believed to be an immunoglobulin showing different degrees of heat-lability in different sera and requiring complement for its action (Strannegard, 1967 and Feldman, 1968). Mice apparently lack both the "Toxoplasma-hostile factor" and the "accessory-factor" and this may account for the unusual susceptibility of these animals to Toxoplasma infections.

The influence of age on susceptibility of dogs to both experimental and natural infection with T. gondii was investigated by Capen and Cole (1966). These workers reported that acutely fatal disease with characteristic lesions was usually observed in dogs under one year of age. Older dogs appeared more resistant and had mild clinical signs and lesions.

Laboratory animals that survive an acute infection with Toxoplasma generally develop immunity (premunity) with cysts persisting for months or years (Frenkel, 1971). The reduction in numbers of parasites

following infection is brought about by cellular and humoral factors and it is thought that circulating antibodies contribute to the elimination of parasite, from the blood (Levine, 1961).

Recent evidence indicates that Toxoplasma is sensitive to interferon and the interferon-inducers, pyran and polyinosinio-polycytidylic acid, confer protection against this parasite in vivo and in vitro.

Where antibodies to T. gondii are present in the human before pregnancy, congenital infection does not usually occur (Feldman, 1968 and Jacobs, 1970).

CHAPTER III

MATERIALS AND METHODS

Geographical area covered

Kaduna and Kano States of Nigeria were covered by the investigation. The two states lie between latitude 9 and 13 degrees and longitude 6 and 9 degrees in the savanna vegetation zone.

Specimens

Blood samples were randomly collected from cattle, sheep, goats and swine where-ever possible. Tissue samples for *Toxoplasma* isolation were collected from similar animals that had been bled. A total of 762 animals were sampled comprising of 200 cattle, 200 goats, 200 sheep and 162 swine.

Sources of specimens

In Kaduna State, specimens were collected from the Animal Products Laboratory (A.P.L.) of the Department of Animal Science, Ahmadu Bello University, Zaria, and from the major abattoirs in Zaria and Kaduna townships. In

Kano State, specimens were collected from the main township abattoir. Approximately, about 300 cattle, 400 sheep and goats are slaughtered in the Zaria abattoir, while in Kaduna township abattoir, about 500 cattle, 600 sheep and goats and 20 swine are slaughtered daily. In the Kano township abattoir, about 400 cattle, 600 sheep and goats, and 40 camels are slaughtered daily.

Blood samples for serology work were drawn by venipuncture just prior to slaughter of each animal into labelled sterile bottle. The age, sex and breed of each animal was recorded. The samples were kept in an ice-box during transportation to the laboratory.

The diaphragm was the tissue of choice for parasite isolation for economic reasons and because Jacobs and Melton (1957a) have earlier shown that in chronically infected rats, the diaphragms were infected as frequently as other striated muscles. These samples were kept in labelled polyethylene plastic bags such that blood sample from the animals corresponded with the tissue sample from the same animals. The tissue samples were also kept in an ice-box while in transit (usually less than 2 hours) to the laboratory and

finally kept in the refrigerator at 4°C pending the serological examination of the blood samples. Toxoplasma isolation was attempted mainly on tissues from serologically positive animals.

Serological tests

Two serological tests were employed. The Indirect Haemagglutination (IHA) and the Indirect Fluorescent Antibody (IFA) tests.

IHA Test

This was carried out using the ToxHA(R) test kit (Wellcome Reagents Limited, Wellcome Research Laboratories, Beckenham, England, BR 3 3BS) and following the manufacturers instructions (Anon, 1977). Results were recorded in worksheets. Each serum sample was inactivated at 56°C for 30 minutes except the bovine serum samples which were inactivated at 60°C for 1 hour as recommended by Dubey and Streitel (1976).

All serum samples were tested as described by Jacobs and Lunde (1957) and modified by Karim and Ludlam (1975). Positive and negative controls were incorperated into each day's run and cut off titre was

1/64. Results were read after an hour in the V-bottomed microtitration plate placed on a plate mirror viewer. The titre of each serum sample was taken as the highest dilution showing clear haemagglutination.

IFA Test

This was carried out as described by Coons et al. (1941) and modified by Karim and Ludlam (1975). Killed commercial Toxoplasma antigens (Wellcome Research Laboratories, England) as well as the anti-species immunoglobulins were used.

Teflon-coated slides were used to aid viewing of test spots. Templates were used to apply the sera dilutions onto the slides. Evans Blue (1:1000) was incorperated into the immunoglobulin as counter stain to mask non-specific staining materials. Positive and negative controls were included in each test run. The cut off titre was 1/32.

SF Test

Anti-pig immunoglobulin was not available hence IFA was not conducted on pig serum samples, rather, the

samples were tested by the Sabin-Feldman Dye test (SF) at the St. George's Hospital Public Health Laboratory London, United Kingdom where the cut off titre was 1/16.

Parasite Isolation

A batch of laboratory-bred Swiss mice was employed. Prior to isolation attempt, the mice were checked for the presence of antibodies to Toxoplasma by randomly picking and sacrificing a few litter mates for serology by the IHA and IFA while histopathological sections for Toxoplasma were examined. The mice were negative to Toxoplasma infection both serologically and histologically.

The tissue samples were stored at 4°C from 1 to 2 days before isolation attempts. The method developed by Jacobs et al. (1960a) for the isolation of Toxoplasma by means of a peptic digestion technique which concentrates parasites from relatively large samples was used in this present work. After stripping off the fat and fascia, the muscles were ground three times in an ordinary household meat grinder. The ground meat was then placed in a 100 ml. Erlenmeyer flask and covered with about 10 times its

weight of a pepsin-hydrochloric acid solution with the following composition:

Pepsin	5 gm
HCl (Conc)	7 ml
Distilled water to make	1000 ml

(The pH was approximately 1.3)

The flask was placed on a magnetic stirrer and incubated at 37°C for 2 hours. The suspension was then filtered through a double layer of gauze and centrifuged at 2000 r.p.m. for 15 minutes. The supernatant was poured off and the sediment resuspended in physiological saline and re-centrifuged. The resulting sediment was suspended in 10 ml of saline and 1 ml of this suspension was injected intraperitoneally (i.p.) into each of 3 mice. A mixture of 1000 units of penicillin and 100 mg of streptomycin per ml of saline solution was injected into each mouse (i.p.) after the inoculation to prevent bacterial infection. The mice were observed for a 4-6 week period during which any that died was examined for proliferative and cyst forms in histological sections. At the end of the observation period, all surviving mice were bled by cardiac puncture

and examined serologically by the IHA and IFA tests for antibodies to Toxoplasma. Their tissues were also examined histologically for Toxoplasma cysts.

CHAPTER IV

RESULTS

IHA Test

The results of the IHA test on sera of 200 cattle, 200 goats, 200 sheep and 162 swine are presented in Table I. The highest rate of positivity was found among sheep where 18 (9.0%) were serologically positive, followed by that of swine with 11 (6.8%) positive; proportion in goats and cattle were 9 (4.5%) and 6 (3.0%) respectively. IHA titre obtained varied between 1/64 and 1/512 for sheep and swine and between 1/64 and 1/128 for goats and cattle.

IFA and SF Dye Tests

The IFA and SF dye test results are shown in Table II. Among the 200 sheep examined by IFA 19 (9.5%) were positive, while 6 (3.0%) goats and cattle were positive. With the SF dye test, 13 (8.0%) of 162 swine were positive. IFA titre varied between 1/32 and 1/512 for sheep and between 1/32 and 1/128 for goats and cattle. SF dye test titres, on the other hand,

varied between 1/16 and 1/512 for swine.

Influence of age on seropositivity

The influence of age on the response of the animals to Toxoplasma infection was analysed. The results are shown in Tables III and VI inclusive. Table III reflects the findings in the cattle population, and it shows that seropositivity by both IHA and IFA tests appear to increase with age. With goats, the situation was similar to that in cattle. The older age-group of $1\frac{1}{2}$ years and above showed a greater seroprevalence to Toxoplasma infection (Table IV). Corresponding results for sheep and swine are shown in Tables V and VI respectively. In these hosts, like cattle and goats, the rate of sero-reaction to Toxoplasma appear to increase with age.

Influence of Animal Breed on Seropositivity

The animals tested were classified according to breed. The results in Table VII appear to show no significant difference in the exposure of each animal breed examined to toxoplasmosis as assessed by serological means $P < 0.05$ (chi square).

Influence of Sex of Animals on Seropositivity

A further grouping of the animals sampled based on sex was done. The result is shown in Table VIII. There was no significant difference between the sexes of the animals sampled $P < 0.05$ (chi square).

Influence of Geographical Location on Seropositivity

A final grouping of the animals was done based on the geographical location from which the samples were collected. The result is shown in Table IX and there was no significant difference in the exposure of food animals in Kano and Kaduna States to toxoplasmosis based on sero-diagnostic method; $P < 0.05$ (chi square). In general, there appeared to be a good correlation between the IHA, IFA and the SF dye tests used in this study.

Results of Toxoplasma Isolation Attempts

Isolation of Toxoplasma was attempted from the diaphragms of 6 cattle, 9 goats, 19 sheep and 12 swine with IHA, IFA or SF dye test titres of 1/64, 1/32 or 1/16 respectively and above. Of the 3 mice per tissue sample innoculated, none died throughout the observation

period of 4-6 weeks. IHA and IFA tests on the sera from these mice at the end of 6 weeks showed a seropositivity to Toxoplasma with titres between 1/64 and 1/256 by IHA test, but histological findings on the tissues (brain, heart, liver and lungs) of the mice revealed no Toxoplasma gondii parasite. The result is summarized in Table X and it shows that none of the diaphragm samples from 6 cattle, 9 goats, 19 sheep and 12 swine yielded Toxoplasma.

TABLE I

SEROLOGICAL EXAMINATION OF CATTLE, GOATS, SHEEP AND SWINE FOR TOXOPLASMOSIS BY THE INDIRECT HAEMAGGLUTINATION (IHA) TES

ANIMAL SPECIES	NO. TESTED	NO. POSITIVE	RECIPROCAL TITRES			
			64	128	256	512
Cattle	200	6(3.0%)	4(2.0%)	2(1.0%)	-	-
Goat	200	9(4.5%)	6(3.0%)	3(1.5%)	-	-
Sheep	200	18(9.0%)	11(5.5%)	2(1.0%)	3(1.5%)	2(1.0%)
Swine	162	11(6.8%)	7(4.3%)	1(0.6%)	2(1.2%)	1(0.6%)

$P < 0.05$ (Chi square)

TABLE II

SEROLOGICAL EXAMINATION OF CATTLE, GOATS AND SHEEP FOR TOXOPLASMOSIS BY THE INDIRECT FLUORESCENT ANTIBODY TEST AND OF SWINE BY THE SF DYE TEST

ANIMAL SPECIES	NO. TESTED	NO. POSITIVE	RECIPROCAL TITRES OF IFA OR SF DYE TEST					
			16	32	64	128	256	512
Cattle	200	6(3.0%)	-	2(1.0%)	3(1.5%)	1(0.5%)	-	-
Goat	200	8(4.0%)	-	3(1.5%)	3(1.5%)	2(1.0%)	-	-
Sheep	200	19(9.5%)	-	7(3.5%)	8(4.0%)	3(1.5%)	-	1(0.5%)
*Swine	*162	13(8.0%)	5(3.1%)	-	5(3.1%)	-	1(0.6%)	2(1.2%)

* TESTED BY THE SF DYE-TEST

$P < 0.05$ (Chi square)

TABLE III

RESULTS OF SEROLOGICAL TESTS ON 200 CATTLE GROUPED ACCORDING TO AGE

TEST	AGE (YRS)	NO. TESTED	NO. POSITIVE	RECIPROCAL TITRES				
				32	64	128	256	512
IHA	2½-3	32	1(3.0%)	ND	1(3.0%)	-	-	-
	3-4	27	0(0.0%)	ND	-	-	-	-
	4-5	69	2(2.9%)	ND	1(1.5%)	1(1.5%)	-	-
	>5	72	3(4.1%)	ND	2(2.8%)	1(1.4%)	-	-
IFA	2½-3	32	1(3.0%)	1(3.0%)	-	-	-	-
	3.4	27	0(0.0%)	-	-	-	-	-
	4-5	69	4(5.8%)	1(1.5%)	3(4.4%)	-	-	-
	>5	72	1(1.4%)	-	-	1(1.4%)	-	-

ND = NOT DONE

P $\angle$ 0.05 (Chi square)

TABLE IV

RESULTS OF SEROLOGICAL TESTS ON 200 GOATS GROUPED ACCORDING TO AGE

TEST	AGE	NO. TESTED	NO. POSITIVE	RECIPROCAL TITRES				
				32	64	128	256	512
IHA	9-12 MONTHS	41	0(0.0%)	ND	-	-	-	-
	$1\frac{1}{2}$-2 YEARS	91	4(4.4%)	ND	2(2.2%)	2(2.2%)	-	-
	>2 YEARS	68	5(7.4%)	ND	4(5.9%)	1(1.5%)	-	-
IFA	9-12 MONTHS	41	0(0.0%)	-	-	-	-	-
	$1\frac{1}{2}$-2 YEARS	91	7(7.7%)	2(2.2%)	3(3.3%)	2(2.2%)	-	-
	>2 YEARS	68	1(1.5%)	1(1.5%)	-	-	-	-

ND = NOT DONE

P $\angle$0.05 (Chi square)

TABLE V

RESULTS OF SEROLOGICAL TESTS ON 200 SHEEP GROUPED ACCORDING TO AGE

TEST	AGE	NO. TESTED	NO. POSITIVE	RECIPROCAL TITRES				
				32	64	128	256	512
IHA	9-12 MONTHS	39	2(5.1%)	ND	1(2.6%)	-	1(2.6%)	-
	1½-2 YEARS	82	5(6.1%)	ND	4(4.9%)	1(1.2%)	-	-
	>2 YEARS	79	11(13.9%)	ND	6(7.6%)	1(1.3%)	2(2.5%)	2(2.5%)
IFA	9-12 MONTHS	39	3(7.7%)	1(2.6%)	-	1(2.6%)	1/2.6%)	-
	1½-2 YEARS	82	9(11.0%)	6(7.3%)	1(1.2%)	1(1.2%)	-	1(1.2%)
	>2 YEARS	79	7(8.9%)	4(5.1%)	1(1.3%)	1(1.3%)	1(1.3%)	-

ND = NOT DONE

P $\angle$ 0.05 (Chi square)

TABLE VI

RESULTS OF SEROLOGICAL TESTS ON 162 SWINE GROUPED ACCORDING TO AGE

TEST	AGE	NO. TESTED	NO. POSITIVE	RECIPROCAL TITRES					
				16	32	64	128	256	512
IHA	6-7 MONTHS	46	1(2.2%)	ND	ND	1(2.2%)	-	-	-
	1-2 YEARS	62	7(11.3%)	ND	ND	4(6.5%)	1(1.6%)	1(1.6%)	1(1.6%)
	3-4 YEARS	54	3(5.6%)	ND	ND	2(3.7%)	-	1(1.9%)	-
SF DYE TEST	6-7 MONTHS	46	2(4.4%)	1(2.2%)	ND	1(2.2%)	-	-	-
	1-2 YEARS	62	10(16.1%)	3(4.8%)	ND	4(6.5%)	-	1(1.6%)	2(3.2%)
	3-4 YEARS	54	1(1.9%)	1(1.9%)	ND	-	-	-	-

ND = NOT DONE

P $\angle$ 0.05 (Chi square)

TABLE VII

A COMPARISON OF SOME BREEDS OF CATTLE, GOATS, SHEEP AND SWINE IN THEIR EXPOSURE TO TOXOPLASMOSIS AS ASSESSED BY THE IHA, IFA AND SF DYE TESTS

ANIMAL SPECIES	TOTAL NO TESTED	BREED & NO. TESTED	NUMBER POSITIVE		
			IHA	IFA	DYE TEST
CATTLE	200	S.G. - 80	4(5.0%)	3(3.8%)	ND
		W.F - 120	2(1.7%)	3(2.5%)	ND
GOAT	200	K.R - 109	2(1.8%)	4(3.7%)	ND
		MIXED - 91	7(7.7%)	4(4.4%)	ND
SHEEP	200	MIXED - 98	8(8.2%)	8(8.2%)	ND
		LOCAL - 102	10(9.8%)	11(10.8%)	ND
SWINE	162	L.W x L.R -46	1(2.2%)	ND	2(4.4%)
		LOCAL - 116	10(8.6%)	ND	11(9.5%)

S.G = SOKOTO GUDALI; W.F. = WHITE FULANI;

L.W = LARGE WHITE; L.R = LAND RACE

K.R = KANO RED ; N.D = NOT DONE

$P < 0.05$ (Chi square)

TABLE VIII

A COMPARISON OF MALE AND FEMALE GENDERS OF CATTLE, GOATS, SHEEP AND SWINE IN THEIR EXPOSURE TO TOXOPLASMOSIS AS ASSESSED BY IHA, IFA AND SF DYE TESTS

ANIMAL SPECIES	NO. SAMPLED	SEXES	NUMBER POSITIVE		
			IHA	IFA	DYE TEST
CATTLE	200	MALES 140	4(2.9%)	4(2.9%)	N.D
		FEMALES 60	2(3.3%)	2(3.3%)	N.D
GOATS	200	MALES 28	2(7.1%)	3(10.7%)	N.D
		FEMALES 172	7(4.1%)	5(2.9%)	N.D.
SHEEP	200	MALES 48	8(16.7%)	6(12.5%)	N.D.
		FEMALES 152	10(6.6%)	13(8.6%)	N.D
SWINE	162	MALES 80	7(8.8%)	N.D	9(11.3%)
		FEMALES 82	4(4.9%)	N.D	4(4.9%)

N.D = NOT DONE

$P < 0.05$ (Chi square)

TABLE IX

COMPARISON OF SOME CATTLE, GOATS, SHEEP AND SWINE FROM KANO AND KADUNA STATES OF NIGERIA IN THEIR EXPOSURE TO TOXOPLASMOSIS AS ASSESSED BY THE INDIRECT HAEMAGGLUTINATION, INDIRECT FLUORESCENT ANTIBODY OR SF DYE TESTS

	KADUNA STATE			KANO STATE		
ANIMAL SPECIES	NO. TESTED	NO. POSITIVE IHA	NO. POSITIVE IFA	NO. TESTED	NO. POSITIVE IHA	NO. POSITIVE IFA
CATTLE	100	4(4.0%)	4(4.0%)	100	2(2.0%)	2(2.0%)
GOAT	100	4(4.0%)	4(4.0%)	100	5(5.0%)	4(4.0%)
SHEEP	100	11(11.0%)	12(12.0%)	100	7(7.0%)	7(7.0%)
SWINE	92	8(8.7%)	10*(10.0%)	70	3(4.3%)	3*(4.3%)
TOTAL	392	27(6.9%)	30(7.7%)	370	17(4.6%)	16(4.3%)

* TESTED BY THE SF DYE TEST

P $\angle$ 0.05 (Chi square

TABLE X

RESULT OF ISOLATION ATTEMPTS OF TOXOPLASMA GONDII FROM SEROLOGICALLY POSITIVE CATTLE, GOATS, SHEEP AND SWINE

ANIMAL SPECIES	IHA TITRE	IFA TITRE	DYE TEST TITRE	NO. POSITIVE FOR TOXOPLASMA
CATTLE	64(4)	32(2)	N.D*	0
GOAT	64(6)	32(3)	N.D*	0
SHEEP	64(11)	32(11)	N.D*	0
SWINE	64(7)	N.D*	16(5)	0
CATTLE	128(2)	64(3)	N.D*	0
GOAT	128(3)	64(3)	N.D*	0
SHEEP	128(2)	64(2)	N.D*	0
SWINE	128(1)	N.D*	64(5)	0
CATTLE	256(-)	128(1)	N.D*	0
GOAT	256(-)	128(-)	N.D*	0
SHEEP	256(3)	128(3)	N.D*	0
SWINE	256(2)	N.D*	256(1)	0
CATTLE	512(-)	256(-)	N.D*	0
GOAT	512(-)	256(-)	N.D*	0
SHEEP	512(2)	256(2)	N.D*	0
SWINE	512(1)	N.D*	512(2)	0
CATTLE	-	512(-)	N.D*	0
GOAT	-	512(-)	N.D*	0
SHEEP	-	512(1)	N.D*	0
SWINE	-	N.D*	-	0

N.D* = NOT DONE.

NB: NO. OF SPECIMENS EXAMINED IN BRACKETS

CHAPTER V

DISCUSSION

Although in Nigeria, toxoplasmosis has been reported in man (Olurin et al., 1972) and buffaloes (Ikede et al., 1978) and there is serological evidence in goats (Falade, 1978), there has been no other reports on the prevalence of the disease in food animals. Accordingly, precise and current information on the presence of the parasite in animals commonly slaughtered for food is particularly important since consumption of raw or undercooked infected meat has been linked with acquisition of T. gondii.

The present investigation showed that antibodies to T. gondii are present in some of the animals slaughtered for meat. Since the presence of antibodies is associated with persistence of infection (Hartley and Moyle, 1974), it can be assumed that the seropositive animals are, in fact, infected and can be considered as a potential source of infection. Attempts to isolate the organism in mice however, failed. This failure may be due to a number of factors, including low antibody

titre. Work (1967) had considerable difficulty in isolating T. gondii from the tissues of cattle, sheep and swine and showed that the probability of isolating T. gondii from an animal apparently increased with increase in antibody titre. In other words, animals with low antibody titre are not likely to yield positive results in mice isolation. Another possibility is that the animals surveyed were able to resist infection or were capable of eliminating the organism which became established (Levine, 1961, Munday and Corbould, 1979). The source of infection to the food animals surveyed is incompletely understood due to the complex epidemiology of the disease. However, it can be speculated that contamination of feed with oocysts from cats and cat family may be the source of infection.

The generally low percentage of seropositive animals contrasts with the finding of Guillo and Desmonts (1960) in France which showed that over 70% of sheep and about 30% of swine were serologically positive for T. gondii and also by the work of Waldeland (1977) in Norway who found a seropositivity of about 42-50% by the SF dye test in mature ewes. This, again may reflect the differences in the epidemiology of the disease and the husbandry practices.

The low percentage of goats carrying antibodies to T. gondii obtained in this study is in consonance with the results of Falade (1978).

From this study, it is evident that only a small percentage of animals slaughtered for meat carry antibodies to T. gondii and suggest that acquisition of infection is not widespread. While this may be the case in the northern part of the country where the dry season is very harsh and may not allow the oocysts to survive long enough to be ingested, the situation may be different in the more southernly part of the country where the weather is warm, moist and humid. Although oocysts are fairly resistant to environmental circumstances, Frenkel (1973) showed that they could be inactivated by high temperature and dryness, the same conditions that are inimical to the oocysts of Eimeria species. On the other hand, warm and moist environmental conditions have been shown to be favourable to the persistence and infectivity of oocysts. Thus, a comprehensive sero-epidemiological survey seems indicated in the southern part of the country.

It is known in human population that the percentage of seropositive individuals increases with age.

Work (1967) examined sheep within the age groups 7 months to 1 year and found no difference in the number of seropositives. It should be noted, however, that very little difference can be expected between animals within this limited age group. The present study shows a trend similar to that observed in human population in cattle, sheep, goats and swine surveyed (Tables III-VI). This may be due to the fact that the older the animals the better are the chances that they would be exposed to infection. Also, the older the animals, the more prone are they to a continuous exposure to T. gondii.

It appears also that based on sexes, there was no significant difference in the proportion of seropositive animals $P < 0.05$ (chi square). This finding is in agreement with that of Garcia, Ruppanner and Behymer, (1979) who found no difference in the sexes using swine. This may not be surprising since the epidemiology of the disease does not appear to put any of the sexes at a disadvantage as far as acquisition of infection is concerned.

It can be concluded based on the present study that slaughter animals in northern Nigeria present minimal public health hazard as far as T. gondii is concerned.

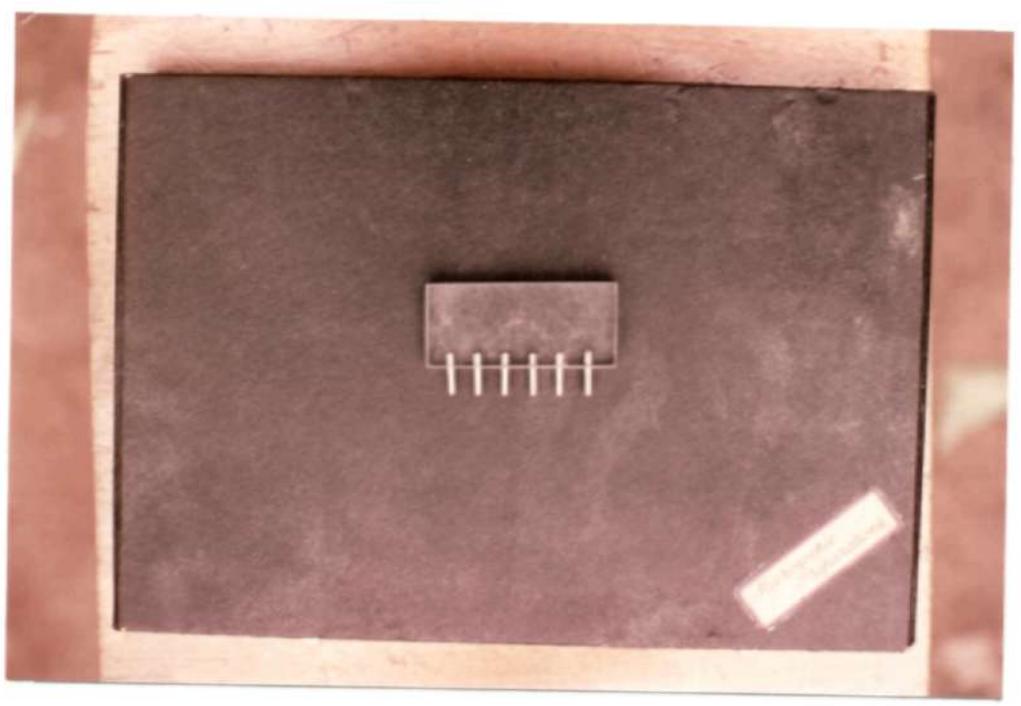

PLATE I: Template used in introducing sera serial dilutions on to teflon-coated slides for IFA tests.

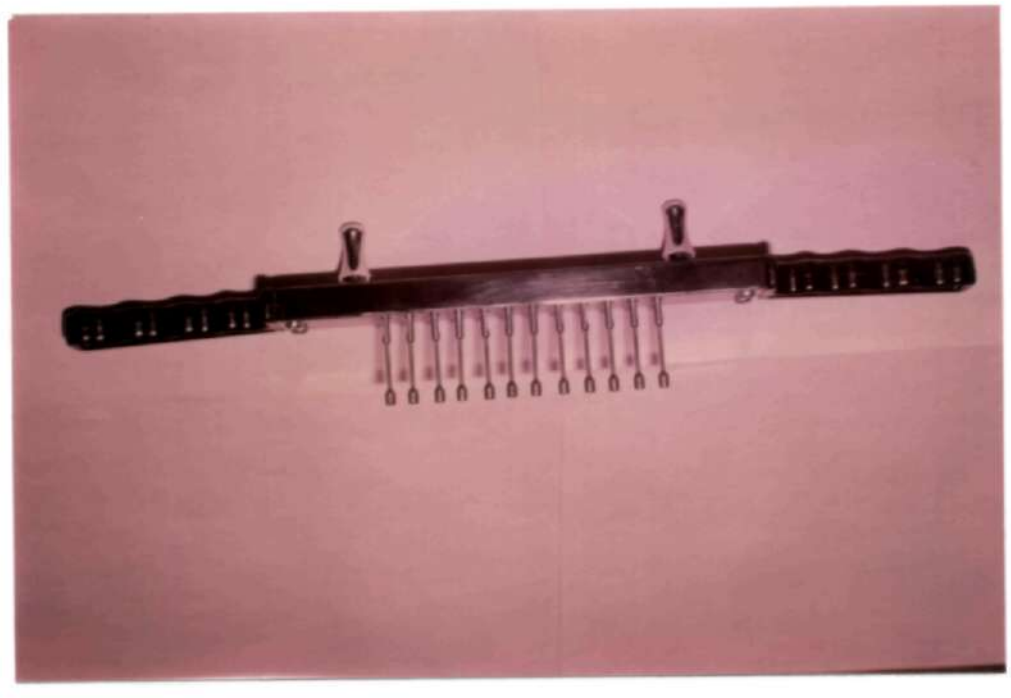

PLATE II: 0.025 ml microdiluters fixed into the handle for manual operation.

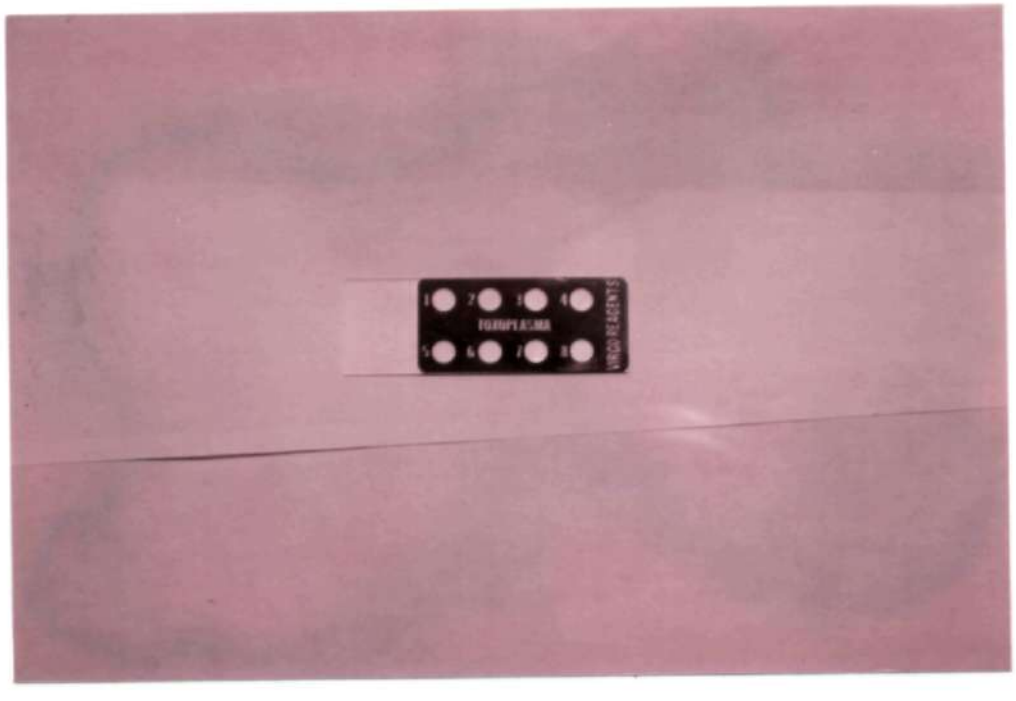

PLATE III: A specially coated slide for Toxoplasma serology by the fluorescent microscopy technique.

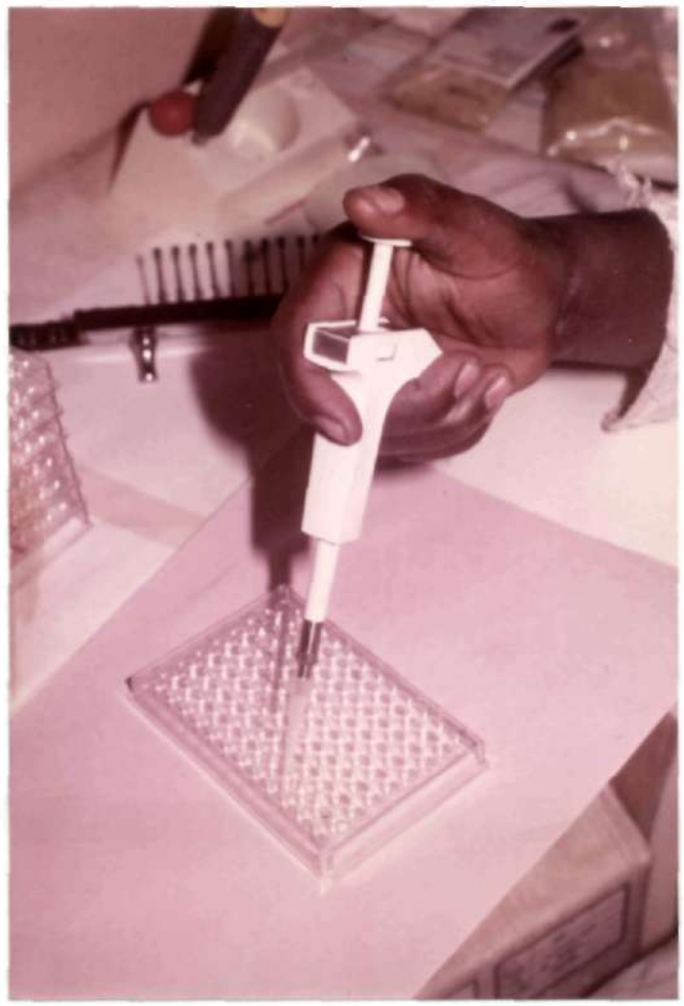

PLATE IV: A 0.025 ml pipette in use during a serum serial dilution in a microtitration plate.

PLATE V: 0.025 ml microdiluter in use for sera serial dilutions in a microtitration plate.

PLATE VI: A microtitration plate placed on a magnifying viewer to observe the results of an IHA test.

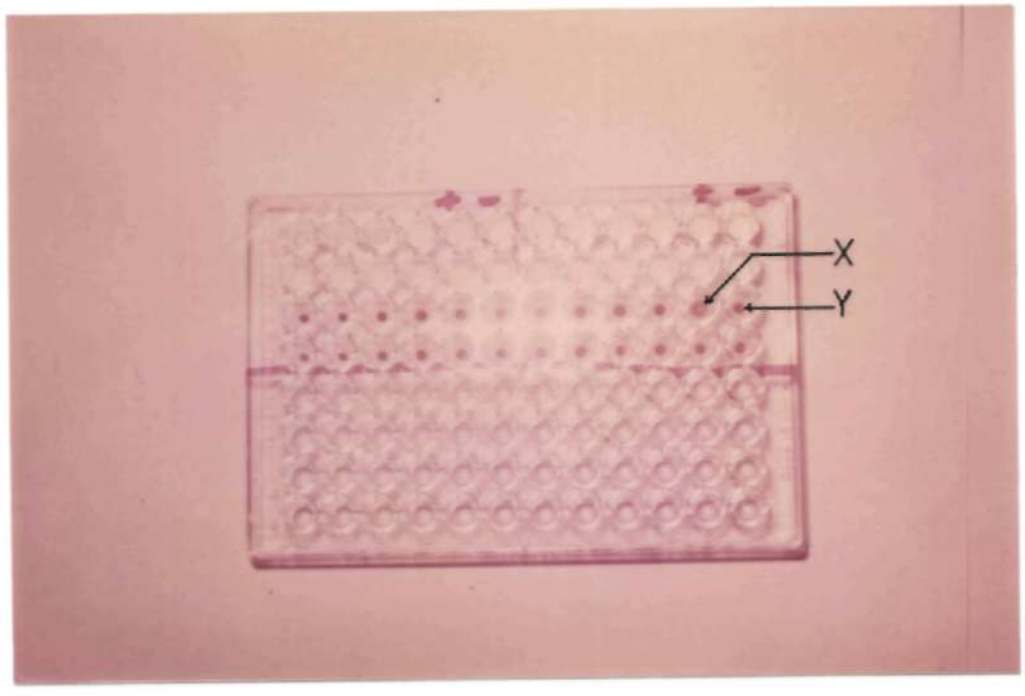

PLATE VII: A microtitration plate showing results of some sera along with the negative control (marked Y) and positive control (marked X) by the IHA test.

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