DEVELOPMENT OF A MICROFLUOROMETRIC IMMUNOASSAY FOR DETECTION OF RECENT EXPOSURES TO *TOXOPLASMA GONDII* IN SHEEP

By

TAMARA J. GEORGE

Bachelor of Science

Oklahoma State University

Stillwater, Oklahoma

1982

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
MASTER OF SCIENCE
May, 1985

DEVELOPMENT OF A MICROFLUOROMETRIC IMMUNOASSAY FOR DETECTION OF RECENT EXPOSURES TO *TOXOPLASMA GONDII* IN SHEEP

Thesis Approved:

Joseph Carl Fox
Thesis Adviser

[illegible]

Jane C. Wright

Norman N. Durham
Dean of Graduate College

ACKNOWLEDGMENTS

I wish to express my appreciation to my research adviser, Dr. J. C. Fox, for his help throughout the study and his suggestions during the preparation of the manuscript. Appreciation is extended to my committee: Dr. J. C. Wright, for his help with the statistical analysis of the serological results; and both he and Dr. S. A. Ewing, for their assistance in the preparation of the final manuscript. Appreciation is also extended to Dr. A. W. Confer for his generous assistance with the histopathological examinations and the use of his laboratory personnel. A note of thanks is given to the Oklahoma Agricultural Experiment Station, Oklahoma State University, for partially funding the investigation.

I would like to thank Mr. and Mrs. Perry Stout for their hospitality during my studies. My appreciation is extended to Robert Norris and Ben Haning for their assistance with the experimental animals. To Dr. Kimberlee McKenzie, I wish to express my thanks for her assistance, and her encouragement, confidence, and friendship. Special thanks is given to Judy Kreps for her invaluable assistance and friendship, without which this project could not have been completed.

To my husband, Wade George, I wish to express my love and gratitude for his encouragement, confidence, and sacrifices throughout my master's program.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Toxoplasma gondii is an intracellular protozoan parasite of the subphylum Apicomplexa. Felids are the only known definitive hosts, but a wide range of intermediate hosts are affected. Intermediate hosts include birds and mammals (Dubey, 1977). Many infections with *Toxoplasma* are asymptomatic in both the definitive and intermediate hosts; however, a host may die as a result of necrosis of the intestine and mesenteric lymph nodes before other organs are severely affected (Dubey, 1977). Toxoplasmosis in human beings may be relatively harmless, or it may result in encephalitis, retinochoroiditis, abortion, or mental retardation (Sabin, 1942; Jacobs, 1967; Dubey, 1977). Common symptoms of acute infections in human beings include lymphadenopathy, fever, headache, muscle pain, anemia, and, occasionally, respiratory complications (Frenkel, 1971; Dubey, 1977). As the host's resistance increases, proliferation of the parasite is depressed and tissue cysts are formed resulting in a chronic infection. The lesions resulting from infections with *Toxoplasma* are influenced by strain virulence, host species susceptibility, individual susceptibility, age, and acquired immunity (Dubey, 1977; Tadros and Laarman, 1982).

In addition to serving as a source of infection for human beings who may ingest undercooked infected meat (Dubey, 1977), infections with *Toxoplasma* in sheep may result in considerable economic losses as a

result of aborted and stillborn lambs (Hartley and Marshall, 1957; Beverley and Watson, 1961; Beverley and Mackay, 1962; Hartley and Moyle, 1968; Beverley and Watson, 1971). Congenital infections occur when sheep become initially infected during gestation; and the severity of the disease in lambs is influenced by the stage of gestation at the time of infection and the size of the infecting dose (Hartley, 1961; Jacobs and Hartley, 1964). Serological diagnosis of toxoplasmosis is important as a result of nonspecific clinical signs which mimic several other infectious diseases (Dubey, 1977). Serological tests include the Sabin-Feldman dye test, the indirect fluorescent antibody test, the indirect haemagglutination test, and the complement-fixation test (Dubey, 1977). In the past several years more emphasis has been placed on the solid-phase immunoassays ELISA (enzyme-linked immunosorbent assay) and FIAX™ (fluorescent immunoassay), which can detect class-specific immunoglobulins. The objective of the present investigation was to develop a quantitative fluorescent immunoassay (FIAX) to detect recent exposure to Toxoplasma gondii in sheep by evaluation of parasite-specific antibody levels.

Review of Literature

Taxonomy of Toxoplasma gondii

Levine (1973a) classified Toxoplasma gondii as follows:

Phylum PROTOZOA: Unicellular; eucaryotic organisms.

Subphylum APICOMPLEXA Levine, 1970: Apical complex present at some stage; micropore(s) generally present; cilia and flagella absent, except for flagellated microgametes in some; cysts often present; all species parasitic.

Class SPOROZOASIDA Leuckart, 1879: Reproduction generally sexual and asexual; oocysts present; flagellated microgametes in some; monoxenous or heteroxenous.

Subclass COCCIDIASINA Leuckart, 1879: Mature gamonts small, intracellular; endodyogeny present or absent; mostly in vertebrates, few in invertebrates.

Order EUCOCCIDIORIDA Léger and Duboscq, 1910: Merogony present.

Suborder EIMERIORINA Léger, 1911: Macrogamete and microgametocyte develop independently; syzygy absent; sporozoites enclosed within a sporocyst; endodyogeny present or absent; monoxenous or heteroxenous.

Family SARCOCYSTIDAE Poche, 1913: Syzygy absent; endodyogeny present; cysts or pseudocysts containing zoites; in veterbrates; heteroxenous.

Subfamily TOXOPLASMATINAE Biocca, 1956: Cysts and pseudocysts present; meronts (schizonts) and gamonts in intestinal cells; facultatively heteroxenous.

Genus Toxoplasma Nicolle and Manceaux, 1908: Cysts in brain not septate; sporogony outside the host; oocysts with two sporocysts, each with four sporozoites.

At present there is general agreement that Toxoplasma gondii belongs in the suborder Eimeriorina. Controversy arises, however, in the classification of family and subfamily. Levine (1973a) places Toxoplasma in the subfamily Toxoplasmatinae, of the family Sarcocystidae. Tadros and Laarman (1982) prefer to synonymize Toxoplasma with the genus Isospora Schneider, 1881, which places it in the family Eimeriidae.

History

Toxoplasma gondii was discovered independently in 1908 by Nicolle and Manceaux in a rodent Ctenodactylus gundi and by Splendore in a laboratory rabbit (Dubey, 1977). Congenital infection in human beings was reported in 1937 by Wolf and Cowen and characterized by Sabin in 1942. Weinman and Chandler (1954) suggested that transmission might occur through the ingestion of undercooked meat and several investigators provided evidence to support the hypothesis (Jacobs et al., 1960a; Wallace et al., 1972).

Transmission studies continued when congenital transmission and carnivorism could not explain widespread infections with Toxoplasma gondii in vegetarians and herbivores. Hutchison (1965) associated Toxoplasma infectivity with feline feces and in 1967 hypothesized that Toxoplasma was transmitted through the eggs of the nematode Toxocara cati. The opinion was discarded as studies by several investigators resulted in the discovery of Toxoplasma oocysts in feline feces (Hutchison et al., 1969, 1970, 1971; Frenkel et al., 1970; Dubey et al., 1970a, 1970b; Sheffield and Melton, 1970; Overdulve, 1970; Weiland and Kuhn, 1970; Witte and Piekarski, 1970; Zaman and Colley, 1970). A life cycle for Toxoplasma which linked the three known methods of transmission--congenital, carnivorism, and fecal--was then proposed by Dubey et al. (1970b) and Frenkel et al. (1970).

Structure and Life Cycle

Transmission of Toxoplasma infections may occur by ingestion of sporulated oocysts from feline feces, carnivorism of infected animals, or congenital transfer of the parasite (see Figure 1). The three infective

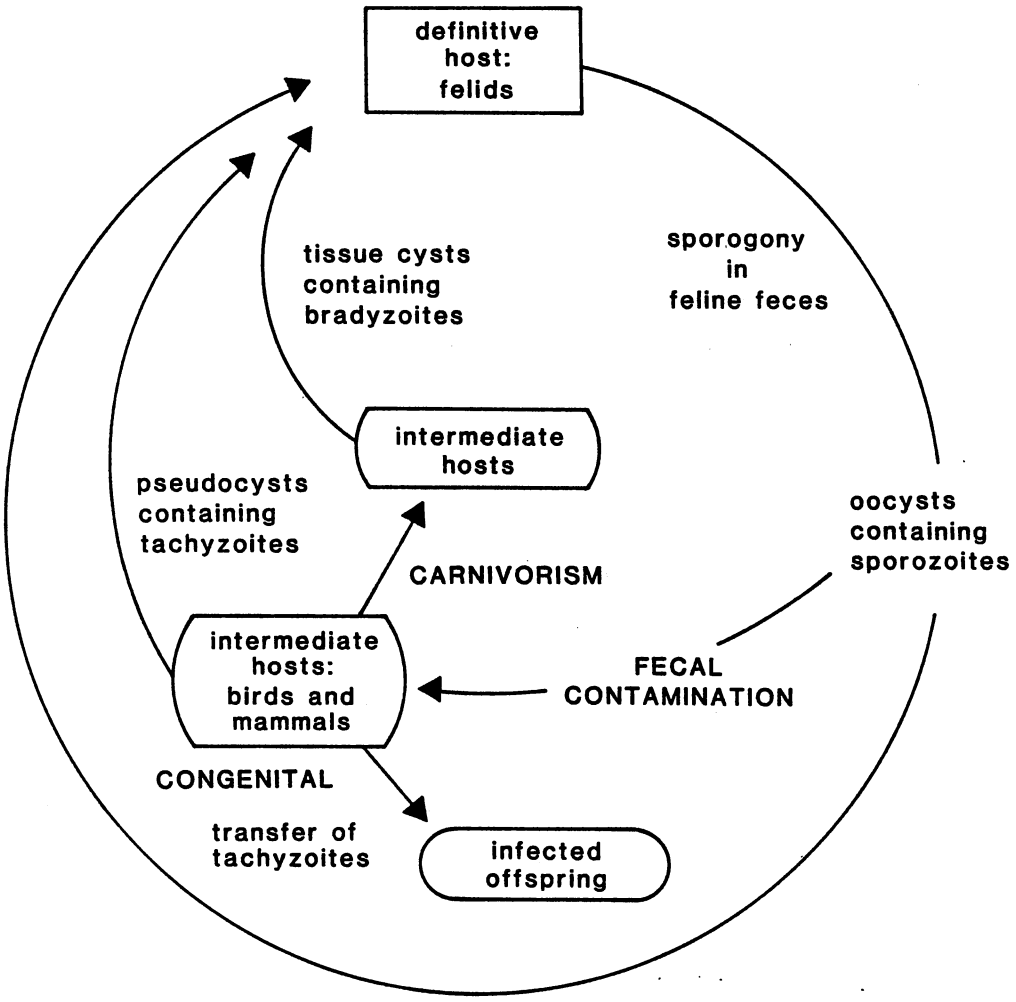

Figure 1. Life Cycle of Toxoplasma gondii Showing the Three Known Methods of Transmission: Fecal, Carnivorism, and Congenital

stages of Toxoplasma are the sporozoites contained within the resistant oocyst, the rapidly multiplying tachyzoites contained within pseudocysts, and the more slowly multiplying bradyzoites contained within tissue cysts. Ingestion of one of the infective stages by the definitive felid host initiates an "enteroepithelial" cycle, which includes both asexual and sexual reproduction of the parasite, and an "extraintestinal" cycle, which consists of asexual reproduction. Ingestion of an infective stage by an intermediate host initiates only the extraintestinal cycle.

After ingestion by the definitive host, the released parasites enter the epithelial cells of the villi of the small intestine and begin to reproduce asexually by endodyogeny. Dubey and Frenkel (1972) have characterized five morphologically distinct types of reproductive forms which develop and apparently multiply by endodyogeny, endopolyogeny, and schizogony before gametogony begins. Female gametocytes mature into macrogametes and the nuclei of male gametocytes divide to produce microgametes. After a motile microgamete penetrates a macrogamete, oocyst wall formation occurs. The cycle is completed with the release of mature oocysts from the ruptured host cells.

Oocyst sporulation occurs outside the felid host within 1 to 21 days depending upon aeration and temperature (Dubey et al., 1970b). The sporulated oocyst contains two sporocysts and each sporocyst contains four sporozoites. Oocysts are subspherical and measure 11 by 13 μm; the sporozoites are 2 by 8 μm (Siim et al., 1963). The tachyzoite has a pellicle, polar ring, conoid, rhoptries, micronemes, mitochondria, subpellicular microtubules, endoplasmic reticulum, Golgi apparatus, ribosomes, micropore, and a well-defined nucleus (Sheffield and Melton,

1968). Tachyzoites are produced by endodyogeny in the extraintestinal cycle. The extraintestinal cycle is initiated simultaneously with the enteroepithelial cycle in the feline definitive host. In the intermediate host only the extraintestinal cycle occurs. Multiplication of tachyzoites occurs in a variety of cell types including fibroblasts, hepatocytes, reticular cells, and myocardial cells (Soulsby, 1982). A group of tachyzoites within a parasitophorous vacuole in a host cell is called a pseudocyst.

As an infection progresses, tachyzoites are replaced by the more slowly multiplying bradyzoites. Bradyzoites differ slightly from tachyzoites in that they contain several glycogen granules and are enclosed within an elastic, argyrophilic cyst wall (Dubey, 1977). The tissue cyst develops within the host cell and becomes larger as the bradyzoites within divide by endodyogeny. Tissue cysts may be as large as 100 μm in diameter and enclose hundreds of organisms (Dubey, 1977).

Tissue cysts are more numerous in the chronic stage of infection after the host has acquired some degree of resistance (Dubey, 1977). The level of resistance does not eradicate the infection but seems to be associated with the presence of living Toxoplasma organisms (premunition). Occasionally, reactivation of chronic disease may occur following rupture of a cyst. The released bradyzoites are probably destroyed by the host's immune response, but there may be formation of new tissue cysts (Frenkel, 1971). The rupture of a cyst in immunosuppressed patients may result in transformation of bradyzoites into tachyzoites, and the host may die from toxoplasmosis unless treated (Frenkel, 1971; Frenkel et al., 1975).

Disease in Sheep

In addition to serving as a source of infection for human beings who may ingest undercooked meat (Dubey, 1977), abortion "storms" which occur in sheep as a result of infections with Toxoplasma gondii are known to be of considerable economic importance in New Zealand and Great Britain (Hartley and Marshall, 1957; Beverley and Watson, 1961; Beverley and Mackay, 1962; Hartley and Moyle, 1968; Beverley and Watson, 1971). Congenital infection occurs when sheep become initially infected during gestation. As seen in Table I, the severity of the disease in lambs is influenced by the stage of gestation at the time of infection. The size of the infecting dose may also influence the severity of the disease (Jacobs and Hartley, 1964). Ewes infected in one pregnancy usually lamb normally in subsequent pregnancies and will also resist re-infection upon subsequent exposure to Toxoplasma (Beverley and Watson, 1971; Watson and Beverley, 1971; Munday, 1972; Blewett et al., 1982).

As cited by Levine (1973b), toxoplasmosis was first reported from sheep in New York, United States of America (U.S.A.) by Olafson and Monlux (1942). It was later found in Australia by Wickham and Carne (1950), in Ohio by Cole et al. (1954), in New Zealand by Hartley and Marshall (1957), and in England by Beverley and Watson (1959). Some animals exhibit no signs of disease (Garnham and Lainson, 1960; Hartley, 1961), while others may show only a brief fever (Jacobs, 1961). Frequently, however, clinical signs may include fever, dyspnea, anorexia, incoordination, opisthotonus, abortion, and stillbirth (Cole et al., 1954; Hartley and Marshall, 1957; Koestner and Cole, 1961).

As seen in Table II, the prevalence of Toxoplasma-specific antibodies reported in sheep ranges from 4% of 699 market lambs in

TABLE I

EFFECTS OF INITIAL INFECTIONS WITH *TOXOPLASMA GONDII* IN EWES AT DEFINED STAGES OF GESTATION

Stage of Gestation at Infection	Percentage Lambs Lost	Number of Ewes Infected	Reference
Early Pregnancy (less than 50 days)	20%	10	Hartley, 1961
	42%	45	Jacobs and Hartley, 1964
Mid-Pregnancy (70 to 90 days)	32%	25	Jacobs and Hartley, 1964
	54%	12	Smith, 1965
	60%	20	Watson and Beverley, 1971
	67%	15	Beverley and Watson, 1961
	71%	7	Hartley, 1961
	85%	13	Beverley et al., 1971
	86%	10	Miller et al., 1982
Late Pregnancy (greater than 100 days)	0%	5	Watson and Beverley, 1971
	22%	15	Blewett et al., 1982
	36%	10	Miller et al., 1982
	69%	10	Smith, 1961

TABLE II

SURVEYS FOR PREVALENCE OF TOXOPLASMA-SPECIFIC ANTIBODIES IN SHEEP FROM BREEDING (br) OR MARKET LAMB (mk) FLOCKS

Location	Prevalence	Number Tested	Test Method	Reference
Australia (br)	30%	239	SFDT	Hartley and Moyle, 1968
Bulgaria (br)	31%	698	IHA	Arnaudov et al., 1977
Colombia (br)	58%	1655	IHA	Perry et al., 1978
Czechoslovakia (mk)	11%	89	SFDT	Prosek and Hejlicek, 1980b
Czechoslovakia	33%	493	IHA	Arnaudov et al., 1977
Czechoslovakia (mk)	40%	280	SFDT	Prosek and Hejlicek, 1980a
Egypt	68%	169	SFDT	Fahmy et al., 1979
England (mk)	21%	100	SFDT	Rawal, 1959
England (br)	31%	77	SFDT	Beverley & Mackay, 1962
England (br)	69%	498	SFDT	Beverley & Watson, 1959
India	9%	514	IHA	Gupta et al., 1981
India	34%	202	IHA	Srivastava et al., 1983
Nigeria (mk)	9%	200	IFA,IHA	Aganga et al., 1981
Nigeria (br)	18%	300	IHA	Okoh et al., 1981
Onterior (br, mk)	65%	273	SFDT	Tizard et al., 1978
Romania	31%	502	IHA	Sharma, 1980
Tasmania (mk)	62%	144	IFA	Munday, 1975
United States of America				
Arizona	5%	66	SFDT	Feldman & Miller, 1956
California (mk)	4%	699	IHA	Riemann et al., 1977
California (br)	13%	405	IHA	Franti et al., 1975
California (br)	15%	1048	IHA	Franti et al., 1976
California (br)	24%	2164	IHA	Riemann et al., 1977
California (mk)	28%	68	IHA	Vanderwagen et al., 1974
Idaho (mk)	8%	147	IHA	Riemann et al., 1977
Idaho (br)	21%	250	IHA	Huffman et al., 1981
Kentucky	56%	9	SFDT	Feldman & Miller, 1956
Maryland (mk)	9%	86	SFDT	Jacobs et al., 1960b
Nevada (mk)	20%	159	IHA	Riemann et al., 1977
Oregon (mk)	22%	51	IHA	Riemann et al., 1977

IFA: Indirect Fluorescent Antibody

IHA: Indirect Hemagglutination

SFDT: Sabin-Feldman Dye Test

California, U.S.A. (Riemann et al., 1977) to 69% of 498 ewes in Yorkshire, England breeding flocks (Beverley and Watson, 1959) indicating widespread exposure of sheep to Toxoplasma gondii. Prevalance rates in sheep in the United States range from 4% (Riemann et al., 1977) to 56% (Feldman and Miller, 1956) indicating that toxoplasmosis may also be an important disease of sheep in this country.

The Research Problem

Toxoplasmosis in sheep is seldom diagnosed in Oklahoma. Judging from studies of experimental infections (Table I) and surveys for Toxoplasma-specific antibodies in sheep in the U.S.A. (Table II), infections with Toxoplasma gondii in sheep could represent a significant disease problem. Indications that infections with Toxoplasma may be common in Oklahoma was provided by a limited preliminary survey for Toxoplasma antibodies in domestic cats. Thirty-eight percent of 144 cats tested positive by the fluorescent immunoassay FIAX™ for Toxoplasma-specific antibodies (George, unpublished data). As seen in Table III, these results were consistent with surveys of other feline populations in the U.S.A.

The proposed research was to develop a FIAX method to detect recent exposures to Toxoplasma gondii in sheep. The FIAX test has been evaluated for detecting Toxoplasma-specific antibodies in human beings by Walls and Barnhart, 1978; Hyde et al., 1980; and Gordon et al., 1981; and is routinely used to detect recent exposures to Toxoplasma gondii in human beings by the Centers for Disease Control (Walls, personal communication). Serum samples are screened for Toxoplasma-specific immunoglobulin G (IgG) and those with positive responses tested for

TABLE III

SURVEYS FOR PREVALENCE OF TOXOPLASMA-SPECIFIC ANTIBODIES IN DOMICILED (d) OR STRAY (s) CATS IN THE UNITED STATES OF AMERICA

Location	Prevalence	Number Tested	Test Method	Reference
California (d, s)	15%	115	IFA, IHA	Behymer et al., 1973
California (d)	20%	86	IHA	Riemann et al., 1978
California	25%	32	SFDT	Soave, 1968
Hawaii (s)	20%	522	SFDT	Wallace, 1971
Iowa-Missouri (s)	58%	157	SFDT	Dubey, 1973
Massachusetts (s)	34%	44	SFDT	Feldman and Miller, 1956
Missouri (d)	38%	128	SFDT	Dubey, 1973
New York (s)	31%	35	SFDT	Feldman and Miller, 1956
South Carolina (s)	28%	35	SFDT	Jones et al., 1958
Tennessee (s)	64%	140	SFDT	Jones et al., 1958
Washington (d, s)	31%	87	SFDT	Ladiges et al., 1982

IFA: Indirect Fluorescent Antibody

IHA: Indirect Hemagglutination

SFDT: Sabin-Feldman Dye Test

Toxoplasma-specific immunoglobulin M (IgM). The demonstration of IgM levels by the indirect fluorescent antibody test has been used in diagnosing toxoplasmosis in humans (Remington et al., 1968), and has been investigated for use in sheep using the indirect haemagglutination test (Blewett et al., 1983). The possibility of a similar approach using FIAX to compare IgM:IgG ratios to detect recent exposures to Toxoplasma in sheep was studied. The objectives for the present investigation were as follows:

1. To follow the antibody responses of gestating ewes experimentally exposed to Toxoplasma gondii using the FIAX™ quantitative fluorescent immunoassay.

2. To determine if the evaluation of levels of parasite-specific antibody classes (IgM and IgG) can be used to detect recent exposures to Toxoplasma gondii in sheep.

CHAPTER II

MATERIALS AND METHODS

Source of Infection

To obtain Toxoplasma oocysts to be used as infective material, three domestic cats were exposed to a Toxoplasma isolate from a Saiga antelope from the Oklahoma City Zoo. Tachyzoites of the Saiga antelope isolate which had been passaged intraperitoneally in mice 19 times and stored at -70 C in 10% glycerin/0.85% saline were inoculated into the peritoneal cavity of laboratory mice. At 14 days post-inoculation (PI), the mice were "boostered" with a second dose of tachyzoites in an attempt to increase the number of tissue cysts. At 51 days PI, the mice were euthanitized and squash mount preparations made of each brain to check for tissue cysts. Several mice, thought to be positive for tissue cysts containing Toxoplasma organisms, were skinned and two carcasses were fed to each of the three cats.

The cats were approximately 9-12 months of age, and had been previously checked for active infections with coccidian parasites and tested for Toxoplasma-specific IgG levels using the FIAX test. Two cats served as nonexposed controls. Fecal samples were monitored daily by coverslip flotation with saturated sodium nitrate (specific gravity 1.4). Blood samples were collected twice weekly for serological testing. The cats were housed in concrete cages with paper cage liners and clay litter in metal pans. The cage liners and clay litter were autoclaved and disposed

of daily and the cages cleaned with a solution of 10% Povidone (Veratex Corp., Troy, MI), 0.5% potassium iodide, and 70% ethyl alcohol. The metal pans were autoclaved and cleaned every other day. The cats were exposed a second time to Toxoplasma tachyzoites, in a similar manner, 27 days later.

As an alternative, Dr. J. P. Dubey of the United States Department of Agriculture Animal Parasitology Institute, Beltsville, Maryland, provided oocysts of a Toxoplasma gondii isolate from sheep. The oocysts had been collected from a domestic cat infected with Toxoplasma gondii strain TS-2. The strain TS-2 Toxoplasma oocysts were ultimately used as the source of infection for the sheep.

Exposure of Experimental Animals

Five bred Ramboillet and Ramboillet-cross ewes approximately five years old were purchased from a local producer. The ewes were screened for Toxoplasma-specific antibodies using FIAX serology and then tested for pregnancy using an ultrasonic pregnancy tester (Pregnosticator Deluxe Electronic Pregnancy Tester, Animark Inc., Aurora, CO). At approximately 60 days gestation, three ewes were exposed to 200,000 Toxoplasma gondii (strain TS-2) oocysts. The remaining two ewes served as nonexposed controls.

To expose the ewes to the Toxoplasma oocysts, two ml volumes of inoculum containing approximately 200,000 oocysts were prepared in disposable syringes. The ewes were allowed to obtain a mouthful of grain, the oocysts were expressed at the back of the mouth, and then the ewes were allowed to continue chewing and to swallow the grain. This procedure was an attempt to mimick the normal method of acquisition.

Maintenance of Experimental Animals

The exposed animals were housed in a concrete enclosure for seven days following exposure to the Toxoplasma oocysts. The enclosure was cleaned daily as a precaution against any oocysts that might have passed through the digestive tract of the sheep without excysting. To clean the enclosure, waste material was removed for special disposal; the concrete walls and floor were sprayed with a solution of 10% Povidone (Veratex Corp., Troy, MI), 0.5% potassium iodide, and 70% ethyl alcohol; and hosed down after allowing the solution to stand a minimum of 15 minutes. After seven days, the exposed animals were moved to an outdoor pen with the nonexposed, control animals. The outdoor pen consisted of a wire-enclosed, dirt-floored, 20 x 45 foot area surrounding a small shed with straw bedding for protection against the weather. The sheep were maintained on prairie grass hay supplemented with Baby Beef Ration (Stillwater Mill, Stillwater, OK) throughout the experiment.

Diagnostic Procedures

Collection of Blood Serum Samples

Blood samples were collected from the sheep three times weekly during weeks 1-2 post exposure (PE) and twice weekly during weeks 3-4 PE. Throughout the remainder of the investigation, samples were collected weekly. Serum was recovered from the blood samples and stored with 0.1% sodium azide at 4 C and -20 C.

Serological Examinations

Serological responses in the ewes were measured with the FIAX™ fluorescent immunoassay system (International Diagnostic Technology, San Jose, CA). The FIAX test system is a solid-phase microfluorometric immunoassay which relies on fluorescein isothiocyanate (FITC)-conjugated antisera to detect bound antibody. An advantage of this system over others in common use is that the conjugated-antisera can be made to recognize only specified antibody classes. The system also allows testing of a large number of samples with relative ease.

Equipment and Supplies. Equipment and supplies used in the FIAX test system included a microdiluter, shaking apparatus, FIAX 100™ Fluorometer (International Diagnostic Technology, San Jose, CA), and Texas Instruments Programmable 58C Calculator (Texas Instruments Inc., Lubbock, TX). The StiQ™ samplers--surface technique for immunoquantitation (International Diagnostic Technology, San Jose, CA)--consisted of cellulose-acetate-nitrate disks attached to plastic handles. FITC-conjugated rabbit antisera specific for sheep immunoglobulin G (IgG) or immunoglobulin M (IgM) (Cooper Biomedical, Malvern, PA) was used to detect bound antibody. Secondary supplies included a micropipettor, disposable glass 12 x 75 mm test tubes, and tube racks.

Antigen Preparation. The test antigen was prepared from *Toxoplasma gondii* (strain TS-2) tachyzoites. After testing several antigen preparation techniques (Dempster, 1984), the procedures of Walls and Barnhart (1978), Carlier et al. (1980), Naot et al. (1981), and Dempster (1984) were combined and modified. Tachyzoites were inoculated into the peritoneal cavity of laboratory mice and allowed to proliferate. At three

days post inoculation, the organisms were harvested by peritoneal lavage of each mouse with five ml sterile 0.85% saline containing 10,000 units penicillin and 25 mg streptomycin. The peritoneal washings were combined and stored overnight at 4 C.

The combined peritoneal washings were centrifuged at 2,500 g for 20 minutes, and the sediment homogenized with a Brinkmann polytron (Brinkmann Instruments, Houston, TX) to loosen the clumps of parasites and host cells. After three alternate 20-minute washings with 15 ml sterile saline and centrifugation at 2,500 g, the sediment was resuspended and sonicated with a Biosonik IV sonicator (VWR Scientific, Irving, TX) at the maximum setting. The solution was kept on ice and sonicated for two minutes, with a 30-second rest, and a final one-minute sonication.

After sonication, the solution was centrifuged at 35,000 g in a Sorvall Superspeed RC2-B refrigerated centrifuge (DuPont Col., Newtown, CT) for one hour at 4 C. The sediment was resuspended in phosphate buffered saline and centrifuged again at 35,000 g for one hour. All supernatants and the sediment were assayed for protein concentration with a Bio-Rad Protein Assay (Bio-Rad Laboratories, Richmond, CA). The sediment and the final wash supernatant were combined and used as a particulate antigen for the FIAX test. The antigen was stored in aliquots at -70 C until used.

FIAX Test. FIAX test conditions were determined for optimum performance and reproducibility by cross titration of known positive antisera and antigen. Conjugate concentration was also varied to find the optimum proportion for the test. The optimum antigen concentration was determined, the volumes adjusted, and 25 µl applied to the front surface

of the StiQ samplers. The StiQs were allowed to dry overnight at room temperature and then stored at 4 C until used. Phosphate buffered saline (PBS) at pH 7.3 with 0.15% Tween 20 was used for the serum dilutions, washes, and conjugate dilutions. Serum samples, which had been collected from the experimental animals and stored with 0.1% sodium azide at -20 C, were diluted 1:100 for the test. The StiQs were incubated in 1.0 ml of the diluted serum samples for 30 minutes and then washed in 0.6 ml of 0.15% Tween 20/PBS for 10 minutes. The StiQs were transferred into 0.5 ml of a 1:200 dilution of either FITC-conjugated rabbit antisheep IgG or FITC-conjugated rabbit antisheep IgM and incubated for 20 minutes. After a final wash, the StiQs were inserted into the FIAX 100™ Fluorometer to determine the fluorescent signal units (FSUs) from bound conjugate.

Negative, transition level, low-positive, and high-positive serum samples were chosen to serve as controls. FIAX values were assigned to these samples based on their pregain fluorescent signals and were used to calculate a standard curve by plotting the FSU values of the control sera against their assigned FIAX values using a log-log data transformation. Values for the remaining samples were then extrapolated from the standard curve using regression analysis. The resulting FIAX values were used as an indirect measurement of the antibody levels in the Toxoplasma-exposed and control ewes.

Statistical Analysis. The antibody responses, as represented by FIAX values, were statistically analyzed by paired t-testing and regression analysis. The analysis was performed by the Statistical Analysis System (SAS Institute, Inc., 1982) using the university's IBM 3021 Mainframe Computer. Differences between mean values of exposed and nonexposed ewes were tested at the 0.05 level of significance.

Monitoring of Clinical Signs

The experimental animals were monitored daily for rectal temperatures for two weeks post exposure (PE). Temperatures were monitored once or twice weekly during weeks 3-5 PE to establish baseline values.

Histopathological Examinations

The experimental animals were euthanitized at 121 days PE and samples of the following tissues were taken for histopathological examination: brain, heart, lung, prescapular lymph node, adrenal gland, spleen, liver, and mesenteric lymph node. Samples were collected from the three Toxoplasma-exposed ewes; one nonexposed, control ewe; one aborted lamb; and the three healthy lambs. Histopathological sections were prepared by staff members of the Department of Veterinary Pathology at Oklahoma State University, Stillwater, OK. The tissues samples were cut into 2.5 cm square pieces, fixed in 10% neutral-buffered formalin, thin-sectioned, and stained with hematoxylin and eosin (H&E) or periodic acid-Schiff (PAS) hematoxylin stain. The sections were examined microscopically for the presence of Toxoplasma organisms.

Mouse Inoculations

Isolation of the parasite by mouse inoculations was attempted using foetal fluid from an aborted lamb of one of the Toxoplasma-exposed ewes, and samples of brain tissues taken from the experimental animals at necropsy. The samples of brain tissue were minced with scissors in sterile 0.85% saline (1 part tissue, 9 parts saline) and expelled through a syringe and 14 gauge needle several times to make a homogenous suspension. Approximately 1.5 ml of the suspension was inoculated

intraperitoneally into laboratory mice. Peritoneal washings were subpassaged several times in mice and impression smears made from the visceral surfaces of the mice. The impression smears were stained with Diff-Quik (American Scientific Products, McGraw Park, IL) and viewed microscopically for the presence of Toxoplasma tachyzoites.

CHAPTER III

RESULTS

Production of Oocysts in Cats

Although slight increases in Toxoplasma-specific antibody levels were detected in the three Toxoplasma-exposed cats using FIAX serology, no oocysts were shed within 27 days after ingestion of tissue cysts or within 42 days after ingestion of tachyzoites. The two nonexposed, control cats remained negative, by both serological and fecal examinations.

Exposure of Sheep to Toxoplasma gondii

Serological Examination

A functional FIAX test for the indirect measurement of Toxoplasma-specific immunoglobulin G (IgG) levels was developed to measure serological responses in the experimental animals. Attempts to measure Toxoplasma-specific immunoglobulin M (IgM) levels in sheep were unsatisfactory as a result of interference from non-specific binding.

Serum samples from the experimental animals were tested for Toxoplasma-specific IgG and the resulting FIAX values statistically analyzed by paired t-testing and regression analysis. Increases in antibody titers provided evidence that infections with Toxoplasma occurred in the exposed sheep. Regression lines for the Toxoplasma-exposed

group versus the nonexposed, control group are shown in Figure 2. Plots of the standardized residuals showed a curvilinear relationship for the Toxoplasma-exposed group and a linear relationship for the nonexposed controls for the variables time and FIAX value.

Figure 2 shows the mean IgG class antibody response, as represented by FIAX values, for the exposed group versus the nonexposed, control group. Figure 3 shows the responses for the individual animals. As seen in Figure 2, the mean IgG level (FIAX value) for the ewes exposed to Toxoplasma oocysts began to increase between 9 and 11 days post exposure (PE) and was significantly higher than the mean IgG level for the nonexposed, control ewes by 14 days PE. The peak antibody response occurred at 88 days PE. Although one of the control ewes showed a considerable variation in baseline FIAX values (see Figure 3), the mean antibody level for the control animals did not change significantly during the course of the study (Figure 2).

Abortion occurred in one of the Toxoplasma-exposed ewes at 9 days PE. As seen in Figure 3, the antibody level in this ewe continued to increase after the abortion. The second exposed ewe delivered a healthy lamb at 66 days PE, but the third exposed ewe had a stillborn lamb at 83 days PE. Both nonexposed, control ewes bore healthy lambs on days 77 and 90 PE. The lambs were tested for the presence of Toxoplasma-specific antibodies and the results are shown in Figure 4. The lamb of the Toxoplasma-exposed ewe exhibited a high level of Toxoplasma-specific IgG, and the two control lambs exhibited low levels indicative of negative responses.

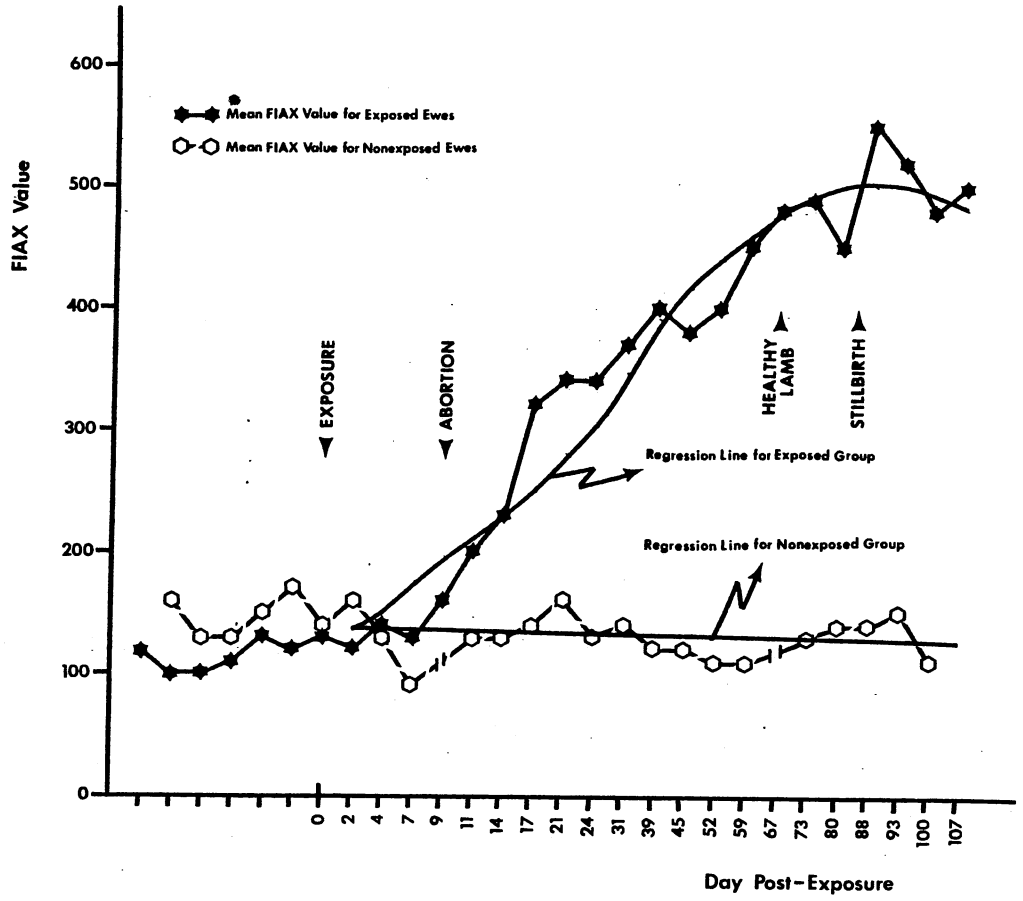

Figure 2. Toxoplasma-Specific Immunoglobulin G Levels, as Represented by Mean FIAX Values, in Sheep Experimentally Exposed to 200,000 Toxoplasma gondii Oocysts

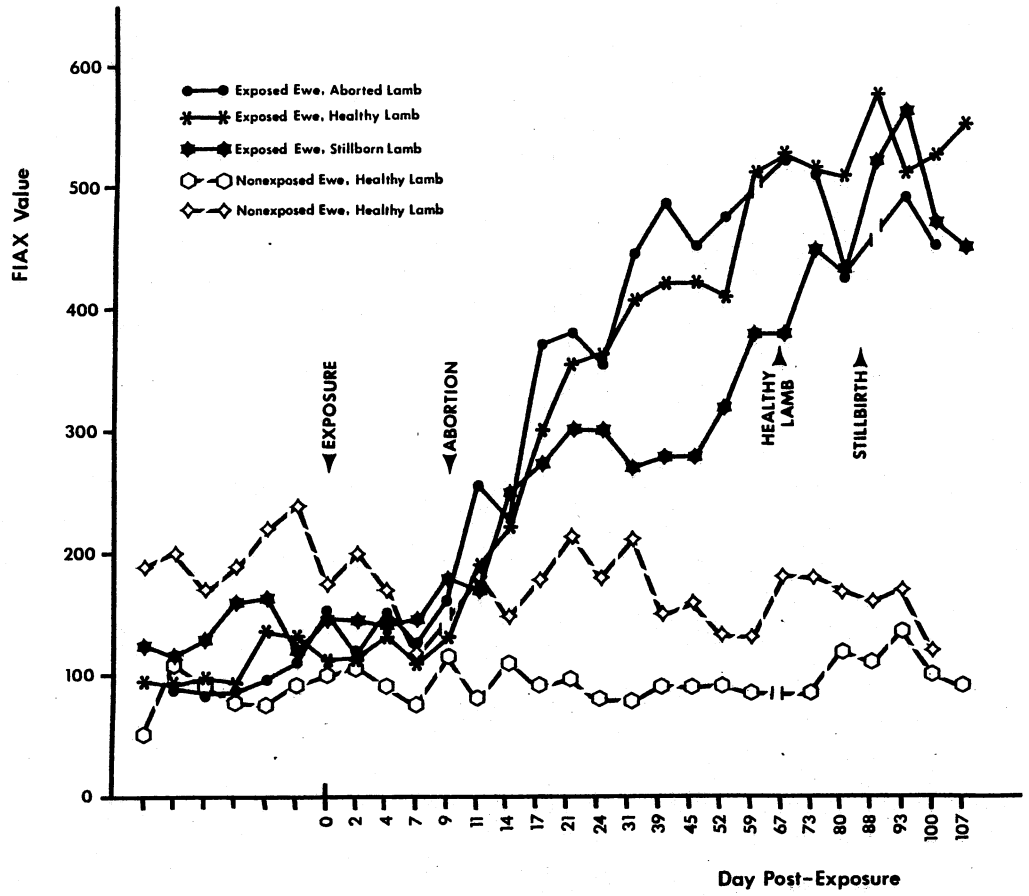

Figure 3. Individual Toxoplasma-Specific Immunoglobulin G Levels, as Represented by FIAX Values, in Sheep Experimentally Exposed to 200,000 Toxoplasma gondii Oocysts

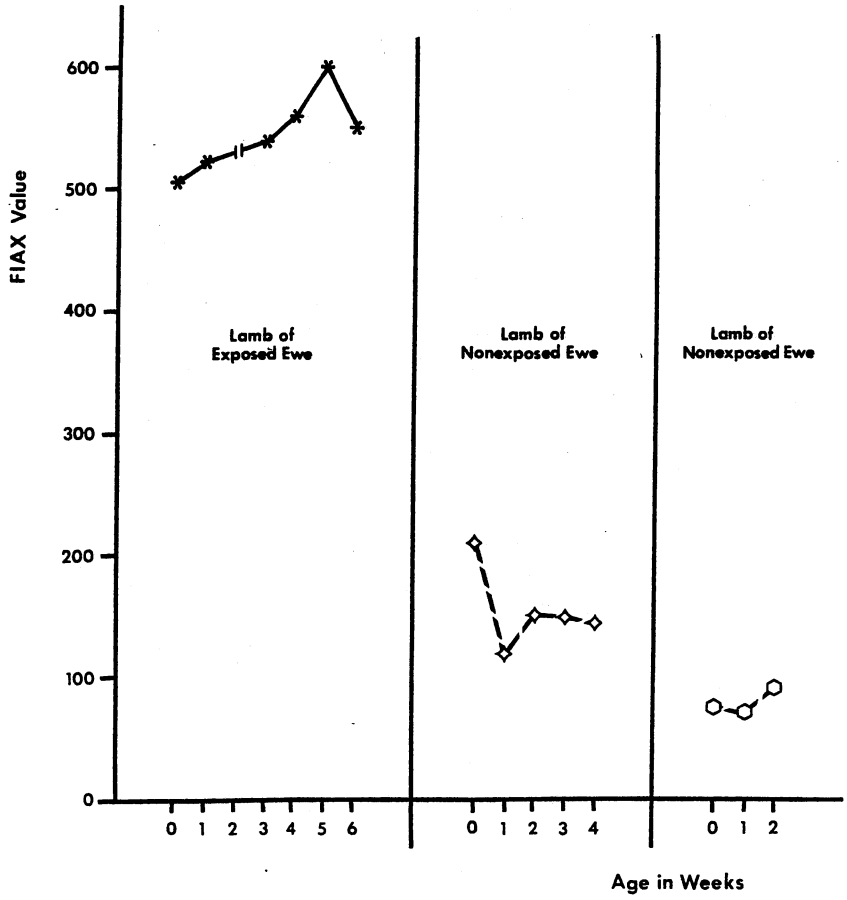

Figure 4. Levels of Toxoplasma-Specific Immunoglobulin G, as Represented by FIAX Values, in Lambs of Toxoplasma-Exposed and Nonexposed Sheep

Clinical Signs

Two of the three (67%) *Toxoplasma*-exposed sheep aborted. One of the ewes aborted at 9 days PE and the other had a stillborn lamb at 83 days PE. The aborting ewe showed signs of ataxia and temporary lameness which appeared on the day of the abortion and continued for five days. The ewe retained some placental membranes for several days and was treated with Combiotic™ (Pfizer Inc., New York, NY) to prevent secondary bacterial infection. The remaining two ewes exhibited no signs other than fever and anorexia during the first week post exposure.

The parasite was not revealed in tissues of the aborted lamb by histopathological examination or mouse inoculations. The stillborn lamb appeared to have developed normally. No gross developmental defects were observed. Tissues from the stillborn lamb were too decomposed for examination. Congenital transmission of *Toxoplasma* in the healthy lamb was confirmed by isolation of the parasite from brain tissue by mouse inoculations.

All three exposed sheep exhibited fevers which began between 2 and 4 days PE. Figure 5 shows the mean rectal temperatures for the exposed and nonexposed ewes. Temperatures in the exposed ewes rose significantly between 2 and 4 days PE, peaked at day 7 PE, and then quickly dropped back to baseline levels. The mean temperatures for the control animals did not change significantly during the course of the study.

Histopathological Examinations

Histopathological examination of the tissue samples collected from the experimental animals at 121 days PE did not reveal *Toxoplasma* organisms. Tissue samples were previously taken from one control ewe which

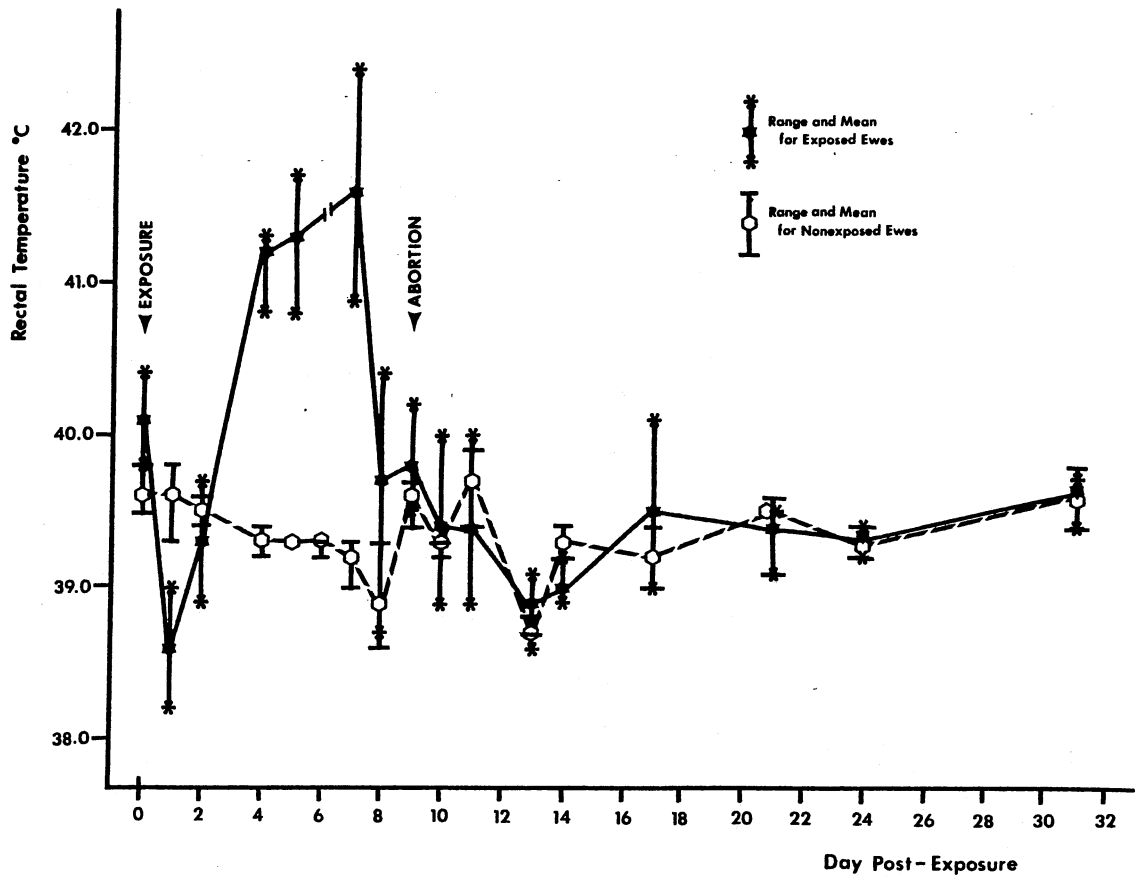

Figure 5. Rectal Temperatures of Sheep Experimentally Exposed to 200,000 Toxoplasma gondii Oocysts

had died of bloat at 105 days PE. Parasites were not observed in either the H&E or PAS-hematoxylin stained sections. Tissues from the stillborn lamb were so decomposed that examination was not attempted.

Mouse Inoculations

Toxoplasma tachyzoites were observed in impression smears of visceral surfaces of mice inoculated with brain tissue from the healthy lamb of a *Toxoplasma*-exposed ewe. Parasites were not observed in mice inoculated with brain tissue from the exposed ewes or the nonexposed controls. Attempts to isolate the parasite from fetal fluid of the aborted lamb were unsuccessful. Tissues from the stillborn lamb had frozen and were so decomposed that isolation of the parasite was not attempted.

CHAPTER IV

DISCUSSION

Production of Oocysts in Cats

Attempts to produce Toxoplasma oocysts in cats were unsuccessful. Although slight increases in antibody levels were detected, cats did not shed oocysts after ingestion of mice inoculated intraperitoneally with tachyzoites of the Saiga antelope isolate. Prepatent periods, in cats, of 3-10 days after ingestion of tissue cysts have been reported by Dubey and Frenkel (1976) and Overdulve (1978). Dubey and Frenkel (1976) reported a prepatent period of 19 days before oocysts were shed by cats after ingestion of tachyzoites. The Saiga antelope isolate of Toxoplasma had been passaged 19 times in laboratory mice possibly resulting in modification of the parasite's ability to produce oocysts in the feline definitive host. Such a phenomenon was previously reported by Dubey and Frenkel (1973).

Exposure of Sheep to Toxoplasma gondii

The effects of exposure to Toxoplasma gondii found in the present investigation were consistent with those of other experimental studies (Table I). Infection of ewes with Toxoplasma early in pregnancy (less than 50 days gestation) commonly results in death of the fetus and resorption, leaving apparently barren ewes (Hartley, 1961; Jacobs, 1961; Jacobs and Hartley, 1964). Disease with abortion, stillbirth, or

neonatal death typically occurs in ewes infected between 70 and 90 days gestation (Watson and Beverley, 1971; Beverley et al., 1971; Miller et al., 1982). Ewes infected during late pregnancy (greater than 110 days gestation) usually lamb normally, but the lambs may be congenitally infected (Jacobs and Hartley, 1964; Watson and Beverley, 1971; Blewett et al., 1982; Miller et al., 1982). In the present investigation, exposure to Toxoplasma in the ewes resulted in one aborted lamb, one stillborn lamb, and one congenitally infected lamb. The birthing date of the surviving lamb indicates that the ewe may have been further into gestation at the time of exposure.

Clinical signs, comparable to those reported by other investigators (Cole et al., 1954; Hartley and Marshall, 1957; Jacobs, 1961; Koestner and Cole, 1961), were observed in the infected animals. A brief period of anorexia and fever was exhibited by the exposed ewes, and the aborting ewe exhibited ataxia and temporary lameness in one limb. The exposed lamb appeared to be healthy.

The inconclusive findings from histopathological examination of tissues from the experimental animals is inconsistent with reports from other studies. Dubey and Sharma (1980) reported patent infection of numerous tissues for at least 64 days post-infection, and isolated the parasite from brain, heart, diaphragm, skeletal muscle, and intestine from animals up to 118 days post infection. The small number of sections viewed may explain the negative findings. The failure of attempts to isolate Toxoplasma from brain tissue of the exposed ewes by mouse inoculations seems to indicate that few parasites were present in the brain tissue.

The antibody responses to Toxoplama measured with FIAX serology

were similar to those reported by other investigators. Miller et al. (1982) reported a rapid rise in indirect haemagglutination (IHA) titers in infected sheep between 10 and 20 days post infection. Hunter et al. (1982) found a significant rise in dye test titers by 28 days post infection. Waldeland (1977) and Blewett et al. (1983) observed that antibody titers in infected sheep may remain high for over two years post infection. The rapid rise and persistence of high titers indicates that serological diagnosis of abortion and stillbirth in sheep as a result of infections with Toxoplasma may depend upon the detection of Toxoplasma-specific IgM.

The demonstration of IgM levels has been used in diagnosing toxoplasmosis in human beings (Remington et al., 1968), and has been investigated for use in sheep. Blewett et al. (1983) reported that IgM levels detected by IHA were greater than IgG levels during the first month post infection. Hunter et al. (1982) used the indirect fluorescent antibody (IFA) test to demonstrate IgM class antibodies in ovine fetal fluid. In the present investigation, measurement of Toxoplasma-specific IgM levels with the FIAX system was unsatisfactory as a result of interference from nonspecific binding. Unfortunately, identification of the interfering substance(s) was not within the scope of the present investigation. Perhaps purification of the antigen using immunoelectrophoretic studies or fractionation of the serum would result in better performance of the IgM test.

The high level of Toxoplasma-specific IgG exhibited by the infected lamb was probably a result of passive transfer of maternal antibody in the colostrum; however, since very little decrease in response occurred within six weeks after birth, part of the antibody may have been

replaced through a response by the lamb itself. Levels of maternally-derived antibodies usually decrease to minimal levels after approximately three months (Waldeland, 1977). Although the presence of Toxoplasma-specific antibodies in lambs during this time period may be of little significance, the absence of specific antibody may be of value in eliminating toxoplasmosis as the cause of neonatal death. Increases in antibody titers after three months of age may indicate congenital transmission of the parasite (Blewett et al., 1982).

The FIAX system proved to be a reliable test for detecting IgG responses to Toxoplasma in sheep. A disadvantage of the system was the need for the FIAX 100™ Fluorometer. Advantages of the test included the capacity of the system to detect class-specific immunoglobulins and the relative ease of testing large numbers of serum samples. Increases in IgG levels could be detected relatively early in infection. At present, serological diagnosis of toxoplasmosis in sheep using FIAX serology would be best accomplished by testing paired serum samples collected within three months post exposure. The FIAX test also may be used to survey flocks for the prevalence of Toxoplasma-specific antibodies.

Conclusions

Infections with Toxoplasma gondii in the experimentally exposed sheep were indicated by seroconversion, and congenital transmission confirmed by isolation of the parasite from brain tissue by mouse inoculations. As a result of the infection, two of three (67%) infected sheep aborted. Although quantitative measurement of Toxoplasma-specific IgM levels was unsatisfactory as a result of nonspecific binding, the FIAX system proved to be a reliable test for detecting IgG responses to

Toxoplasma in sheep. Large numbers of serum samples may be screened with relative ease to determine the prevalence of Toxoplasma-specific antibodies in flocks. The FIAX test may be used to detect recent exposures to Toxoplasma gondii in sheep by testing paired serum samples, collected within three months post exposure, for increases in Toxoplasma-specific IgG levels.

LITERATURE CITED

Aganga, A. O., Belino, E. D., Adegboye, D. S., and Ilemobade, A. A. 1981. A serological survey of toxoplasmosis in food animals (cattle, sheep, goats, and swine) in two northern states of Nigeria. Int. J. Zoon. 8:57-62.

Arnaudov, D., Kozoed, V., Ira, I., Zitova, D., and Shtourach, L. 1977. Immunological and epizootiological studies on toxoplasmosis in sheep. Veterinarano-Meditsinski Nauki. 14(4):31-39.

Behymer, R. D., Harlow D. R., Behymer, D. E., and Franti, C. E. 1973. Serologic diagnosis of toxoplasmosis and prevalence of Toxoplasma gondii antibodies in selected feline, canine and human populations. JAVMA. 162(11):959-963.

Beverley, J. K. A., and Mackay, R. R. 1962. Ovine abortion and toxoplasmosis in the East Midlands. Vet Rec. 74(17):499-501.

Beverley, J. K. A., and Watson, W. A. 1959. Ovine abortion due to toxoplasmosis. Nature. 184:2041.

Beverley, J. K. A., and Watson, W. A. 1961. Ovine abortion and toxoplasmosis in Yorkshire. Vet Rec. 73:6-11.

Beverley, J. K. A., and Watson, W. A. 1971. Prevention of experimental and of naturally occurring ovine abortion due to toxoplasmosis. Vet Rec. 88:39-41.

Beverley, J. K. A., Archer, J. F., Watson, W. A., and Fawcett, A. R. 1971. Trial of a killed vaccine in the prevention of ovine abortion due to toxoplasmosis. Brit Vet J. 127:529-535.

Blewett, D. A., Bryson, C. E., and Miller, J. K. 1983. Studies of antibody titres in experimentally induced ovine toxoplasmosis. Res Vet Sci. 34:163-166.

Blewett, D. A., Miller, J. K., and Buxton, D. 1982. Response of immune and susceptible ewes to infection with Toxoplasma gondii. Vet Rec. 111(9):175-177.

Carlier, Y., Bout, D., Dessaint, J. P., Capron, A., Van Knapen, F., Ruitenberg, E. J., Bergquist, R., and Huldt, G. 1980. Evaluation of the enzyme-linked immunosorbent assay (ELISA) and other serological tests for the diagnosis of toxoplasmosis. Bulletin of the World Health Organization. 58(1):99-105.

Cole, C. R., Sanger, V. L., Farrell, R. L., and Kornder, J. D. 1954. The present status of toxoplasmosis in veterinary medicine. North Am Vet. 35:265-270.

Dempster, R. P. 1984. Toxoplasma gondii: purification of zoites from peritoneal exudates by eight methods. Exp Para. 57:195-207.

Dubey, J. P. 1973. Feline toxoplasmosis and coccidiosis: A survey of domiciled and stray cats. JAVMA. 162(10):873-877.

Dubey, J. P. 1977. Toxoplasma, Hammondia, Besnoitia, Sarcocystis, and other tissue cyst-forming coccidia of man and animals. In Parasitic Protozoa (Kreier, J. P., ed.). Academic Press, New York, NY. 3:101-237.

Dubey, J. P., and Frenkel, J. K. 1972. Cyst-induced toxoplasmosis in cats. J Protozool. 19:155-177.

Dubey, J. P., and Frenkel, J. K. 1973. Experimental Toxoplasma infection in mice with strains producing oocysts. J Para. 59(3):505-512.

Dubey, J. P., and Frenkel, J. K. 1976. Feline toxoplasmosis from acutely infected mice and the development of Toxoplasma cysts. J Protozool. 23:537-546.

Dubey, J. P., and Sharma, S. P. 1980. Parasitemia and tissue infection in sheep fed Toxoplasma gondii oocysts. J Para. 66(1):111-114.

Dubey, J. P., Miller, N. L., and Frenkel, J. K. 1970a. Characterization of the new fecal form of Toxoplasma gondii. J Para. 56:447-456.

Dubey, J. P., Miller, N. L., and Frenkel, J. K. 1970b. The Toxoplasma gondii oocyst from cat feces. J Exp Med. 132:636-662.

Fahmy, M. A. M., Arafa, M. S., Mandour, A. M., and Abdel Rahman, A. M. 1979. Toxoplasmosis in ruminants of Assiut Governorate, Upper Egypt. J Egyptian Vet Med Assoc. 39(2):119-126.

Feldman, H. A., and Miller, L. T. 1956. Serological study of toxoplasmosis prevalence. Am J Hyg. 64:320-335.

Franti, C. E., Connolly, G. E., Riemann, H. P., Behymer, D. E., Ruppanner, R., Willadsen, C. M., and Longhurst, W. 1975. A survey for Toxoplasma gondii antibodies in deer and other wildlife on a sheep range. JAVMA. 167(7):565-568.

Franti, C. E., Riemann, H. P., Behymer, D. E., Suther, D., Howarth, J.A., and Ruppanner, R. 1976. Prevalence of Toxoplasma gondii antibodies in wild and domestic animals in northern California. JAVMA. 169(9):901-906.

Frenkel, J. K. 1971. Toxoplasmosis. Mechanisms of infection, laboratory diagnosis and management. Current Topics Path. 54:28-75.

Frenkel, J. K., Dubey, J. P., and Miller, N. L. 1970. Toxoplasma gondii: Fecal stages identified as coccidian oocysts. Science. 167:893-896.

Frenkel, J. K., Nelson, B., and Arias-Stella, J. 1975. Immunosuppression and toxoplasmic encephalitis: clinical and experimental aspects. Human Path. 6:97-112.

Garnham, P. C. C., and Lainson, R. 1960. Sheep as a potential reservoir of Toxoplasma for man. Lancet. II:71-74.

George, T. J. 1982. Unpublished data.

Gordon, M. A., Duncan, R. A., and Kingsley, L. T. 1981. Automated immunofluorescence test for toxoplasmosis. J Clin Micro. 13:283-285.

Gupta, S. L., Gautam, O. P., and Bhardwaj, R. M. 1981. Note on toxoplasmosis: serological survey of antibodies in sheep of Hissar area by indirect haemagglutination test (microtitre system). Indian J Animal Sci. 51(3):381-382.

Hartley, W. J. 1961. Experimental transmission of toxoplasmosis in sheep. N Z Vet J. 9:1-6.

Hartley, W. J., and Marshall, S. C. 1957. Toxoplasmosis as a cause of ovine perinatal mortality. N Z Vet J. 5:119-124.

Hartley, W. J., and Moyle, G. 1968. Observations on an outbreak of ovine congenital toxoplasmosis. Aust Vet J. 44:105-107.

Huffman, E. M., Kirk, J. H., Winward, L., and Gorham, J. R. 1981. Relationship of neonatal mortality in lambs to serologic status of the ewe for Toxoplasma gondii. JAVMA. 178(7):679-682.

Hunter, D., Chadwick, P., Balfour, A. H., and Bridges, J. B. 1982. Examination of ovine foetal fluid for antibodies to Toxoplasma gondii by the dye test and an indirect immunofluorescence test specific for IgM. Brit Vet J. 138(1):29-34.

Hutchison, W. M. 1965. Experimental transmission of Toxoplasma gondii. Nature. 206:961-962.

Hutchison, W. M. 1967. The nematode transmission of Toxoplasma gondii. Trans Roy Soc Trop Med Hyg. 61:80-89.

Hutchison, W. M., Dunachie, J. F., Siim, J. C., and Work, K. 1969. Life cycle of Toxoplasma gondii. Brit Med J. 4:806.

Hutchison, W. M., Dunachie, J. F., Siim, J. C., and Work, K. 1970. coccidian-like nature of Toxoplasma gondii. Brit Med J. 1:142-144.

Hutchison, W. M., Dunachie, J. F., Work, K., and Siim, J. C. 1971. The life cycle of the coccidian parasite, Toxoplasma gondii, in the domestic cat. Trans Roy Soc Trop Med Hyg. 65:380-399.

Hyde, B., Burgett, N. W., and Maggio, E. T. 1980. A solid-phase fluoroimmunoassay for the quantitation of antibodies to Toxoplasma gondii. Clin Chim Acta. 103:393-398.

Jacobs, L. 1961. Toxoplasmosis in man and animals. N Z Vet J. 9:85-91.

Jacobs, L. 1967. Toxoplasma and toxoplasmosis. In Advances in Para (Dawes, B., ed.). Academic Press, New York, New York. 5:1-45.

Jacobs, L., and Hartley, W. J. 1964. Ovine toxoplasmosis: Studies on parasitaemia, tissue infection, and congenital transmission in ewes infected by various routes. Brit Vet J. 120:347-364.

Jacobs, L., Remington, J. S., and Melton, M. L. 1960a. The resistance of the encysted form of Toxoplasma gondii. J Para. 46:11-21.

Jacobs, L., Remington, J. S., and Melton, M. L. 1960b. A survey of meat samples from swine, cattle, and sheep for the presence of encysted Toxoplasma. J Para. 46:23-28.

Jones, F. E., Eyles, D. E., and Gibson, C. L. 1958. The prevalence of toxoplasmosis in the domestic cat. Am J Trop Med Hyg. 6:820-826.

Koestner, A., and Cole, C. R. 1961. Neuropathology of ovine and bovine toxoplasmosis. Am J Vet Res. 22:53-66.

Ladiges, W. C., DiGiacomo, R. F., and Yamaguchi, R. A. 1982. Prevalence of Toxoplasma gondii antibodies and oocysts in pound-source cats. JAVMA. 180(11):1334-1335.

Levine, N. D. 1973a. Introduction, history and taxonomy. In The Coccidia. (Hammond, D. M. and Long, P. L., eds.) Univ Park Press, Baltimore, Maryland. 1-22.

Levine, N. D. 1973b. Protozoan Parasites of Domestic Animals and of Man. 2nd ed. Burgess Publishing Co., Minneapolis, Minnesota. 294-307.

Miller, J. K., Blewett, D. A., and Buxton, D. 1982. Clinical and serological response of pregnant gimmers to experimentally induced toxoplasmosis. Vet Rec. 111 (6):124-126.

Munday, B. L. 1972. Transmission of Toxoplasma infection from chronically infected ewes to their lambs. Brit Vet J. 128:1xxi.

Munday, B. L. 1975. Prevalence of toxoplasmosis in Tasmanian meat animals. Aust Vet J. 51:315-316.

Naot, Y., Desmonts, G., and Remington, J. S. 1981. IgM enzyme-linked immunosorbent assay test for the diagnosis of congenital Toxoplasma infection. J Ped. 98(1):32-36.

Okoh, A. E. J., Agbonlahor, D. E., and Momoh, M. 1981. Toxoplasmosis in Nigeria - a serological survey. Trop Animal Health Prod. 13(3):137-140.

Olafson, P., and Monlux, W. S. 1942. Toxoplasma infection in animals. Cornell Vet. 32:176-190.

Overdulve, J. P. 1970. The identity of Toxoplasma Nicolle and Manceaux, 1909 with Isospora Schneider, 1881. I. Proc Kon Ned Akad Wetensch Ser C. Biol Med Sci. 73:129-151.

Overdulve, J. P. 1978. Excretion of Toxoplasma gondii by non-immunized and immunized cats: its role in the epidemiology of toxoplasmosis. Proc Kon Ned Akad van Wetensch Ser C. Biol Med Sci. 81(1):1-18.

Perry, B. D., Grieve, A. S., Mogollon, J. D., and deGalvis, A. L. H. 1978. Serological study of ovine toxoplasmosis in Columbia: prevalence of haemagglutinating antibodies to Toxoplasma in sheep. Vet Rec. 103:584-585.

Prosek, F., and Hejlicek, K. 1980a. Detection of antibodies against toxoplasmosis in slaughter animals from private farms. Veterinarstvi. 30(7):321-322.

Prosek, F., and Hejlicek, K. 1980b. Detection of antibodies against toxoplasmosis in slaughter animals on farms from the socialist sector. Veterinarstvi. 30(9):405-406.

Rawal, B. D. 1959. Toxoplasmosis in sheep in England. Lancet. I:881-882.

Remington, J. S., Miller, M. J., and Brownlee, I. 1968. IgM antibodies in acute toxoplasmosis: I. Diagnostic significance in congenital cases and method for their rapid demonstration. Pediatrics. 41(6):1082-1091.

Riemann, H. P., Kaneko, J. J., Haghighi, S., Behymer, D. E., Franti, C. E., and Ruppanner, R. 1978. The prevalence of antibodies against Toxoplasma gondii among hospitalized animals and stray dogs. Can J Comp Med. 42:407-413.

Riemann, H. P., Willadsen, C. M., Berry, L. J., Behymer, D. E., Garcia, Z. V., Franti, C. E., and Ruppanner, R. 1977. Survey for Toxoplasma antibodies among sheep in western United States. JAVMA. 171(12):1260-1263.

Sabin, A. B. 1942. Toxoplasmosis, a recently recognized disease of human beings. Adv Pediat. 1:1-56.

SAS Institute Inc. SAS User's Guide: Statistics, 1982 Edition. Cary, NC: SAS Institute Inc. 1982. 1-584.

Sharma, S. P. 1980. Prevalence of Toxoplasma infection in sheep in Romania. Vet Para. 7(1):19-23.

Sheffield, H. G., and Melton, M. L. 1968. The fine structure and reproduction of Toxoplasma gondii. J Para. 54:209-226.

Sheffield, H. G., and Melton, M. L. 1970. Toxoplasma gondii: The oocyst, sporozoite, and infection of cultured cells. Science. 167:892-893.

Siim, J. C., Biering-Sorensen, U., and Moller, T. 1963. Toxoplasmosis in domestic animals. Adv Vet Sci. 8:335-429.

Smith, I. D. 1961. Ovine toxoplasmosis as a cause of reproductive wastage: preliminary observations. Aust Vet J. 37:18-21.

Smith, I. D. 1965. Pathogenicity to pregnant ewes of two strains of Toxoplasma gondii isolated from the water rat (Hydromys chrysogaster). Aust Vet J. 41:282-284.

Soave, O. A. 1968. Serologic survey for Toxoplasma antibodies in a colony of research dogs and cats in California. Am J Vet Res. 29(7):1505-1506.

Soulsby, E. J. L. 1982. Helminths, Arthropods and Protozoa of Domesticated Animals. 7th ed. Lea & Febiger, Philadelphia, Pennsylvania. 670-682.

Srivastava, A. K., Bhoop, S., and Gupta, S. L. 1983. Prevalence of Toxoplasma antibodies in sheep and goats in India. Trop Animal Health Prod. 15(4):207-208.

Tadros, W., and Laarman, J. J. 1982. Current concepts on the biology, evolution and taxonomy of tissue cyst-forming eimeriid coccidia. In Advances in Para. (Lumsden, W. H. R., Muller, R. and Baker, J. R., eds.). Academic Press, New York, New York. 20:293-468.

Tizard, I. R., Harmeson, J., and Lai, C. H. 1978. The prevalence of serum antibodies to Toxoplasma gondii in Ontario mammals. Canadian J Comp Med. 42(2):177-183.

Vanderwagen, L. C., Behymer, D. E., Riemann, H. P., and Franti, C. E. 1974. A survey for Toxoplasma antibodies in northern California livestock and dogs. JAVMA. 164(10):1034-1037.

Waldeland, H. 1977. Toxoplasmosis in sheep: influence of various factors on the antibody contents. Acta Vet Scand. 18:237-247.

Wallace, G. D. 1971. Isolation of Toxoplasma gondii from the feces of naturally infected cats. J Inf Dis. 124(2):227-228.

Wallace, G. D. Marshall, L., and Marshall, M. 1972. Cats, rats, and toxoplasmosis on a small Pacific island. Am J Epi. 95:475-482.

Walls, K. W. 1981. Personal communication. Centers for Disease Control, Atlanta, GA.

Walls, K. W., and Barnhart, E. R. 1978. Titration of human serum antibodies to Toxoplasma gondii with a simple fluorometric assay. J Clin Micro. 7(2):234-235.

Watson, W. A., and Beverley, J. K. A. 1971. Ovine abortion due to experimental toxoplasmosis. Vet Rec. 88:42-45.

Weiland, G., and Kuhn D. 1970. Experimentelle Toxoplasma-infektionen bei der Katze. II. Entwicklungstadien des Parasiten im Darm. Berlin Muench Tieraerztl Wochenschr. 83:128-132.

Weinman, D., and Chandler, A. H. 1954. Toxoplasmosis in swine and rodents. Reciprocal oral infection and potential human hazard. Proc Soc Exp Biol Med. 87:211-216.

Wickham, N., and Carne, H. R. 1950. Toxoplasmosis in domestic animals in Australia. Aust Vet J. 26:1-3

Witte, H. M., and Piekarski, G. 1970. Die oocysten-Ausscheidung bei experimentel infizierten Katzen in Abhangigkeit vom Toxoplasma-Stamm. Parasitenk. 33:358-360.

Wolf, A., and Cowen, D. 1937. Granulomatous encephalomyelitis due to an encephalitozoon (encephalitozic encephalomyelitis). A new protozoan disease of man. Bull Neurol Inst New York. 6:306-335.

Zaman, V., and Colley, F. C. 1970. Observations on the endogenous stages of Toxoplasma gondii in the cat ileum. I. Light microscopic study. Southeast Asian J Trop Med Publ Health. 1:457-464.

VITA 2

Tamara J. George

Candidate for the Degree of

Master of Science

Thesis: DEVELOPMENT OF A MICROFLUOROMETRIC IMMUNOASSAY FOR DETECTION OF RECENT EXPOSURE TO TOXOPLASMA GONDII IN SHEEP

Major Field: Veterinary Parasitology

Biographical:

Personal Data: Born in Stillwater, Oklahoma, September 3, 1960, the daughter of Mr. G. Earnest Isch and Ms. Paula C. Isch.

Education: Graduated from Hobart High School, Hobart, Oklahoma, in May, 1978; received Bachelor of Science degree in Agricultural Economics from Oklahoma State University in 1982; enrolled in master's program at Oklahoma State University, 1982-85; completed requirements for Master of Science degree at Oklahoma State University in May, 1985.

Professional Experience: Laboratory Technologist, Department of Veterinary Parasitology, Microbiology, and Public Health, Oklahoma State University, 1980-84.