SEROLOGICAL SURVEY OF TOXOPLASMOSIS IN SOME WILD ANIMALS FROM OKLAHOMA AND TEXAS

By

PATCHIMA INDRAKAMHANG CHOTTIPANICH

Doctor of Veterinary Medicine

Chulalongkorn University

Bangkok, Thailand

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SEROLOGICAL SURVEY OF TOXOPLASMOSIS IN SOME WILD ANIMALS FROM OKLAHOMA AND TEXAS

Approved by:

Helen E. Jordan
Major Advisor

John T. Homer

Joseph Carl Fox

Norman N. Durham
Dean of the Graduate College

PREFACE

This serological survey was designed to be an exploratory report for the prevalence of toxoplasmosis in some wild animals in parts of Oklahoma and Texas. Experience in conducting the serological technic was also expected to be useful for the author's career.

Completing this Master's degree program would not have been possible without the encouragement, patience, and advice of the author's major advisor, Dr. Helen E. Jordan, Professor of Department of Parasitology, Microbiology and Public Health. Her understanding and willing help in every kind of problems throughout the program have been gratefully appreciated. Sincere appreciation is extended to the advisory committee, Dr. John T. Homer and Dr. Carl J. Fox, Associate Professors of Department of Parasitology, Microbiology and Public Health, for their unforgettable knowledge, patience and counsel as well as laboratory equipment availability. This manuscript would not have been completed without their valuable comment and corrections.

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CHAPTER I

INTRODUCTION

Toxoplasma gondii, a coccidium which can encyst in many tissues, infects a variety of animal hosts, including man. The infection is manifested by various clinical signs, but most frequently is asymptomatic. It may produce congenital defects in infants. Diagnosis of *T. gondii* is difficult; thus, immunological methods for laboratory diagnosis have been developed and are routinely used for this purpose.

The organism was first described by Nicolle and Manceaux in 1908 in a gondi (*Ctenodactylus gondi*) from northern Africa. The epidemiology of toxoplasmosis has been studied in part by serological surveys in different host species from various geographical regions of the world. In Oklahoma, the first and only report of toxoplasmosis was in horses (Riemann et al., 1975a). The present study was conducted to determine the prevalence of toxoplasmosis in coyotes, deer and Canada geese from Okahoma and Texas sandhill cranes, believed to have spent time in Oklahoma.

CHAPTER II

REVIEW OF LITERATURE

Several serological technics are now routinely used in diagnosis of both symptomatic and asymptomatic T. gondii infections as well as past infections; these are useful methods for studying prevalence and other epidemiologic features of these infections (Fife, 1971; Lunde, 1973; Kagan and Norman, 1975). Each technic has definite advantages and disadvantages.

The first sero-diagnostic test for T. gondii was the complement fixation test (CF), described by Nicolau and Ravelo in 1937 (Fulton, 1963). This test is suitable for detecting only recent infections because complement fixing antibodies disappear within a few years after infection (Lunde, 1973).

In the past, the most commonly used technic was the methylene blue dye test (MBD), devised by Sabin and Feldman (1948). Because of the hazard in working with live organisms and the difficulty in technical procedures other methods have been developed. However, this test is considered to be a reference standard in the serology of toxoplasmosis by the United States Public Health Service, Center for Disease Control (Kagan and Norman, 1975).

The indirect fluorescence antibody test (IFA) is another reliable test for toxoplasmosis. It was developed by Weller and Coons in 1954 (Gracheva, 1970) and is widely used to detect congenital infections.

The parasites become covered with specific antibody which in turn reacts with fluorescence-labeled antiglobulin. A fluorescence microscope is used to detect the fluorescence reaction. The test requires specific antiserum against each animal species to be tested. This limits the use of the test when an antiserum to a particular species is not available.

The indirect hemagglutination test (IHA) was introduced for detecting Toxoplasma antibodies by Jacobs and Lunde (1957). This test uses tanned erythrocytes which have been sensitized with soluble antigen. These cells will agglutinate with homologous antibody, forming characteristic patterns that can be seen within a few hours. Antibodies detected by IHA test will appear about seven to ten days after those detected by MBD test. This IHA titer increases rapidly and remains high for a long time, probably for life (Lunde, 1973). The IHA test is therefore useful in detecting past infections whether they were symptomatic or not. Since the IHA test uses soluble antigen, it is safer for the personnel performing the examination. The development of microtitration systems and the availability of the commercial test kits allow widespread use of the IHA test for routine diagnosis and serological survey of toxoplasmosis.

Other serologic technics are being developed for detecting T. gondii antibodies, but they are still in the experimental stages and have not been evaluated for routine use.

Serological surveys have been used in the United States to determine the prevalence of Toxoplasma infections in some wild and domestic animals. Several toxoplasmosis surveys have been conducted in California. Dogs and five species of livestock were surveyed in northern California (Vanderwagen et al., 1974). The prevalence was 28% in 353 food animals (cattle, swine, sheep and goats) and 10% in 217 nonfood animals (horses

and dogs) surveyed. In northern and central California, five species of wild carnivores were surveyed (Riemann et al., 1975). In this study, bobcats, raccoons, coyotes and badgers were sero-positive for Toxoplasma antibodies, and the highest prevalence (71%) was in bobcats (21 tested). All the gray foxes were sero-negative. Another survey in northern California was done using 37 species of wild mammals, 35 species of wild birds and 5 species of domestic animals (Franti et al., 1976). Wild mammals had the highest prevalence of toxoplasmosis (45% in 229 carnivores). This included 69% in 86 bobcats, 48% in 25 raccoons, 27% in 26 gray foxes, 22% in 32 striped skunks, one civet cat and one mink. A lower prevalence was found in wild rodents (mice, rats, squirrels), wild herbivores (black-tailed deer), and wild omnivores (feral pigs). Among wild birds, the highest prevalence was in crows (13% in 75), and prevalence was lower in magpies (11% in 9), robins (5% in 20), and mudhens (2% in 38). T. gondii was isolated from the heart of one in two ravens. A recent survey for toxoplasmosis among wild carnivores in California (Riemann et al., 1978) showed high prevalence in Felidae (bobcats and mountain lions); 72% in 43 in the coastal region, 55% in 44 in the central valley, and 50% in 20 in the mountain region. In contrast, prevalence among Canidae (coyotes and gray foxes) was 23% in 35, 28% in 67, and 23% in 102 in the respective geographic regions.

A few surveys have been conducted in the midwestern states. In Iowa, the prevalence of Toxoplasma antibodies was reported as 6.8% to 85.7% in swine, 12.5% to 36.8% in sheep, 16.1% in dogs, and 4.8% in cats. No positive sera were detected in any of 32 horses or 129 cattle (McCullock et al., 1964). Domestic and wild animals from Illinois and adjacent areas of Kentucky and Missouri were tested for T. gondii

antibodies (Paine, 1969). Cattle (7% in 138), swine (4% in 74), and cats (12% in 8) were sero-positive, but three horses tested were sero-negative. Among the wild animals tested (17 raccoons, 14 opossums, 7 skunks, and 9 woodchucks) only 1 woodchuck was positive. A survey for T. gondii antibodies in cattle raised in Indiana, Kentucky, Illinois and Ohio and slaughtered at an Ohio abattoir revealed that all 352 cattle were negative by both serological and parasitological technics (Dubey and Streitel, 1976). However, Toxoplasma may be in this region because more recently toxoplasmosis has been reported in a four-week-old pig from Indiana (Dubey et al., 1979).

A nationwide survey for Toxoplasma antibodies in horses from the United States showed the average prevalence ranged from 16% in the South Atlantic states to 35% in the East South Central states. In some of the West South Central states, the average prevalence was 26%, based upon values of 21% in 14 from Louisiana, 18% in 11 from Oklahoma, and 30% in 43 from Texas. In some of the East South Central states the combined prevalence was 35%, based upon values of 36% in 25 horses in Kentucky, 50% in 2 from Alabama, and 25% in 4 from Mississippi (Riemann et al., 1975a). Another report showed T. gondii antibodies in 34% of 200 horses from Texas (Eugster and Joyce, 1976).

A southwestern states survey for toxoplasmosis included New Mexico, Arizona, and Colorado where some wild and domestic animals as well as a few laboratory animals were tested (Marchiondo et al., 1976). The prevalence of Toxoplasma antibodies from New Mexico wild animals was 45% in 279 carnivores, 21% in 63 herbivores, and 28% in 103 omnivores. Among New Mexico pets tested, 8% of 91 cats and 75% of 4 dogs were sero-positive. None of 29 rhesus monkeys tested showed Toxoplasma antibodies.

One bobcat from Arizona was sero-negative, whereas 20% of 5 coyotes and 20% of 50 dogs from that state were sero-positive. Only seven dogs from Colorado were tested, but two were sero-positive for T. gondii.

Several additional reports of toxoplasmosis are available from the southeastern states. A seven-year survey of a variety of domestic and wild animals was conducted in Memphis, Tennessee, using both serological and parasitological methods (Eyles et al., 1959). Toxoplasma antibodies were demonstrated in cats, dogs, chickens, white peking ducks, pigeons, wild rats, and mice collected from urban slum areas; swine, horses, and lambs from slaughter houses; and cottontail rabbits from rural areas. Cattle and mule samples collected at the abattoir were all sero-negative. A variety of wild birds tested (house sparrow, white-crowned sparrow, cardinal, mourning dove, turkey vulture, and black vulture) were negative by both serological and parasitological methods. In this survey, cats had the highest prevalence. Among 64 cats from slum areas, 77% were sero-positive, which was a higher prevalence than among cats tested from the city pounds (44% in 102 in early 1956 and 40% in 116 during 1956-1957). The pound subjects were mostly young cats. Dog sera collected from the Memphis pounds showed less than half the prevalence (15% in 137 dogs in 1952-1953 and 16% in 663 in 1953-1954) in cats from the same pounds.

Another study of the prevalence of toxoplasmosis in wild animals in the southern states was conducted using wild carnivores from Fort Stewart, Georgia (Walton et al., 1964). This study used the methylene blue dye test and mouse inoculations. The prevalence of Toxoplasma antibodies detected serologically was 33% in 67 raccoons, 9% in 76 opossums, 70% in 47 gray foxes, 73% in 15 bobcats, 19% in 16 cottontails, 12% in 8 gray

squirrels, 100% in 2 red foxes, 50% in 2 deer, and 20% in 5 striped skunks. The prevalence detected by mouse inoculation was 8% in 50 raccoons, 3% in 30 opossums, 7% in 45 gray foxes, 6% in 16 bobcats, 8% in 13 cottontails, and 22% in 9 gray squirrels. Antibodies of Toxoplasma were not detected serologically in 10 deermice, 8 cottonrats, and 9 feral pigs and were not detected by mouse inoculation in 14 deermice, 7 cottontails, 8 feral pigs, 2 red foxes, 2 deer, and 4 striped skunks.

There are not many reports from the northeastern states for the prevalence of toxoplasmosis. T. gondii was isolated and MBD test antibodies were found in 1 of 50 wild pigeons trapped in Syracuse, New York (Manwell and Drobeck, 1951). Toxoplasmosis in wild pigeons also was reported by Jacob et al., 1952. Ten of eighty birds from Washington, D.C. were positive by either the MBD test or mouse inoculation (four by inoculation and seven by serology). Only one was positive by both methods.

CHAPTER III

MATERIALS AND METHODS

Serum Samples

Sera[1] were collected from 51 coyotes and 48 deer from 14 counties in Oklahoma during March 1978 and February 1979 (Figure 1). The number of animals, sex, date of serum collection, and counties from which they were caught are listed in Tables I and II for coyotes and deer respectively. Sera[1] were collected from 50 Canada geese from the Great Salt Plains National Wildlife Refuge, Alfalfa County, Oklahoma on February 6, 1977. Serum samples were collected from 48 sandhill cranes[1] from Brownsville, Texas between January and March 1976. The sandhill cranes had spent some time in Oklahoma since it is in the major flyway for these birds (Drewien and Kuyt, 1979). In all cases, whole blood was collected and left to clot. Serum was separated from the clotted blood by centrifugation, and it was frozen until the time of examination.

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[1]Sera were obtained from Dr. Alan A. Kocan, Department of Parasitology, Microbiology and Public Health, College of Veterinary Medicine, Oklahoma State University.

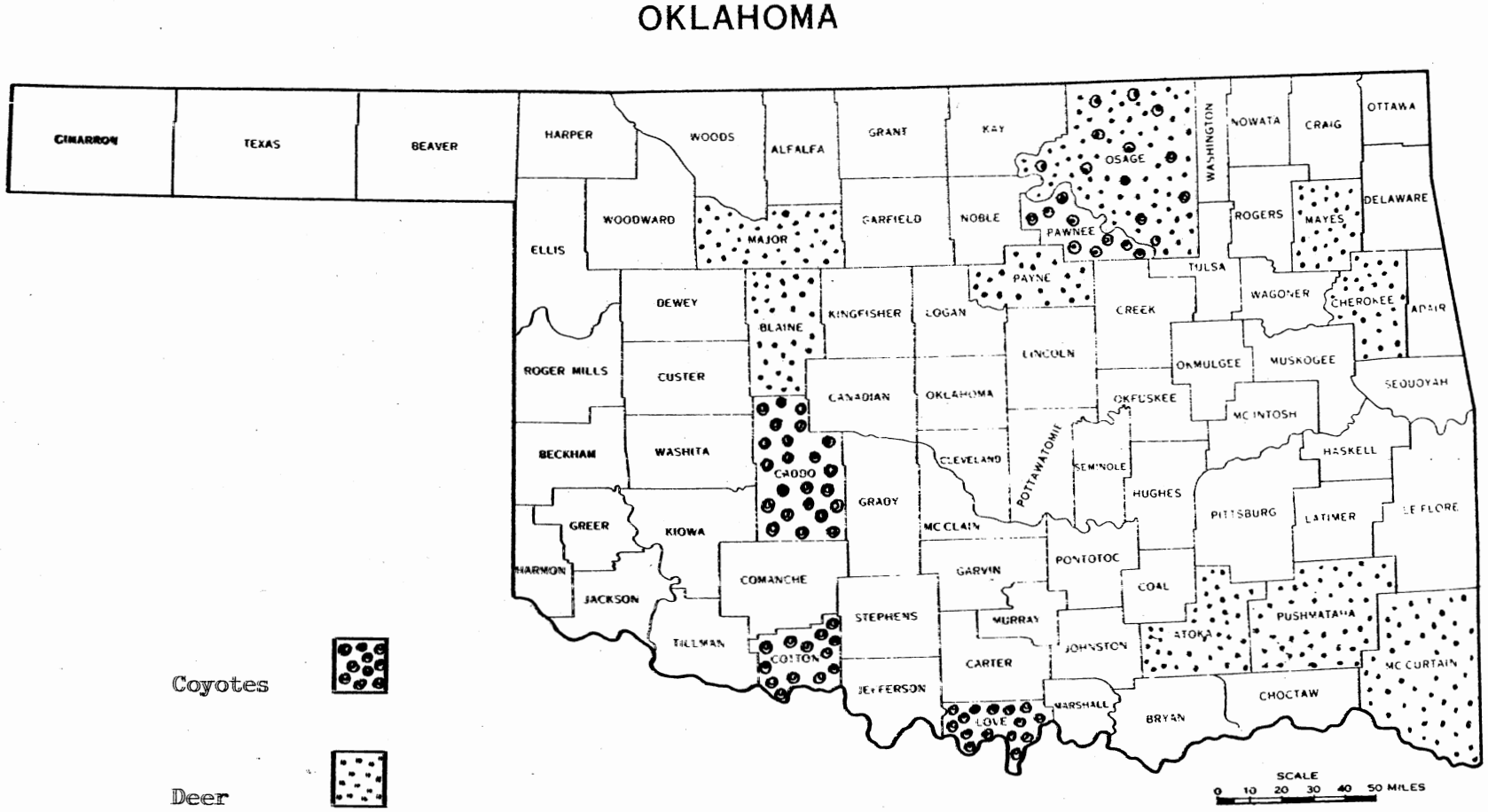

Figure 1. Counties in Oklahoma Where Coyotes and Deer Were Surveyed for Toxoplasma gondii Antibodies During 1978-1979.

TABLE I

COYOTES COLLECTED IN OKLAHOMA FOR TOXOPLASMOSIS ANTIBODY DETECTION BY THE INDIRECT HEMAGGLUTINATION TEST

	Numbers of Animals	Sex		Date Collected	County
		Male	Female		
	5	2	3	March 9, 1978	Love
	7	6	1	March 14, 1978	Cotton
	4	1	3	March 15, 1978	Cotton
	8	5	3	March 15, 1978	Caddo
	5	3	2	April 4, 1978	Logan
	14	9	5	April 6, 1978	Pawnee
	8	6	2	April 13, 1978	Osage
Total	51	22	19		

TABLE II

DEER COLLECTED IN OKLAHOMA FOR TOXOPLASMOSIS ANTIBODY DETECTION BY THE INDIRECT HEMAGGLUTINATION TEST

	Numbers of Animals	Sex		Date Collected	County
		Male	Female		
	2	--	2	August 7, 1978	Major
	2	--	2	August 18, 1978	Osage
	4	--	4	August 22, 1978	Atoka
	6	--	6	August 22, 1978	Pushmataha
	5	--	5	August 29, 1978	Mayes
	2	--	2	August 29, 1978	Payne
	2	--	2	September 6, 1978	Major
	8	--	8	September 6, 1978	Payne
	3	1	2	September 6, 1978	McCurtain
	4	--	4	September 10, 1978	Blaine
	3	--	3	September 16, 1978	Blaine
	2	--	2	February 15, 1978	Cherokee
	5	2	3	February 18, 1978	Payne
Total	48	3	45		

Indirect Hemmaglutination Test Kits

Commercial kits for the IHA test for T. gondii antibodies were purchased from two companies. One, the Tox HA kit[2], utilized freeze-dried, formalin-treated, tanned, turkey erythrocytes coated with a soluble sonicate of T. gondii as the test cells. Prior to use, the cells were adjusted to a 2.25% cell suspension using distilled H_2O. The diluent for the test was phosphate-buffered saline, pH 7.2, containing 1.5% normal rabbit serum and 0.1% sodium azide. Positive and negative control sera were from sheep and cattle, respectively.

The other, TPM-Test kit[3], used as test cells, T. gondii-sensitized, tanned, sheep erythrocytes. The 0.5% cell suspension in buffered saline contained 1% normal rabbit serum and a preservative. The test diluent was buffered saline, pH 7.0-7.4, containing 1% normal rabbit serum and a preservative. Both the positive and negative control sera were of human origin.

Reagents were prepared according to the instructions provided with each kit.

At the beginning of the study, samples were tested using only the Tox HA kit. Later, the TPM-Test kit was used for comparison purpose.

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[2]Wellcome Reagents Division, Burroughs Wellcome Company, Research Triangle Park, North Carolina 27709.

[3]Wampole Laboratories, Dist., Division of Carter-Wallace, Inc., Cranbury, New Jersey 08512.

Indirect Hemagglutination Test

Sera were thawed at room temperature immediately before use. Hemolyzed samples were centrifuged to remove the lysed cells. A 0.025 ml volume of the required buffer was added to each well of a disposable U-bottom microtiter plate[4]. To wells 1 and 6 of each row was added 0.025 ml serum, which was then diluted, using a titration device[5] equipped with 0.025 ml microdiluters, to a final dilution of 1:64. Positive and negative control sera were included on each plate. Control cells were added to the 1:32 dilution and test cells to the 1:64 dilution according to the kit procedures. The plates were tapped gently to mix the contents, covered, and incubated at room temperature for a minimum of one hour. The agglutination patterns were read with the aid of a mirror and scored as positive or negative by comparison with the control sera.

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[4]Linbro Scientific Company, Ind., Hamden, Connecticut 06514.

[5]Cooke Laboratory Products, Division of Dynatech Laboratories Incorporated, 900 Slaters Lane, Alexandria, Virginia 22314.

CHAPTER IV

RESULTS

Six of fifty-one coyotes tested were positive for T. gondii antibodies at the 1:64 dilution. Five of them were detected using the Tox HA kit and four by the TPM-Test kit. Only three of the samples were positive by both kits. Five of the six counties sampled had at least one coyote serum sample that was positive. Two positive specimens were from Love County and one each from Cotton, Caddo, Pawnee, and Osage counties. These specimens came from three females and three males. The ages of animals ranged between one to three years. Results are given in Table III.

Five of the forty-eight deer were sero-positive for T. gondii at the 1:64 dilution. Of these, two were detected using the Tox HA kit and four by the TPM-Test kit. Only one was positive using both kits. Sero-positive samples came from only four of the eight counties sampled, one each from Atoka, Pushmataha, and Osage counties and two from McCurtain county. Four of the deer were female, and one was male. The ages of animals varied from two to ten years. Table IV lists results on deer serum samples.

All sera from Canada geese and sandhill cranes were negative for Toxoplasma antibodies using both kits.

TABLE III

COMPARISON OF *TOXOPLASMA* ANTIBODIES IN COYOTES FROM OKLAHOMA USING TWO COMMERCIAL INDIRECT HEMAGGLUTINATION TEST KITS

Animal Number	Sex	Age in Years	Date Collected	Location County	ToxHA 1:64	TPM-Test 1:64
1	Male	3	March 9, 1978	Love	+	+
2	Female	2	March 9, 1978	Love	+	+
5	Female	3	March 9, 1978	Cotton	+	-
11A	Female	2	March 15, 1978	Caddo	+	-
36B	Male	2	April 6, 1978	Pawnee	+	+
40	Male	1	April 13, 1978	Osage	-	+

TABLE IV

COMPARISON OF *TOXOPLASMA* ANTIBODIES IN DEER FROM OKLAHOMA USING TWO COMMERCIAL INDIRECT HEMAGGLUTINATION TEST KITS

Animal Number	Sex	Age in Years	Date Collected	Location County	ToxHA 1:64	TPM-Test 1:64
3	Female	3	August 18, 1978	Osage	+	+
2	Female	9	August 23, 1978	Pushmataha	-	+
5	Male	2	August 23, 1978	Atoka	-	+
78-0466	Female	10	September 13, 1978	McCurtain	+	-
78-8465	Female	2	September 13, 1978	McCurtain	-	+

The summarized results for all animal sera are listed in Table V. Locations where coyotes and deer having positive T. gondii antibodies were collected are shown in Figure 2.

TABLE V

PREVALENCE OF *TOXOPLASMA GONDII* ANTIBODIES IN SOME WILD ANIMALS FROM OKLAHOMA AND TEXAS

Animal	Number Tested	Number of Positive at 1:64 Dilution				
		Tox HA	TPM-Test	Both Tox HA and TPM-Test	Total Number	(%)
Coyote	51	4	5	3	6	(11.76%)
Deer	48	4	2	1	5	(10.42%)
Canada Geese	50	0	0	0	0	
Sandhill Crane	48	0	0	0	0	

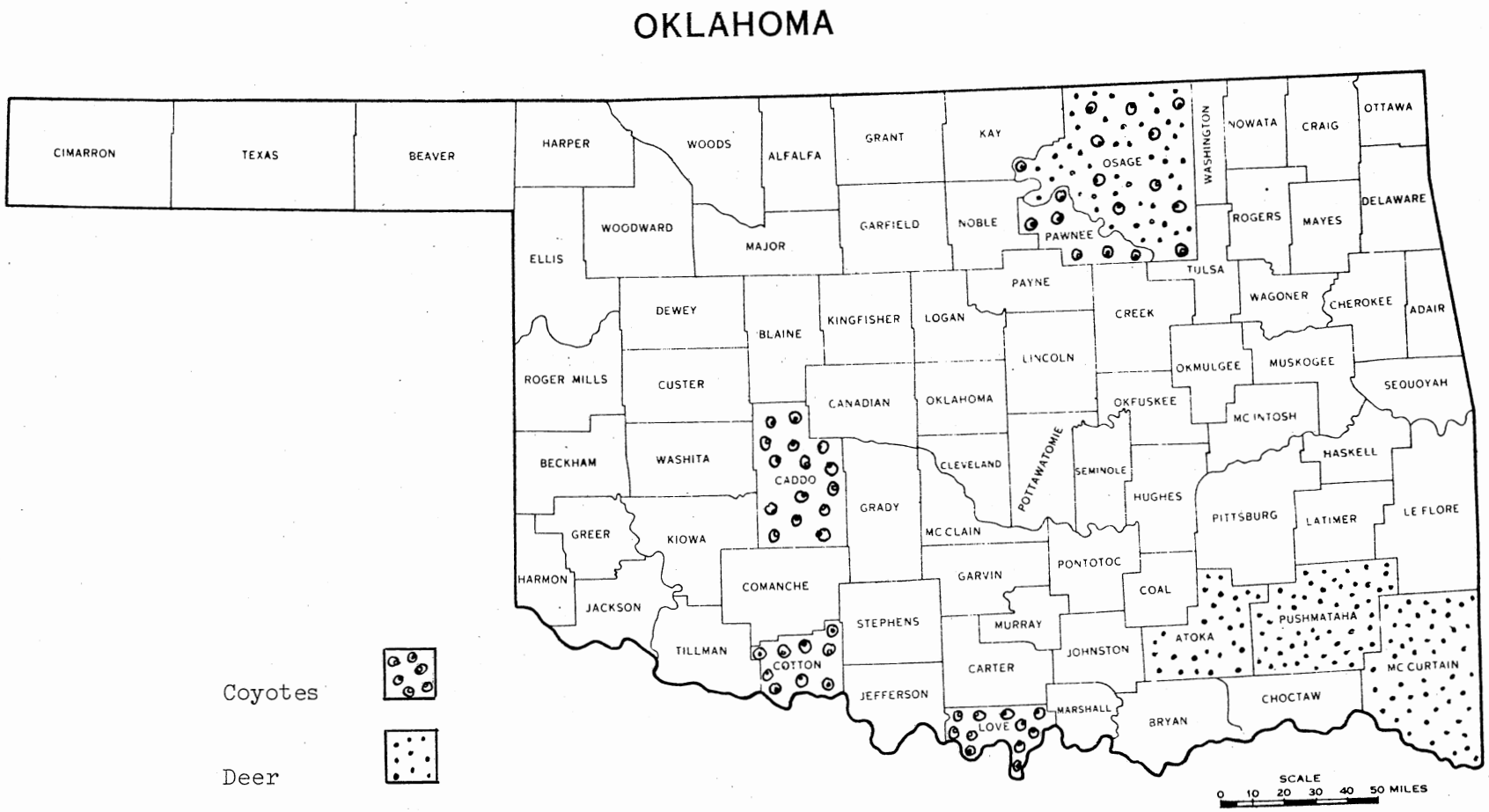

Figure 2. Counties in Oklahoma Where Toxoplasma gondii-Positive Coyotes and Deer Were Collected in 1978-1979.

CHAPTER V

DISCUSSION

Infections with Toxoplasma gondii are widely distributed both in host range and geographic region in the United States. Wolf et al. (1937) first showed the transmission of the disease in man, 29 years after the original description of the organism. Although many studies have been conducted to attempt to describe the epidemiology of this zoonotic disease, the details are still unresolved. Several investigators incriminated Felidae, both wild and domestic, as the only definitive hosts that pass the oocysts into the environment (Dubey et al., 1970; Frenkel et al., 1970; Hartley and Munday, 1974). These workers found that within a few days under favorable conditions, the oocysts became infective for a variety of animal hosts but oocysts were not passed by these animals in turn. Other investigators who have conducted surveys to determine the prevalence of T. gondii in man and animals also have speculated that Felidae are the only definitive hosts (Jewell et al., 1972; Miller et al., 1972; Songandares-Bernal et al., 1975; Riemann et al., 1975; Franti et al., 1976; Marchiondo et al., 1976). Teutsch et al. (1979), in their investigation of an epidemic of toxoplasmosis in man, claimed that cat feces as the source of infections. Dubey et al. (1979) also suggested cat feces as the source in a recent report of enzootic toxoplasmosis on a pig farm in Indiana. Other investigations have suggested that T. gondii oocysts might be shed by animals other than Felidae.

In one study, T. gondii oocysts were passed in the feces of Puerto Rican land snails for two days after being fed cat feces with oocysts. (Miller et al., 1972).

Felidae may not be the only source of T. gondii for humans. For example, some studies showed that the prevalence of T. gondii antibodies in persons exposed to cats was about the same as that of those who were not exposed to cats (Behymer et al., 1973; Sengbusch, 1976; Tizard and Caoili, 1976). Other studies show that prevalence was even higher in persons who were not exposed or less exposed to cats than in those with frequent exposure to cats (Comstock and Ganley, 1973; Riemann et al., 1974). The most probable mode of infection in man is considered to be ingestion of raw or undercooked infected meat rather than oocysts (Weinman and Chandler, 1956; Jacob et al., 1960).

The source of toxoplasmosis for animals other than man is considered by some investigators to be their foodstuffs i.e. via their feeding habits (Hartley and Munday, 1974; Franti et al., 1976). Carnivores also may acquire T. gondii by ingestion of raw or undercooked infected meat and, in fact, the high prevalence of toxoplasmosis in carnivores indicated by several surveys (Franti et al., 1976; Riemann et al., 1975; Quinn et al., 1976) suggests a route of transmission similar to that for man. Herbivore infections are assumed to acquire by the ingestion of oocysts contaminated food. Results from the present study showed a similar pattern of prevalence for Oklahoma animals and therefore suggest similar modes of transmission for the carnivore and herbivore under study. The ingestion of prey by coyotes (Gier, 1975) certainly would facilitate the acquisition of Toxoplasma gondii, however, determination of the source of infection was not within the scope of this study.

Deer, which are herbivores, could have acquired the infection by ingestion of oocysts from contaminated soil, forage, or water. The survival of oocysts in soil of warm, moist climates of Kanasa and Costa Rica (Frenkel et al., 1975) suggests that oocysts could also survive in some regions of Oklahoma. The present study therefore showed that Toxoplasma was found in some of the wild animal populations (deer and coyote) of Oklahoma. Franti et al. (1976) reported that bird species known to consume meat as part of their feed showed relatively high prevalence of T. gondii (13% in 74 crows; 11% in 9 magpies, and 50% in 2 ravens). Those bird species that are grain or aquatic feeders showed a lower prevalence (5% in 20 robins; 2% in 38 mudhens and none in 2 Canada geese). That Canada geese and sandhill cranes were sero-negative in this survey could indicate that these animals had minimum exposure to Toxoplasma. More extensive study of toxoplasmosis in wild and domestic animals from Oklahoma and Texas will be valuable in the understanding of T. gondii infections for this region.

The indirect hemagglutination technic used for this survey is widely used at present for routine diagnosis of toxoplasmosis as well as in serological surveys. It is accepted as a reliable and practical test. The disagreement between results obtained from the two kits used in this study could have been caused by several factors:

1. Experimental error could account for some of the variation. This could be minimized by running the samples in duplicate, simultaneously with both kits.

2. False positive reactions may occur because many antibodies adhere to carrier red blood cells. These antibodies may be the result of prior exposure to the red blood cell or a cross-reacting antigen. False

positive reactions may be a problem, especially with sera from wild carnivores, such as the coyote, whose diets may include sheep and turkeys. However, these antibodies perhaps could be detected by including unsensitized carrier cells in the test procedure, or by absorbing the antibodies with homologous cells.

3. False negative reactions result from inability to detect low levels of antibody. The sensitized cells used in the two tests may have contained differences in the quantity of antibody required to produce a positive agglutination pattern. Also, a known positive serum from the species under the study would help in evaluating the sensitivity of the two kits.

4. The endpoint was easier to read in one kit (TPM-Test) than in the other (Tox HA). This could result from differences in cell type (sheep cells, TPM-Test; turkey cells, Tox HA), cell concentrations (0.5%, TPM-Test; 2.5%, Tox HA), or settling characteristics of the cells.

Both the kits were designed for detecting Toxoplasma antibodies in human sera, and the application of the kits to animal sera may have yielded aberrant results. Perhaps, the adjustment of the test may not have been optimum for the animal sera, and the results may not have been properly standardized for animals other than man. Good positive and negative control sera for each species are needed to provide the proper standard.

CHAPTER VI

SUMMARY

Serum samples from 51 coyotes, 48 deer, 50 Canada geese from Oklahoma and 48 sandhill cranes from Texas were screened at 1:64 dilution using two commercial indirect hemagglutination kits to detect Toxoplasma antibodies. Six of fifty-one coyotes were positive; five were positive using the Tox HA kit, four were positive using the TPM-Test kit, and three were positive using both kits. Five of forty-eight deer were positive; two were positive using the Tox HA kit, four were positive using the TPM-Test kit, and only one of them was positive using both kits. None of the Canada geese or sandhill cranes were sero-positive. The results of the study revealed a Toxoplasma prevalence of 11.76% in 51 coyotes and 10.42% in 48 deer.

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VITA 2

Patchima Indrakamhang Chottipanich

Candidate for the Degree of

Master of Science

Thesis: SEROLOGICAL SURVEY OF TOXOPLASMOSIS IN SOME WILD ANIMALS FROM OKLAHOMA AND TEXAS

Major Field: Veterinary Parasitology and Public Health

Biographical:

Personal Data: Born in Petchburi, Thailand, November 18, 1947, the daughter of Amorn and Pui Indrakamhang.

Education: Graduated from Mahavajiravudh, Songkhla, Thailand, in February, 1965; graduated Pre-vet from Kasetsart University, Bangkok, Thailand, in March, 1967; received Doctor of Veterinary Medicine from Chulalongkorn University, Bangkok, Thailand, in March, 1971; enrolled in Master's program at Department of Entomology, Purdue University, Indiana, 1976-1978; complete requirements for Master of Science degree at Oklahoma State University in July, 1979.

Professional Experience: Veterinarian of Laboratory Animals Care Center, Faculty of Science, Mahidol University, Bangkok, Thailand, from April, 1971 - March, 1973; 2nd Grade Veterinary Officer in Parasitology, Livestock Department, Ministry of Agriculture, Bangkok, Thailand, from April, 1973 - July, 1976; graduate research assistant, Department of Parasitology, Microbiology and Public Health, College of Veterinary Medicine, Oklahoma State University, from October, 1978 - May, 1979.