EVALUATION OF THE VECTOR POTENTIAL OF *AMBLYOMMA AMERICANUM*, *DERMACENTOR VARIABILIS*, AND *IXODES SCAPULARIS* AS VECTORS FOR THE LYME DISEASE AGENT *BORRELIA BURGDORFERI*

By

STANLEY WANYANGU MUKOLWE

Bachelor of Veterinary Medicine
University of Nairobi
Nairobi, Kenya
1984

Master of Science
Oklahoma State University
Stillwater, Oklahoma
1987

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
DOCTOR OF PHILOSOPHY
May, 1991

EVALUATION OF THE VECTOR POTENTIAL OF AMBLYOMMA AMERICANUM, DERMACENTOR VARIABILIS AND IXODES SCAPULARIS AS VECTORS FOR THE LYME DISEASE AGENT BORRELIA BURGDORFERI

Thesis Approved:

Thesis Adviser

Katherine M. Kocan

R D Wilkinson

R. W. Barker

Norman N. Durham

Dean of the Graduate College

ACKNOWLEDGEMENTS

I wish to express my sincere thanks to Dr. Alan Kocan, Dr. John H. Wyckoff III, Dr. Robert Barker, Dr. Ronald Welsh and Dr. Kathy Kocan for accepting to serve on my graduate committee. Special thanks to Dr. Alan Kocan, my major adviser for his diligent guidance and for his continual encouragement throughout the thesis research. I appreciate Dr. Wyckoff's efforts in stirring up a real interest in immunology in me. Dr. Welsh's willingness to serve on the committee at very short notice is appreciated, and his contribution as a microbiologist to my research was invaluable. I would also like to thank Dr. Welsh and the management of the Oklahoma Animal Disease Diagnostic Laboratory (OADDL) for allowing me to use their facilities for Leptospira serology and for all IFA work conducted in this study. Dr. Barker provided research facilities in which all the transmission studies were conducted, and was always willing to stop what he was doing to give me guidance. His expert knowledge on tick ecology and tick trapping methods contributed to the success of this work. Dr. Kathy Kocan's expertise as an electron microscopist rubbed off on me as she worked téte-à-téte with me, helping me with every little

thing and making my training more rounded and meaningful. She was always excited when I got positive results and very supportive when I got negative results. Dr. George Murphy contributed to my training by teaching me how to conduct the Polymerase Chain Reaction (PCR). He was very patient and showed no irritation when I asked him foolish questions about the assay.

I would like to thank Dr. Rebecca Morton and Ms. Sandra Rodgers for their advice on *in vitro* cultivation of spirochetes and for providing me with sera samples that helped me develop the ELISA used in this study.

I acknowledge gratefully The International Education and Research Division of the Ministry of Foreign Affairs, Government of the Netherlands, The Haig, for providing me with financial support throughout my training, and the Kenya Agricultural Research Institute (KARI), my present employer, for granting me study leave to undertake study for the Ph.D degree. Dr. James K. Lenahan, Training Officer, ILRAD, was very efficient at coordinating my training and I am grateful to him. My wife was very patient with me as I spent many hours at school getting this study completed, and I am very grateful for her support. I feel honored to be married to Patience. My parents, brothers and sisters supported me throughout this study giving me the necessary encouragement to go on even when the going got tough.

This study was supported, in part, by the Oklahoma

Center for the Advancement of Science and Technology (OCAST) Project number HR9-012.

I wish to dedicate this thesis to my wife Patience, my daughter Melinda and my son Michael Blakely.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Lyme disease is a zoonotic tick-borne spirochetosis caused by the bacterium *Borrelia burgdorferi* (Burgdorfer *et al.*, 1982; Steere *et al.*, 1983a; Johnson *et al.*, 1984a, b). The disease has been described as the most commonly reported tick-borne illness in the United States (Centers for Disease Control, 1985; Ciesielski *et al.*, 1988), and as the only tick-borne spirochete thus far isolated in the eastern United States (Anderson *et al.*, 1989).

Historical Background and Geographical Distribution

Lyme borreliosis is thought to have a worldwide distribution (Burgdorfer, 1986). The earliest known reporting of a Lyme borreliosis-like syndrome occurred in 1909, when Arvid Afzelius demonstrated to the Swedish Dermatological Society that a migrating annular skin lesion, which he referred to as *erythema migrans*, developed at the site of a bite by *Ixodes reduviidus* (Burgdorfer, 1986; Dammin, 1989). It was subsequently noticed that the symptom had a long duration and was thus renamed *erythema chronicum migrans* (ECM) in 1913. Today, ECM is considered one of the initial and

pathognomonic symptoms of Lyme disease. In cases in which the ECM develops, it is often seen as a rash that begins as a flat or slightly raised red macule or papule, which expands over time, often with a partially clear center (Steere *et al.*, 1986). In 1923, Lipschultz postulated that ECM was caused by toxins or infectious agents acquired by the tick and transmitted while ticks fed (Burgdorfer, 1986). Other theories explained ECM as being an allergic reaction or being caused by an infective agent with allergizing properties (Burgdorfer, 1986). By 1955, reports from Europe indicated that ECM may be caused by a bacterial agent that was susceptible to penicillin. Although an etiological agent had not been isolated, reports existed as early as 1948 describing spirochete-like elements in sections of skin from a patient with ECM (Burgdorfer, 1986).

Reports from Germany further complicated the understanding of ECM when it was linked to an inflammatory skin condition called *acrodermatitis chronicum atrophicans* (ACA) which was first described by Alfred Buchwald in 1883. Additional information from Germany reported 6 out of 7 cases of ACA tested positive for *Treponema pallidum*, suggesting that ACA had a spirochetal etiology (Burgdorfer, 1986).

The worldwide distribution of Lyme borreliosis includes reports from France, Sweden, Austria, Switzerland and Germany (Stanek *et al.*, 1988). In the Soviet Union, cases occur from the Baltic republics to the Pacific Ocean (Dekonenko *et*

al., 1988). Lyme disease has also been diagnosed in Australia, Japan and China (Steere, 1989). The presence of Lyme disease in Africa and South America has not been well documented. Reports from South Africa indicate that there are sero-reactors in the human population as well as in horses and dogs (Fivaz and Petney, 1989; Fivaz, Botha and Cairns, 1990). There has, however, been no isolation of spirochetes from either the hosts or suspect vectors in any of these cases. There are various species of *Borrelia* in humans and domestic livestock in Africa including *B. theileri, B. recurrentis*, (Spitz and Ash, 1945), and *B. duttoni* (Burgdorfer *et al.*, 1989), but the significance of these and other borreliae in relation to Lyme disease has not been evaluated.

The history of human Lyme disease in the United States is recent; only dating back to 1969 when it was first reported in a Wisconsin hunter who was also a physician (Scrimenti, 1970; Burgdorfer, 1986). In 1976, what had been described earlier as Lyme arthritis was renamed Lyme disease after the disease was noticed to affect various organ systems in the body (Steere, 1986). The disease was initially found to be localized in Lyme Connecticut and was shown to be correlated with a history of tick bite (Burdorfer *et al.*, 1986; Steere *et al.*, 1986). The annual number of cases of Lyme disease in the U.S.A. has risen steadily; there were 491 cases in 1982, 600 in 1983, 1500 in 1984, 1520 in 1985, and 1394 in 1986 (Ciesielski *et al.*, 1988). In 1988,

there were over 4,000 cases of the disease reported (Piesman, 1989; unpublished data). Until 1982, the majority of cases were reported in the north-eastern and north-central U.S.A. from seven states, (New York, New Jersey, Massachussets, Rhode Island, Connecticut, Wisconsin and Minnesota). By 1986 the disease had been reported from 32 states (Ciesielski *et al.*, 1988), while in 1989 this number had risen to 43 (Steere, 1989). It is not known whether there is an actual yearly increase in the number of new Lyme disease cases or whether the increase in numbers is due to improved diagnostic techniques and better reporting systems. It is expected that when Lyme disease becomes a reportable disease in most states, the number of documented cases will sharply increase.

Isolation of Etiological Agent

Successful *in vitro* cultivation of spirochetes was demonstrated as early as the 1920s (Barbour, 1986). More recently successful cultivation of *Borrelia hermsii*, *B. hispanica*, *B. parkeri*, and *B. turicatae* was achieved by Richard Kelly (Kelly, 1971; 1976). The media used for cultivation of these spirochetes was referred to as Kelly's media. This media was later fortified by Stoenner by addition of a yeast preparation, amino acids, nucleotides and growth factors (Stoenner *et al.*, 1982). Barbour first isolated *B. burgdorferi*, the etiological agent of Lyme disease, in fortified Kelly's media (Barbour, 1984; Benach

et al., 1983; Burgdorfer *et al.*, 1982 and Steere *et al.*, 1983a). This media is now referred to as Barbour-Stoenner-Kelly (BSK) media and continues to undergo modification (Barbour, 1984).

Borrelia burgdorferi has been isolated from or detected in humans (Steere *et al.*, 1983b; Benach *et al.*, 1983), cotton-tail rabbits (Anderson *et al.*, 1989), rodents (Anderson *et al.*, 1985, 1986a and 1987), raccoons, *Procyon lotor* (Anderson *et al.*, 1983), wild foxes, coyotes, the gray wolf (Kazmierczak *et al.*, 1988; Kazmierczak and Burgess, 1989), domestic dogs (Kornblatt *et al.*, 1985, Burgess 1986a), cows and horses (Burgess, 1988; Burgess *et al.*, 1987). *Borrelia* spp. have been detected in white-tailed deer (*Odocoileus virginianus*) although they have not been characterized as *B. burgdorferi* (Anderson, 1988). Numerous species of infected birds have also been identified (Anderson *et al.*, 1986a).

Clinical Manifestations and Diagnosis

Early in the course of an infection, Lyme disease manifests itself with signs akin to flu symptoms, including fatigue, muscle pain, headache, fevers and chills as well as the characteristic skin rash (Steere, 1989). In the second phase, the nervous and musculoskeletal systems are involved with resulting lymphocytoma, meningo-polyneuritis, acute or subacute arthritis and carditis (Asbrink and Hovmark, 1988). Myocarditis and palpitations may be experienced at this stage as well. In the third and last phase of the disease,

also referred to as late Lyme disease, there is chronic arthritis of large joints as well as chronic neurologic manifestations (Steere *et al.*, 1977; 1983a; Asbrink and Hovmark, 1988). Sixty percent of untreated patients experience arthritis months to years following tick bite and presumed infection (Steere *et al.*, 1984). In some patients, arthritis clinically similar to rheumatoid arthritis may be the only manifestation of Lyme disease (Steere *et al.*, 1977; Habicht *et al.*, 1988). Death as a direct sequel of Lyme is very rare.

Although ECM is a pathognomonic sign of Lyme disease, the lesion may be atypical or absent altogether in certain cases making it hard to interpret the clinical picture based on presenting symptomology (Steere *et al.*, 1983b). When accompanied by a history of tick bite at the site of skin lesion, presumptive diagnosis of Lyme disease becomes easier. Studies have shown that less than 50% of patients recall having had a tick bite (Steere *et al.*, 1983b; Asbrink and Hovmark, 1988) possibly because they are bitten by immature stages of the tick vector which are very small and hard to see, or when seen, are often mistaken for other arthropods. Serologic diagnosis has therefore become a useful tool in conjunction with clinical symptoms. Serologic tests commonly used include the indirect immunofluorescent antibody test (IFA), enzyme-linked immunosorbent assay (ELISA) and fluorescence immunoassay (FIAX) (Magnarelli, 1988, Pennell *et al.*, 1987). In one study where the IFA

and ELISA were compared for diagnostic purposes, both methods were in agreement in detection of positive cases, but the ELISA was found to yield less variable results, was more sensitive and was more easily standardized (Magnarelli *et al.*, 1987). Successful serological tests are best obtained from untreated patients who have suffered neurologic and arthritic disorders (Magnarelli, 1988). Blood samples taken within 3 weeks of tick bite or onset of ECM often test negative (Russel *et al.*, 1984; Shrestha *et al.*, 1985) regardless of assay method or conjugate used. This apparent lack of immunological response is due to an increase in spontaneous activity of host suppressor cells in the initial weeks of infection, with a resulting antibody response to only the most immunogenic peptides (Moffat *et al.*, 1984). If antibiotic therapy is sought during early disease, antibody production may be curtailed. It is possible, therefore, to find clinical Lyme disease cases that test negative serologically (Magnarelli and Anderson, 1986). In one study where horses were experimentally inoculated with *B. burgdorferi* and later serologically tested by IFA and ELISA, it was found that those horses that were exposed to the organism by repeated inoculations tested strongly positive while those that were exposed by a single inoculation had variable results ranging from negative to suspicious (Cohen *et al.*, 1988). In this study horses showed varied symptoms including edema on all 4 legs, conjuctivitis, nasal discharge, cough and at times dermatitis on the white areas

of the lower leg similar to a photosensitivity reaction (Cohen *et al.*, 1988). When Syrian hamsters were inoculated with a human isolate of *B. burgdorferi*, no obvious signs of illness were observed although organisms were later recovered from spleen and kidney as early as 14 days and as late as 14 months post inoculation (Johnson *et al.*, 1988).

Isolates of *B. burgdorferi* from different sources have shown notable variations. Isolates from humans (Benach *et al.*, 1983; Steere *et al.*, 1983a), *I. dammini* (Anderson *et al.*, 1983, 1987; Burgdorfer *et al.*, 1982; Steere *et al.*, 1983a), *Ixodes pacificus* (Bisset and Hill, 1987; Burgdorfer *et al.*, 1985), rodents (Anderson, 1983; 1985; 1986b; 1987; Bosler *et al.*, 1983; Burgess, 1986b; Godsey *et al.*, 1987; Levine *et al.*, 1985; and Loken *et al.*, 1985) and a raccoon, *Procyon lotor* (Anderson, *et al.*, 1983) had outer surface membrane protein A (OspA), and OspB as well as flagellin proteins with approximately similar molecular weights (Barbour *et al.*, 1983, 1984, 1985, 1986). One isolate from *I. pacificus* lacked Osp A and Osp B but had an abundance of a protein with relative molecular mass of 25,000 daltons (Bisset and Hill, 1987) while a strain from a bird (Anderson *et al.*, 1986a) had Osp A protein with a slightly lower molecular weight and appeared to lack Osp B protein altogether (Barbour *et al.*, 1985). Isolates from *Ixodes dentatus* lacked the major 31,000 and 34,000 dalton proteins commonly found on other isolates (Anderson *et al.*, 1989). When an isolate from *I. dentatus* was compared with

one from a rabbit on which these ticks had previously fed, even these were found to be different (Anderson *et al.*, 1989). In culture, Osp B may at times not be produced at all (Schwan and Burgdorfer, 1987; Schwan *et al.*, 1988; Wilske *et al.*, 1988). The lack of production of Osp B in culture may be responsible, in part, to the lack of infectivity of many isolates following a several passages *in vitro*.

Vectors, Reservoirs and Transmission

The principal wild vertebrate reservoir for *B. burgdorferi* in the United States is the white-footed mouse (*Peromyscus leucopus*) (Bosler *et al.*, 1984; Levine *et al.*, 1985). Although initially thought to be a reservoir for Lyme disease, white-tailed deer now appear to be incidental in the maintenance of the spirochete agent. White-tailed deer, however, do play a major role in maintaining the adult stage of the principal tick vector, *I. dammini*, in the northeastern and midwestern parts of the U.S.A (Wilson *et al*,. 1986; Burgdorfer *et al.*, 1982; Anderson *et al.*, 1983; Steere, *et al.*, 1983a; and Johnson *et al.*, 1984b). It has been shown that on certain islands, the presence of *I. dammini* correlates with abundance of white-tailed deer (Spielman, 1988), and that when deer were absent, so were the ticks and Lyme disease. Infection rates among *Ixodes* spp. in endemic areas along the northeastern Atlantic coastline can be as high as 90% as compared to 3% on the west coast (Ciesielski *et al.*,

1988). Larval and nymphal *I. dammini* feed on small-, medium- and large-sized mammals belonging to the orders Marsupalia, Insectivora, Lagomorpha, Rodentia, Carnivora, Artidactyla, Perissodactyla and Primates (Anderson, 1988). Many of these animals can therefore be potential reservoirs for Lyme disease. A large variety of birds are also parasitized by immature stages of *I. dammini*. Birds may therefore also act as reservoirs of Lyme disease (Anderson, 1988) and may actually play a significant role in disseminating the spirochete and/or tick over long distances, even, perhaps between continents during bird migrations. It is interesting to note the correlation between the distribution of Lyme disease in the U.S.A. and migration routes of migrating birds, especially waterfowl.

Other vectors for *B. burgdorferi* include *I. pacificus* in the western part of the U.S.A. (Burgdorfer, *et al.*, 1985), *I. ricinus* in Europe (Krampitz, 1986) and *I. persulcatus* in Asia (Dekonenko *et al.* 1988). The bacteria have also been isolated from or detected in various other ixodid tick genera including *Ixodes scapularis* (Say), *I. pacificus, I. dentatus* (Anderson *et al.*, 1989), *Dermacentor variabilis* (Say) (Anderson *et al.*, 1987) and *Amblyomma americanum* (L) (Schulze *et al.*, 1984). Although a bite from an infected tick is the recognized mode of transmission of *B. burgdorferi*, human transmission through the bite from a seropositive horse has been reported in Belgium (Marcels *et al*, 1987; Cohen *et al.*, 1988). Contact transmission be-

tween infected and uninfected *Peromyscus* spp. housed together has been demonstrated under experimental conditions in the absence of tick vectors (Burgess *et al.*, 1986). If this latter type of transmission takes place in nature, it may be a means by which *B. burgdorferi* is maintained between mice in the wild during seasons when tick numbers are low or absent on mice.

Laboratory studies on transovarial and transstadial transmission of *B. burgdorferi* have been conducted. In one such study, spirochetes were transovarially transmitted to 100% of F_1 larvae from a single female *I. pacificus* that was infected as an adult (Lane and Burgdorfer, 1987). Infection of F_2 larvae from four out of five adults examined was 90-97%. Transstadial transmission was demonstrated in 100% of nymphs and adults that molted from infected larvae without re-exposure. Although the above study demonstrated that both transovarial and transstadial transmission of the Lyme disease agent can occur in the western black-legged tick, the epidemiological significance of these results are still unclear. In another study, 21.7% adult *I. ricinus* acquired infection after feeding on an infected rabbit (Lane and Burgdorfer, 1987) with two of the infected females showing generalized infection. The same two females passed the spirochete transovarially to 60% and 100% of their F_1 larvae while infection rates in F_2 larvae were considerably lower. Females showing non-generalized infection did not transmit the spirochete transovarially. In

another study, larval *I. dammini* were collected from vegetation and examined for the presence of *B. burgdorferi* by dark-field microscopy and by indirect fluorescent antibody test (Piesman *et al.*, 1986). Out of 274 larvae examined, only two (0.7%) were infected and this was attributed to transovarial transmission. The results of this study were interpreted to mean that transovarial transmission of *B. burgdorferi* was rare, but that it may have significance in introducing the agent to new sites.

Growth kinetics studies of *B. burgdorferi* in *I. dammini* indicate that multiplication of spirochetes in larvae and nymphs reached peak levels on days 15 and 75 postrepletion with mean spirochete numbers of 2,735 and 61,275, respectively (Piesman *et al.*, 1990).

Lyme Disease in Oklahoma

Literature on Lyme disease in Oklahoma is scanty. Rodgers *et al.*, (1989) tested serum samples submitted from dogs to the Oklahoma Animal Disease Diagnostic Laboratory for Lyme disease by the IFA test between June 1986 and July 1987. A total of 256 samples were tested yielding 45 (18%) positives. Antibody titers $\geq$ 64 were considered positive. Our laboratory recently tested 167 serum samples from cotton tail rabbits collected in Payne county between 1986 and 1988 using the IFA test with FITC-labelled protein G as the conjugate. Six (3.6%) were weakly positive at 1:64 dilution (unpublished data).

Confirmed human Lyme disease reported to the Oklahoma Department of Health between 1988 and 1989 were 8 and 25 respectively (Reiner *et al*, 1991). In 1990, 13 cases were reported. Human Lyme disease was made a reportable disease in Oklahoma in December of 1990. It is expected that the number of reported cases will increase in 1991 due to the mandatory reporting regulations. Most cases seem to occur from the central and eastern parts of the state, but it is not known whether these cases originated from Oklahoma or were contracted elsewhere. Oklahoma is classified as a non-endemic area for Lyme disease with the number of cases reported so far being less than 0.01 - 0.1 per 100,000 people (Piesman, 1989, unpublished data).

An ELISA for antibodies to *B. burgdorferi* was conducted on sera from white-tailed deer and dogs in Oklahoma (Mukolwe *et al.*, 1991 in press). Preliminary results show that 4.5% of the deer and 11.7% of the dogs were positive with the first deer reactors dating back to 1978. By January 1991 over 8,500 field-collected ticks including *I. scapularis, D. variabilis, Dermacentor albipictus* (Packard), and *A. americanum*, and over 90 rodents including *Peromyscus leucopus, Mus musculis, Sigmodon hispidus* and *Neotoma floridana* from Oklahoma had been examined for presence of spirochetes. Spirochetes identified as *B. burgdorferi* were recovered from the urinary bladder of one *P. leucopus* (unpublished data). Spirochetes have been recovered from *I. scapularis* and *D. albipictus* but remain to be characterized.

The principal vector for Lyme disease, *I. dammini* does not naturally occur in Oklahoma. It appears, however, that there is an inland movement of this tick from the east coast into continental U.S.A. (Spielman, 1988), and it is therefore not unthinkable that *I. dammini* could invade Oklahoma. At present, Oklahoma has an abundance of other ixodid ticks including *A. americanum*, *D. variabilis*, *I. scapularis* and *Rhipicephalus sanguinius* (Latreille). The first three species mentioned are known to feed on humans during some stage in their life cycle, and may therefore pose a danger of transmitting *B. burgdorferi* should they carry the agent. If tick-transmitted Lyme disease occurs naturally in Oklahoma, it is likely that one or more of these three tick species are involved.

The purpose of this project was be to measure the vector potential of *A. americanum, D. variabilis* and *I. scapularis* in the transmission of *B. burgdorferi*. Studies bearing some similarity to this have been conducted by Piesman (1988), and by Piesman and Sinsky (1988) with varying results. In the study by Piesman, (1988), none of the 356 *A. americanum* larvae (from Gillespie Co., Texas) exposed to infected hamsters became infected as compared to 56% of the *I. dammini* that fed on the same hamsters. When comparing *D. variabilis* and *I. dammini* that fed on the same hamster, the infection rates were 12% and 76% respectively. In the study by Piesman and Sinsky, (1988), *A. americanum* from two different locations (Alabama and Texas) were used.

The Alabama ticks were susceptible to the agent (13% infection) while the ticks from Texas were refractory. All ticks were exposed as larvae and evaluated for infection postrepletion by the indirect fluorescent antibody test (IFA). No transmission studies were tried with nymphal *A. americanum* and *D. variabilis* that were exposed as larvae based on the observation that the few nymphs that were examined did not appear to have spirochetes. The results of this last study indicate that there is a variation in susceptibility of *A. americanum* from different geographical locations to infection with *B. burgdorferi*. The difference in susceptibility may be related to the isolate of the spirochetes used and/or differences in ticks from different geographical areas. *A. americanum* naturally infected with *B. burgdorferi* have been found both in New Jersey and in Texas (Schulze *et al.*, 1984; Rawlings, 1986) suggesting that *A. americanum* can become infected and maintain the spirochete for some time under natural conditions. In the New Jersey study, 5.9% of adults and 22.2% of nymphal *A. americanum* were found to be infected . Naturally infected *D. variabilis* have also been identified in Texas (Rawlings, 1986).

Since it appears from the studies described above that there is a variation in susceptibility of *A. americanum* to *B. burgdorferi*, there was need for a study to evaluate the susceptibility of indigenous Oklahoma *A. americanum* to the spirochete. The present study differed from those described previously in that the efficacies of the three tick species,

A. americanum, *D. variabilis* and *I. scapularis* to transmit *B. burgdorferi* as adults previously exposed to infected rabbits as larvae were evaluated. In this project, we chose to use rabbits rather than *P. leucopus* because of the large number of ticks that one can feed on rabbits as compared to mice.

Specific Objectives

1. To evaluate the ability of larval *A. americanum*, *D. variabilis* and *I. scapularis* to acquire *B. burgdorferi* organisms after feeding on experimentally infected rabbits or white-footed mice.

2. To compare the infection rate of larval *A. americanum*, *D. variabilis* and *I. scapularis* allowed to feed on experimentally infected rabbits.

3. To compare the ability of nymphal *A. americanum*, *D. variabilis* and *I. scapularis* that molted from larvae that were exposed to infected rabbits to transmit *B. burgdorferi*.

4. To compare the ability of adult *I. scapularis*, *A. americanum* and *D. variabilis*, molted from nymphs, that were exposed as larvae to rabbits experimentally infected with *B. burgdorferi* to transmit the spirochete.

CHAPTER II

MATERIALS AND METHODS

General Procedures

Animals

Rabbits (New Zealand white) used in this study were reared at Oklahoma State University in the Entomology Tick Research Laboratory (ETRL). All rabbits were maintained in a tick-free state until used. White-footed mice used in the study were laboratory raised in a tick-free environment at the Oklahoma State University College of Veterinary Medicine Laboratory Animal Resources facility.

Ticks

Laboratory-raised ticks used for this study were maintained at the OSU Department of Entomology EFAW tick laboratory. Tick species used were *Amblyomma americanum* (L.), *Dermacentor variabilis* (Say) and *Ixodes scapularis* (Say). Ticks were maintained in humidity chambers (90-98% relative humidity) at 25° C with a 14-hour photoperiod. Tick-rearing procedures followed those described by Patrick and Hair, (1975).

Feeding immature Ticks on Experimental Animals

Rabbits were exposed to ticks by placing laboratory reared ticks into nylon stocking that were made to fit onto each of the rabbit's ears. The open ends of the stockings were sealed with masking tape. Rabbits were then placed in wire cages measuring approximately 17 X 40 X 20 cm (LXWXH). The cages were large enough to allow rabbit movement but small enough to restrict grooming. Rabbit cages were suspended above the bottom of open topped plastic tubs measuring approximately 60L X 60W X 40H cm. Rabbits were fed lettuce and carrots during tick feeding. Rabbit cages were placed about 5 cm above the floor of the tub on metal rods in plastic open-topped tub. Water was poured on the bottom of the tubs below the suspended cages to catch ticks as they became engorged and dropped off. The top edges of the plastic tub were taped as an additional preventive measure against tick escape.

Spirochete Agent

The spirochete agent used in this study was the JD-1 isolate of *B. burgdorferi*, originally isolated from *I. dammini* and provided to us by the Centers for Disease Control, Ft. Collins, Colorado. The spirochetes were tested for pathogenicity by inoculating and re-isolating from laboratory raised *P. leucopus* before they were used in this study.

Media for Spirochete Growth

Spirochetes were cultured in modified BSK media. The media was prepared according to the following formula:

900 ml.	Distilled water
6.0 g	HEPES (Sigma Cat # H 9136)
0.7 g	Sodium citrate
5.0 g	Neopeptone (Difco Cat # 0119-02)
5.0 g	Glucose (Sigma Cat # G 6138)
2.2 g	Sodium bicarbonate (Sigma Cat # S 5761)
2.53 g	TC Yeastolate (Difco Cat # 5577-15-5)
0.8 g	Pyruvic acid (Sodium Salt) (Sigma Cat # P 3662)
0.4 g	N-acetyl glucosamine (Sigma Cat # A 3286)
50 g	Bovine serum albumin, Fraction V. (Sigms Cat# A-4503 Lot # 69F0713)

The above components were mixed in a flask using a magnetic stir bar, care being taken to avoid excessive frothing. The pH was adjusted to 7.5 using sodium hydroxide. The mixture was filtered through a 0.45 u filter before sterilization using a 0.22 u filter. To this mixture, 100 ml. of sterile CMRL 1066 tissue culture media (10X) without glutamine (Difco) was added. A 1.0 ml. portion of the freshly made media was removed and incubated at 37° C overnight to check for sterility. The remainder of the media was stored at 4° C. After sterility was confirmed, 64 ml of sterile heat-inactivated rabbit serum (Pel Freez, Rogers, Arkansas) was added to the media followed by a gelatin mixture prepared by autoclaving 14 g of gelatin in 185 ml distilled water. Reducing agents [superoxide dismutase (Sigma Cat # 2515), (75 units per ml), dithiothreitol (Sigma (Cat# D-0632), (154 ug/ml or 1 uM) and cysteine

hydrochloride, (Sigma Cat # C 8277) (241 ug /ml or 2 uM)] were mixed in 15 ml distilled water and filter-sterilized into the media. Media was stored at 4° C until used.

Maintenance of spirochetes in *in vitro* cultures

B. burgdorferi (JD-1 isolate) were maintained in BSK media at 33-34° C. When spirochete density reached approximately 500 spirochetes per high power field they were subcultured by dispensing 100 ul from the original culture into each of 10 tubes containing 6.5 ml of fresh BSK media. These cultures were maintained at 33° C until they reached a spirochete density similar density to the original tube (approximately 10 days). At this time, 8 tubes were centrifuged at 9000 X g for 20 minutes and the sediment resuspended in phosphate-buffered saline (PBS), pH 7.4. Spirochetes from these cultures were used for animal inoculations.

Spirochete Isolation Attempts from Experimental Animals

Experimental animals were euthanatized with chloroform. Liver, spleen, kidney, heart and urinary bladder were removed aseptically and placed in sterile containers. The containers were transferred to a sterile laminar flow hood where the surface of each organ (except bladder) was seared with a red-hot spatula. A sterile portion, the size of a

dime, was then removed from inside the organ and placed in a sterile mortar. Approximately 2 ml of fresh BSK media was added and the tissue gently ground with a pestle. A 100 ul portion of media containing ground-up tissue were inoculated into a tube containing 6.5 ml BSK media. The tubes were incubated at 33-34° C and examined weekly for six weeks by dark-field microscopy for presence of spirochetes. The urinary bladder was cut open and a portion cut from the inside and placed in media. When culturing from mice, the dead mice were sprayed with 70% ethanol and placed in the hood. Whole organs were removed asceptically, ground up and put in culture as described above. Whole mouse urinary bladder was also put in culture without grinding.

When attempting to culture from skin, the portion of skin to be used (usually the ear) was shaved and thoroughly scrubbed. The area was disinfected with 70% ethanol and allowed to air dry. A skin biopsy was then taken using a sterile ear notching instrument, and put in culture. Rifampicin (Sigma Cat # R3501) was dissolved in a small volume of absolute methanol and added into BSK media (50 ug/ml) in which skin biopsies were cultured.

Animal inoculation

Experimental rabbits were divided into 3 main groups A,B,C with 7 rabbits per group. The control group consisted of 6 rabbits. Each of the main groups were divided further into two subgroups ; A-L, A-N, A-A, B-L, B-N, B-A ,C-L, C-

N, C-A. The "L", "N" or "A" at the end of the group letter indicated the stage of ticks that were feed on the rabbits, (*i.e.* larvae, nymphs or adults respectively).

Rabbits in subgroups A-L, B-L and C-L were inoculated intraperitoneally with 1.0 ml. of BSK media containing approximately 2-9 X 10^6 spirochetes. The organisms were allowed to incubate in the rabbits for 7 -10 days before rabbits were exposed to larvae. Larval ticks were allowed to feed on experimentally exposed rabbits to repletion.

The Enzyme-Linked Immunosorbent Assay (ELISA)

Antigen Preparation

Borrelia burgdorferi were grown in 10 tubes each containing 45 ml. of BSK media as described above. After 10 days, the spirochetes had reached a density of approximately 10^8 per ml. The tubes were centrifuged at 10,000 X g for 30 minutes at 4° C. The supernatant was discarded and the sedi ment resuspended in PBS pH 7.2 and centrifuged at 10,000 X g for 30 minutes. After washing a second time in PBS, the resuspended sediment was exposed to gamma irradiation (1.4 Mrad from a ^{60}Co source) to kill spirochetes. The spirochetes were examined under dark-field microscopy to determine motility and then resuspended in 10 ml of 0.1 M sodium citrate, 1.0 M sodium chloride and sonicated in a Branson Sonifer Cell Disruptor 200 (VWR Scientific, Connecticut) in 3 one-minute pulses. The resulting sonicate was centrifuged at 1000 X g for 10 minutes. The supernatant was

harvested and its protein concentration determined using the BCA assay method (Pierce Chemical Co.). The supernatant had a protein concentration of 338 ug/ml and was used as antigen for ELISA.

Checkerboard titrations were conducted using different antigen concentrations (1.25 ug/ml, 2.5 ug/ml, 5 ug/ml and 10 ug/ml) to determine the optimum antigen concentration to use for sensitizing plates for the ELISA. Different primary sera dilutions (1:125, 1:250, 1:500, 1:1000) were also tested to determine optimum serum dilution. Antigen concentration of 2.5 ug/ml and serum dilution of 1:250 were optimum.

To sensitize plates, *B. burgdorferi* antigen was diluted to 2.5 ug/ml in 0.6 M carbonate buffer, pH 9.6. Ninety-six well flat bottom plates were sensitized with 100 ul per well, sealed and incubated at 4° C overnight to 24 hours.

Plates were washed with PBS (pH 7.2) containing 0.05% Tween 20 (PBS-Tween) using a Nunc Immunowash apparatus and slapped dry on paper towels. Test sera were diluted 1:250 in PBS-Tween and added to the wells in duplicate at 100 ul per well. Two known positives and 10 known negatives were run on each plate and the last two wells on each plate were left as blanks, *i.e.*, no test serum was added to them. Positive control sera were obtained from animals immunized with whole dead spirochetes in Freund's complete adjuvant followed by two inocula in Freund's incomplete adjuvant 2 and 4 weeks later. Sera were collected one week after second

booster. Plates were sealed and incubated at 37° C in a water bath for 45 minutes followed by a wash with PBS-Tween. Rabbit antisera diluted 1:200 in PBS-Tween was added to all wells at 100 ul per well. The plates were sealed and incubated for 45 minutes followed by a wash with PBS-Tween. Substrate was prepared according to the following recipe: 10 ml. 0.5M sodium acetate buffer pH 5 + 830 ul tetramethyl benzidine solution (10 mg/ml) + 10 ul hydrogen peroxide (30%) and made up to 100 ml. with distilled water (the hydrogen peroxide was added last, the components were mixed well, and the substrate was immediately added to the wells at 100 ul per well). A blank pattern was run on an ELISA reader (Vmax, Molecular Devices) and the plate read at 690 nm. The enzyme kinetics (mOD/min excursion 1) were run for one minute with the automix on. Samples were run in duplicate and examined for correlation in numerical values and if the values were more than five units apart, the samples were assayed again. To determine the cut-off point between positive and negative values, a mean of the kinetics values of the 10 known negative samples was calculated and the standard deviation determined. Samples showing readings above mean + 3SD were considered positive while those showing readings below mean + 3SD were considered negative.

The Indirect Fluorescent Antibody (IFA) test

Suspect ticks to be evaluated for the presence of spirochetes by the IFA were dissected using Personna^{R} razor

blades and their midguts removed. Midguts from individual ticks were smeared on separate slides and air-dried for 30 minutes. Slides were then fixed in 100% acetone for 10 minutes. Slides were stored at -20° C until used. The test was conducted by covering the smeared surface on the slide with serum from a rabbit previously immunized with *B. burgdorferi* as described previously. The serum was diluted 1:64 using PBS pH 7.4. Positive controls were *B. burgdorferi* (JD-1 isolate) washed twice in PBS-Tween and fixed on slides as described above for tick midguts. Slides were incubated at 37° C in a humidity chamber for 30 minutes after which they were washed with cold PBS, pH 7.4. The smeared surface was then covered with fluorescein isothiocyonate-labelled goat anti- rabbit IgG (H + L) (Vector Laboratories) diluted 1:20 with PBS and incubated at 37° C for 30 minutes in a humidity chamber. Slides were rinsed well in cold PBS and allowed to dry in a dark room. When the slides were dry, a drop of glycerol (pH 9.6) was placed on the smeared surface and a glass cover slip placed on top. The slides were examined using epifluorescence ultraviolet light on a Lietz Orthoplan IFA Microscope, [Xenon Arc Lens (400 HBO) Exciter (KP 500), Barrier (KP 515) filters, E. Leitz Rockleigh, NJ] at the Oklahoma Animal Disease Diagnostic Laboratory, and the slides scored positive or negative for presence or absence of spirochetes.

Objective 1

<u>To evaluate the ability of larval ticks to acquire</u>

B. burgdorferi infection after exposure to infected rabbits

Rabbit 53 (belonging to group A-L) was inoculated intraperitoneally with 2.6 X 10^6 spirochetes. At 10 days postexposure, approximately 500 laboratory raised uninfected larval *I. scapularis* were allowed to feed to repletion on rabbit 53. Larvae were collected as they became replete and divided into two groups. One group (80 larvae) was placed in 2 culture tubes (30 in one and 50 in another) as described above, while the second portion (about 200) was placed in a humidity chamber and allowed to molt (see Figure 1). Seven days after the *I. scapularis* larvae were collected, approximately 1000 uninfected laboratory- raised larval *A. americanum* were allowed to feed on the same rabbit. Engorged larval *A. americanum* were placed in culture in 3 tubes each with 50 larvae and approximately 300 larvae were placed in a humidity chamber to molt. One week after *A. americanum* became replete, 1000 laboratory-raised uninfected larval *D. variabilis* were allowed to feed on the same rabbit. A total of 550 larvae were recovered of which 125 were placed in culture and the remainder allowed to molt to nymphs.

Two weeks after the *D. variabilis* larvae were collected, rabbit 53 was reinfested with *I. scapularis* larvae. Once tick feeding was complete, blood from rabbit 53 was collected in heparin, and 300 ul inoculated into one tube of BSK media. Two laboratory-raised *P. leucopus* were also inocu-

lated intraperitoneally with 0.5 ml of the blood. Mouse organs were cultured in BSK media as previously described approximately 4 weeks after the inoculation.

Culture of spirochetes from larval ticks

Larvae were divided into groups as described above and surface sterilized with 3% hydrogen peroxide for 70 seconds and with 70% ethanol for a further 70 seconds. Larvae were then ground in a sterilized tissue grinder with 1 ml of BSK media. Three tubes of BSK media were each inoculated with 200 ul portions of the tick stabilate and incubated at 34 °C. One of the 3 tubes contained rifampicin at 50 ug/ml. Cultures were examined by dark-field microscopy every 3 days for 42 days or until spirochetes were detected.

Nymphs that molted from replete larvae were used in experiments described under objective 3. A control for this experiment included rabbit 1, which was not inoculated with spirochetes but was exposed to clean larval *I. scapularis*, *D. variabilis* and *A. americanum*. Engorged larvae from rabbit 1 were cultured and the cultures monitored for spirochetes every 3 days for 42 days. Approximately 100 unfed larvae of the three tick species served as unexposed controls and were placed into culture as previously described.

Objective 2

To compare the infection rate of larval ticks fed on infected rabbits

The experiment described under objective 1 was repeated by allowing clean larval *A. americanum*, *D. variabilis* and *I. scapularis* to feed on rabbits 2,3 and E, respectively. In addition to attempting to culture spirochetes from replete larvae, 100 engorged larvae of each of the three tick species were dissected and the midguts examined under dark-field microscopy for presence of spirochetes. Ticks that were found to harbor spirochetes in their midgut were fixed on slides and the IFA test was conducted as described previously. Nymphs that molted from replete larvae in this experiment were saved and used in the experiment described under objective 3.

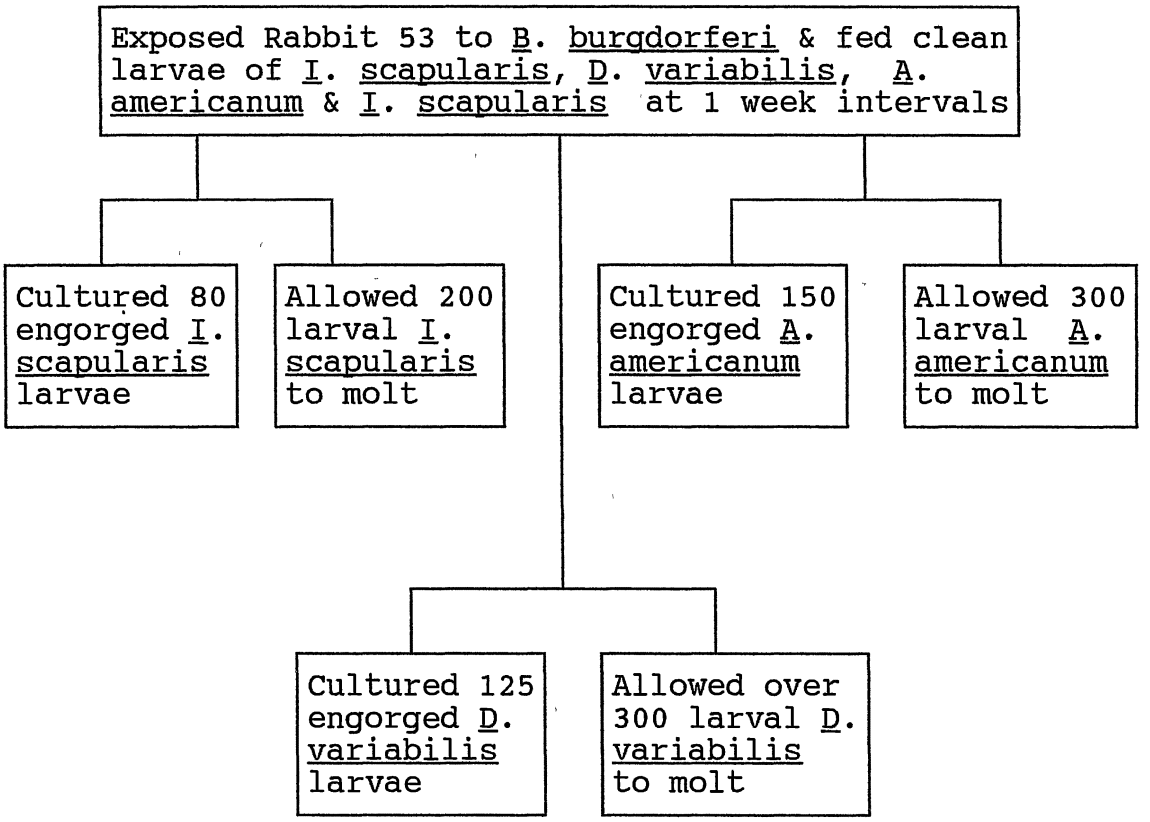
Objective 3

To compare the vector potential of nymphs of the three tick species that were exposed to infected rabbits as larvae

Nymphal ticks that molted from larvae previously exposed to infected rabbits or infected mice (as described under objectives 1 and 2 above) were allowed to feed on uninfected rabbits in subgroups A-N, B-N and C-N to determine if nymphs would transmit *B. burgdorferi*. Pre-exposure sera samples were drawn from all rabbits before exposure to nymphs. Rabbits were bled 10 days after exposure for culture, and every 2 weeks for 8 weeks for serology.

Nymphal *A. americanum* that had molted from larvae fed on rabbit 53 were fed on rabbits 010 and 50 while nymphal

Figure 1: Experimental protocol illustrating exposure of clean larval *I. scapularis*, *A. americanum* and *D. variabilis* to rabbit 53 which was experimentally exposed to *B. burgdorferi* intraperitoneally.


Exposed Rabbit 53 to B. burgdorferi & fed clean larvae of I. scapularis, D. variabilis, A. americanum & I. scapularis at 1 week intervals
Cultured 80 engorged I. scapularis larvae
Allowed 200 larval I. scapularis to molt
Cultured 150 engorged A. americanum larvae
Allowed 300 larval A. americanum to molt
Cultured 125 engorged D. variabilis larvae
Allowed over 300 larval D. variabilis to molt

D. variabilis were fed on rabbit 18. Rabbits C and 005 were infested with nymphal *I. scapularis* that were fed as larvae on an experimentally-infected *P. leucopus*. Between 8 and 10 weeks after exposure to nymphs, rabbits were euthanatized and their organs cultured as described previously in an attempt to isolate spirochetes.

Rabbit infestation with nymphs was repeated on rabbit 16 (exposed to nymphal *D. variabilis* that were fed on rabbit 3 as larvae) and on rabbit 17 which were infested with nymphal *D. variabilis* that had been fed on an experimentally-infected *P. leucopus* as larvae. Rabbits A and B were exposed to *A. americanum* nymphs that were previously exposed to Rabbit 2 as larvae.

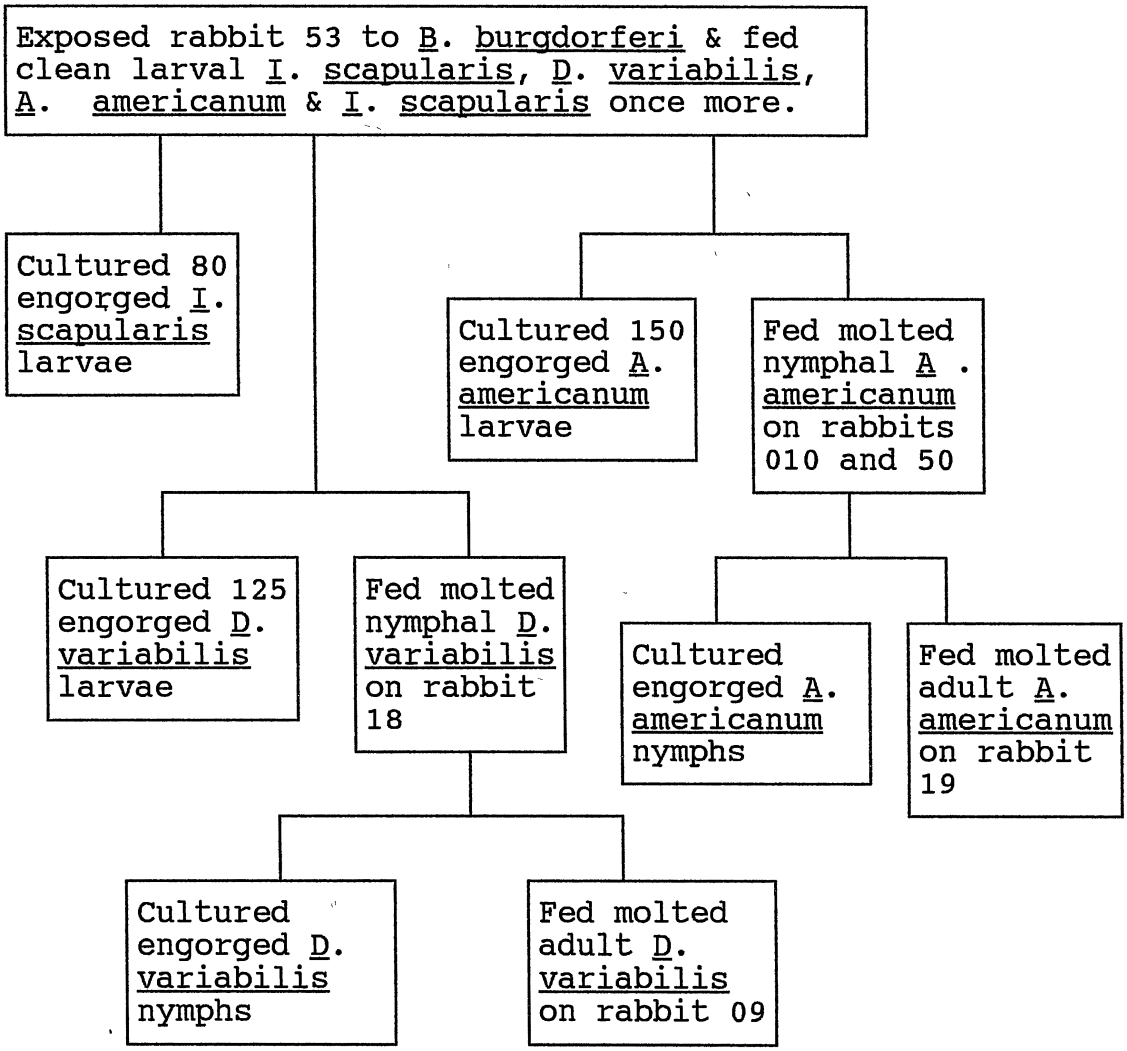
Objective 4

To compare the vector potential of adult ticks that were exposed to infected rabbits as larvae

Fifty adult *D. variabilis* were allowed to feed on rabbit 10 as described previously. These adults had previously fed on infected *P. leucopus* as larvae and on rabbit 17 as nymphs. Rabbit 10 was bled before exposure to the adult ticks. Four weeks after exposure, rabbit 10 was bled, euthanatized and organs were placed in culture as described above.

Rabbit 19 was infested with 50 adult *A. americanum* that were fed on rabbit 53 as larvae and on rabbits 010 and 50 as nymphs. Figure 2 summarizes experiments described above.

Figure 2: Experimental protocol showing tick feeding experiments.


Exposed rabbit 53 to B. burgdorferi & fed clean larval I. scapularis, D. variabilis, A. americanum & I. scapularis once more.
Cultured 80 engorged I. scapularis larvae
Cultured 150 engorged A. americanum larvae
Fed molted nymphal A. americanum on rabbits 010 and 50
Cultured 125 engorged D. variabilis larvae
Fed molted nymphal D. variabilis on rabbit 18
Cultured engorged A. americanum nymphs
Fed molted adult A. americanum on rabbit 19
Cultured engorged D. variabilis nymphs
Fed molted adult D. variabilis on rabbit 09

CHAPTER III

RESULTS

Objective 1

To evaluate the ability of larval *I. scapularis* *A. americanum* and *D. variabilis* to acquire *B. burgdorferi* organisms after feeding on experimentally infected rabbits or white-footed mice

Spirochetes were first seen by dark-field microscopy in two cultures containing 30 and 50 replete larval *I. scapularis* that had fed on rabbit 53. Spirochetes were seen 7 days after larvae were put in culture. Cultures were heavily contaminated with bacteria. There was approximately 1 spirochete per 2 40X fields. Spirochetes ranged in length from 17.6 - 28.6 um. Both motile and non-motile organisms were observed.

No spirochetes were observed in cultures of larval *A. americanum* or *D. variabilis* that were fed on infected Rabbit 53. After 6 weeks, cultures were assumed negative.

Four replete larvae were recovered from the second group of larval *I. scapularis* feed on rabbit 53. No spirochetes were detected in the one larva that was examined by dark-field microscopy. Cultures of the 3 larvae were contam-

inated with other bacteria but also contained spirochetes. The spirochetes were few in number (1 per 4-5 40X fields) and non-motile.

Spirochetes were not observed in control cultures containing 100 unfed larvae of each of the three tick species through 6 weeks of incubation.

In the second trial of objective 1, attempts were made to culture spirochetes from larval *A. americanum, D. variabilis* and *I. scapularis* exposed to infected rabbits 2,3, and E respectively. Spirochetes were again recovered from cultures of *I. scapularis* but none were seen in those of *A. americanum* and *D. variabilis*.

Objective 2

To compare the infection rates of larval *I. scapularis* *D. variabilis* and *A. americanum americanum* fed on an experimentally infected rabbit

Forty percent of replete *I. scapularis* larvae obtained after feeding on rabbit 53 molted through to the nymphal stage after 3 months. Approximately 90% of larval *A. americanum* and *D. variabilis* fed on rabbit 53 molted to nymphs in approximately 35 days. At repletion, 50 *I. scapularis* fed on rabbit 53 were examined for the presence of spirochetes by dark-field microscopy. Spirochetes were seen in 10 (20%) of the ticks. No spirochetes were observed in the 100 replete larval *A. americanum* or *D. variabilis* examined. *I. scapularis* larvae that were determined positive by dark-

field microscopy, and a similar number that were negative were prepared for IFA. Positive fluorescence was seen on all ticks in which spirochetes were previously seen. Fluorescence was weak and the spirochete numbers few (between 20 and 30 per tick). By contrast, no fluorescence was observed in the ticks that were negative by dark-field microscopy.

This study was repeated using 100 larvae from each of the three tick species that fed on infected rabbits 2,3 and E. No spirochetes were observed by dark-field microscopy in larval *A. americanum* or *D. variabilis*. An average of 25 spirochetes per tick were demonstrated by dark-field microscopy in 18 (18%) larval *I. scapularis* that fed on rabbit E. Larval *I. scapularis* in which spirochetes were detected by dark-field microscopy and a similar number in which spirochetes were not observed were prepared for IFA. Fluorescence was observed in all positive ticks. Fluorescence was again weak but spirochetes were easy to identify. On average, 30 spirochetes per tick were seen by IFA. No fluorescence was observed by IFA in ticks that were negative by dark-field microscopy.

This study was repeated with larvae fed on rabbits 55A, 56A, and 57A with similar results to those observed in the first two trials. Twenty one of 100 larval *I. scapularis* were positive for spirochetes by dark-field microscopy. Spirochetes were not detected in any of the larvae of *A. americanum* or *D. variabilis* (Table 1).

Objective 3.

To compare the ability of nymphal *I. scapularis* *A. americanum* and *D. variabilis* exposed to infected rabbits/mice to transmit *B. burgdorferi*.

All pre-exposure sera samples from rabbits tested negative by ELISA for antibodies to *B. burgdorferi*. Rabbits used in the study also tested negative by IFA for antibodies to *Leptospira conicola, L. grippotyphosa, L. hardjo, L. icterohaemorrhagiae, L. pomona* and *L. bratislava*.

All rabbits inoculated with *B. burgdorferi* sero-converted, as determined by ELISA, two weeks after inoculation, and remained positive until they were euthanatized.

Rabbit C, infested with about 50 nymphal *I. scapularis* that had fed on infected *P. leucopus* as larvae, developed detectable antibodies to *B. burgdorferi* at 10 weeks post-exposure. Of the 50 nymphal *I. scapularis* exposed to rabbit C, only 16 were recovered of which 14 were replete and two partially engorged. Rabbit 005 was infested with 30 nymphal *I. scapularis* that fed on a experimentally infected *P. leucopus* as larvae. Antibodies were detected by ELISA in this rabbit 14 weeks after exposure to nymphal *I. scapularis*.

Rabbits A, B, 010 and 50 were exposed to nymphal *A. americanum* that had been fed on infected rabbits as larvae. Over 900 replete nymphs were recovered from each of the four rabbits. Antibodies to *B. burgdorferi* were not detected in

any of these through 12 weeks post-exposure.

Rabbits 16 and 18 were infested with *D. variabilis* that were fed on infected rabbits as larvae while rabbit 17 was infested with nymphal *D. variabilis* that had fed on infected *P. leucopus* as larvae. Over 150 nymphs were recovered from each of rabbits 16 and 18, while over 50 were recovered from rabbit 17. Rabbits 17 and 18 did not seroconvert during 10 weeks post-nymphal repletion. However rabbit 16 showed a positive ELISA titer, 10 weeks after exposure to

TABLE I

INFECTION RATES IN LARVAL *I. SCAPULARIS, A. AMERICANUM* AND *D. VARIABILIS*, AFTER EXPOSURE TO INFECTED RABBITS. INFECTION RATES WERE DETERMINED BY DARK-FIELD MICROSCOPY AND BY IFA

	Number of ticks examined (# positive)		
	I. scapularis	*A. americanum*	*D. variabilis*
53	50(10)	100 (0)	100 (0)
E	100(20)	nd	nd
2	nd	100 (0)	nd
3	nd	nd	100 (0)
55A	100(21)	nd	nd
56A	nd	100 (0)	nd
57A	nd	nd	100 (0)

nd= not determined

nymphal *D. variabilis*.

Attempts were made to culture spirochetes from organs of experimental and control rabbits in this study. Recovery was successful in 3 of 7 rabbits infected with *B. burgdorferi* by intraperitoneal inoculation, and in both rabbits exposed to nymphal *I. scapularis*. In 3 of 5 cases, recov-

ery was made from spleen cultures while in 2 cases, spirochetes were cultured from the urinary bladder and from kidneys.

No spirochete isolations were made from rabbits infested with nymphal *A. americanum* or *D. variabilis* that were previously fed on infected rabbits or mice as larvae.

Objective 4

To evaluate the ability of adult *I. scapularis*, *A. americanum* and *D. variabilis* to transmit *B. burgdorferi* after molting from nymphs that were exposed to infected rabbits/mice as larvae

Rabbit 19 was infested with 50 adult *A. americanum* that had fed on infected rabbit 53 as larvae and on rabbits 010 and 50 as nymphs. A total of 32 replete adults were recovered from rabbit 19. Antibodies were not detected in sera from rabbit 19 through 8 weeks after feeding.

Rabbit 10 was infested with 50 adult *D. variabilis* that had fed on infected *P. leucopus* as larvae and on rabbit 17 as nymphs. Antibodies were not detected in sera from Rabbit 10.

Ixodes scapularis nymphs did not molt to adults, therefore transmission attempts of *B. burgdorferi* using adult *I. scapularis* were not attempted.

CHAPTER IV

DISCUSSION

The inability to recover spirochetes in cultures of *A. americanum* larvae that fed an infected rabbits are consistent with those previously reported. Piesman (1988) recovered 356 engorged larval *A. americanum* from an infected hamster, but failed to detect spirochetes in any of the ticks. In another study using *A. americanum* from Texas and Alabama, Piesman and Sinsky (1988) demonstrated a 13% infection rate in engorged *A. americanum* larvae from Alabama while the Texas ticks were refractory. Occurence of naturally-infected *A. americanum* have been reported in Texas, (Rawlings, 1986)and in New jersey, Shultze *et al.*, (1984). In the New Jersey study, both nymphal and adult *A. americanum* had *B. burgdorferi* as demonstrated by IFA. Reports of transmission of *B. burgdorferi* by *A. americanum* have not been found.

Laboratory-reared larval *D. variabilis* acquired *B. burgdorferi* after feeding on an infected hamster and had an infection rate of 19% (Piesman, 1988). The infection appeared short-lived, dropping from 19% one day after repletion, to 8.3% eight days later, and to 0% at 38 days post repletion. Spirochetes could not be detected in nymphs molting from these larvae. In the present study no spiro-

chetes were recovered by culturing *D. variabilis* larvae that had fed on infected rabbits. It is not known if there is a difference in susceptibility of *D. variabilis* to different isolates of *B. burgdorferi*, or if *D. variabilis* from different geographical locations show differential susceptibility.

The percentage of larval *I. scapularis* in which spirochetes were demonstrated was consistent in the three trials (18% in the first, 20% in the second and 21% in the third). These figures were considerably lower than those reported by Piesman, (1988) and Piesman and Sinsky, (1988) for *I. dammini* where 56% and 76% infection rates were reported respectively. Piesman, (1988) also reported 83% infection rates in larval *I. scapularis* fed on hamsters exposed to the JD-1 isolate of *B. burgdorferi*. Infection rates in field-collected nymphal *I. dammini* acquired as larvae vary with geographical location. Magnarelli and Anderson, (1988) reported infection rates of 36.2% for field-collected immature *I. dammini* in Connecticut while Ciesielski *et al.*, (1988) reported infection rates of 3% from *I. pacificus* on the west coast and of 90% in *I. dammini* along certain parts of the north-eastern atlantic coastline. It is not known whether the difference in infection rates is due to difference in exposure to infected animals, the isolate of *B. burgdorferi*, or differences in tick susceptibility from different geographical regions. The infection rates of *I. scapularis* in this study are comparable with those reported for other species of *Ixodes*.

The factors which determine whether a particular *Ixodes* sp. larva will acquire infection when exposed to an infected animal are not clear. Piesman, (1988) suggested that there may be unique physiological characteristics of ticks in the genus *Ixodes* that enable them to serve as vectors for *B. burgdorferi*. It would appear that any hematophagus arthropod could take circulating spirochetes along with a blood meal. The ability of these arthropods to maintain *B. burgdorferi* biologically appears to be restricted, however. *B. burgdorferi* have been demonstrated in deer flies, mosquitoes, horse flies (Magnarelli and Anderson, 1988) and in fleas (Rawlings, 1986), but transmission by these insects has not been demonstrated.

In the second *I. scapularis* feeding trial on rabbit 53, only four engorged larvae were recovered. Although the sole tick examined by dark-field microscopy was not confirmed to be infected, the three that were cultured yielded spirochetes. The yield of spirochetes from the three larvae was significant in at least two ways; (i) it documented that rabbit 53 was still infected several weeks after initial exposure to *B. burgdorferi* and after feeding by larval *A. americanum* and *D. variabilis*, both of which failed to acquire the infection and (ii) it showed that spirochetes can be recovered from a small number of infected larvae.

Rabbits A, B and 010 infested with large numbers of *A. americanum* nymphs that had fed on infected animals as larvae were sero-negative for the entire 10 week period of testing. These results are consistent with our inability to

detect spirochetes in *A. americanum* larvae exposed to infected animals that had feed on these rabbits.

Rabbits 16,17 and 18 infested with nymphal *D. variabilis* showed mixed results with one of three rabbits exposed (# 16) showing a positive reaction on ELISA. Although rabbit 16 had a positive titer when it was killed 10 weeks post exposure, no spirochetes were detected in any of the ticks examined. Piesman, (1988) showed that *D. variabilis* larvae are capable of acquiring infection with *B. burgdorferi* with infection rates as high as 20%, but that infection was lost by the time larvae molted to nymphs. However, nymphal *D. variabilis* which molted from larvae that were exposed to infected hamsters in that study were not fed on naive animals to evaluate transmission. The results of this study indicate that transmission by *D. variabilis* nymphs previously exposed to infected rabbits as larvae may have occurred although we were unable to detect spirochetes in either the nymphs or the rabbit on which they fed. If *D. variabilis* larvae picked up spirochetes, it is possible that the infection rate was very low (less than 1%) and therefore easy to miss. If this is true, it would explain why only one of three rabbits that were infested with nymphal *D. variabilis* seroconverted despite the use of very large numbers of nymphs (approximately 900 per rabbit). All rabbits were individually caged thus eliminating chances of contact transmission.

Rabbits 10 and 19, which were infested with adult *D.*

variabilis and adult *A. americanum* respectively, did not sero-convert. Organisms were not recovered from *in vitro* organ cultures from these rabbits.

Previous studies on recovery of *B. burgdorferi* from infected mammals have shown variation in recovery rates. In our laboratory, we have had success in recovering spirochetes from the urinary bladder of infected *P. leucopus*, but have variable results from other organs. More studies are needed to determine the optimal time(s) and organs/tissues for isolation of *B. burgdorferi* from infected mammals.

CHAPTER V

SUMMARY AND CONCLUSIONS

This study was conducted to evaluate the ability of larval *Ixodes scapularis, Dermacentor variabilis* and *Amblyomma americanum* to acquire *Borrelia burgdorferi*, the etiological agent for Lyme disease after feeding on laboratory infected rabbits or white-footed mice (*Peromyscus leucopus*). The ability of nymphs and adults that molted from these larvae to transmit *B. burgdorferi* was also evaluated.

Spirochetes were demonstrated in 20% of replete larval *I. scapularis* by dark-field microscopy and by IFA. No spirochetes were demonstrated in larval *A. americanum* or *D. variabilis*. Rabbits inoculated with *B. burgdorferi* seroconverted 2 weeks after exposure and remained positive for approximately 10 weeks at which time they were euthanized and tissues cultured for spirochetes. Antibodies to *B. burgdorferi* were detected by indirect kinetic ELISA in two of two rabbits exposed to nymphal *I. scapularis* approximately 10 weeks post exposure. Antibodies were detected in one of three rabbits exposed to nymphal *D. variabilis*, but none were detected in four of four rabbits on which nymphal *A. americanum* fed. Rabbits infested with adult *D. variabilis*

and with adult *A. americanum* did not sero-convert. Nymphal *I. scapularis* did not molt through to adults and were presumed dead after 7 months.

Spirochetes were cultured from spleen, kidney and urinary bladder in three of seven rabbits inoculated with spirochetes and in two of two rabbits infested with infected *I. scapularis* nymphs. No spirochetes were recovered from rabbits infested with nymphal or adult *A. americanum* or *D. variabilis* that had been fed on infected animals previously.

If the results of our study utilizing the JD1 isolate of *B. burgdorferi* and laboratory reared *A. americanum*, *D. variabilis* and *I. scapularis* are indicative of naturally occuring events, it appears that only *I. scapularis* is capable of acquiring and transmitting *B. burgdorferi* where this species occurs. Although infection rates in field collected *I. scapularis* are much lower than those of *I. dammini*, (probably due to exposure to few infected animals) the former tick appears to be an efficient vector of *B. burgdorferi* and may play a significant role in the epidemiology of Lyme disease in geographical areas in which *I. dammini* does not exist. Further studies are needed to determine the host preferences and susceptibility of each of the life stages of this potential vector. Although there may have been transmission with *D. variabilis*, the role played by this tick in the epidemiology of Lyme disease appears limited and may require further investigation to verify its

significance in Lyme disease transmission.

LITERATURE CITED

Anderson, J.F., L.A. Magnarelli, and W. Burgdorfer. 1983. Spirochetes in *Ixodes dammini* and mammals from Connecticut. Am. J. Trop. Med. Hyg. 32: 818-824.

Anderson, J.F., R.C. Johnson, L.A. Magnarelli, and F.W. Hyde. 1985. Identification of endemic foci of Lyme disease: isolation of *Borrelia burgdorferi* from feral rodents and ticks (*Dermacentor variabilis*). J. Clin. Microbiol. 22: 36-38.

Anderson, J.F., R.C. Johnson, L.A. Magnarelli, and F.W. Hyde. 1986a. Involvement of birds in the epidemiology of the Lyme disease agent *Borrelia burgdorferi*. Infec. Immuno. 51: 394-396.

Anderson, J.F., R.C. Johnson, L.A. Magnarelli, F.W. Hyde, and J.E. Myers. 1986b. *Peromyscus leucopus* and *Microtus pennslyvanicus* simultaneously infected with *Borrelia burgdorferi* and *Babesia microti*. J. Clin. Microbiol. 23: 135-137.

Anderson, J.F., P.H. Duray, and L.A. Magnarelli. 1987. Prevalence of *Borrelia burgdorferi* in white-footed mice and *Ixodes dammini* at Fort McCoy. Wis. J. Clin. Microbiol. 25: 1495-1497.

Anderson, J.F. 1988. Mammalian and avian reservoirs for *Borrelia burgdorferi*. Ann. NY Acad. Sci. 539: 180-191.

Anderson, J.F., L.A. Magnarelli, R.B. LeFebrvre, T.G. Andreadis, J.B. McAninch, G. Perng, and R.C. Johnson. 1989. Antigenically variable *Borrelia burgdorferi* isolated from cottontail rabbits and *Ixodes dentatus* in rural and urban areas. J. Clin. Microbiol. 27: 13-20.

Asbrink, E. and A. Hovmark. 1988. Early and late cutaneous manifestations in *Ixodes*-borne Borreliosis (Erythema Migrans Borreliosis, Lyme Borreliosis). Ann. NY Acad. Sci. 539: 4-15.

Barbour, A.G. 1984. Isolation and cultivation of Lyme disease spirochetes. Yale J. Biol. Med. 57: 521-525.

Barbour, A.G. 1986. Cultivation of *Borrelia*: A Historical Overview. Zbl. Bakt. Hyg. A 263: 11-14.

Barbour, A.G., S.F. Hayes, R.A. Heiland, M.E. Schrumpf, and S.L. Tessier. 1986. A *Borrelia*-specific monoclonal antibody binds to a flagellar epitope. Infect. Immunol. 52: 549-554.

Barbour, A.G., R.A. Heiland and T.R. Howe. 1985. Heterogeneity of major proteins in Lyme disease borreliae; a molecular analysis of North American and European isolates. J. Infect. Dis. 152: 478-484.

Barbour, A.G., S.L. Tessier, and S.F. Hayes. 1984. Variation in major surface protein of Lyme disease spirochetes. Infect. Immun. 45: 94-100.

Barbour, A.G., S.L. Tessier, and W.J. Todd. 1983. Lyme disease spirochetes and ixodid tick spirochetes share a common surface antigenic determinant defined by a monoclonal antibody. Infect. Immuno. 41: 795-804.

Benach, J.L., E.M. Bosler, J.P. Hanrahan, J.L. Coleman, T.F. Bast, G.S. Habicht, D.J., Cameron, J.L., Ziegler, W. Burgdorfer, A.G. Barbour, R. Edelman, and R.A. Kaslow. 1983. Spirochetes isolated from the blood of two patients with Lyme disease. N. Engl. J. Med. 308: 740-742.

Bisset, M.L., and W. Hill. 1987. Characterization of *Borrelia burgdorferi* strains isolated from *Ixodes pacificus* ticks in California. J. Clin. Microbiol. 25: 2296-2301.

Bosler, E. M., J.L. Coleman, J.L. Benach, D.A. Massey, J.P. Hanrahan, W. Burgdorfer, and A.G. Barbour. 1983. Natural distribution of the *Ixodes dammini* spirochete. Science 220: 321-322.

Bosler, E.M., B.G. Ormiston, J.L. Coleman, J.P. Hanrahan, and J.L. Benach. 1984. Prevalence of the Lyme disease spirochete in populations of white-tailed deer and white-footed mice. Yale J.Biol. Med. 57: 651-659.

Burgdorfer, W., Barbour, A.G., Hayes, S.F., Benach, J.L., Grunwaldt, E., and J.P. Davis. 1982. Lyme disease - a tick borne spirochetosis? Science 216: 1317-1319.

Burgdorfer, W., R.S. Lane, A.G. Barbour, R.A. Gresbrink, and J.R. Anderson. 1985. The western Black-legged tick, *Ixodes pacificus*: a vector of *Borrelia burgdorferi*. Am. J. Trop. Med. Hyg. 34: 925-930.

Burgdorfer, W. 1986. Discovery of the Lyme disease spirochete: A historical review. Zbl. Bakt. Hyg. A 263 7-10.

Burgdorfer, W., S.F. Hayes, and D. Corwin. 1989. Pathophysiology of the Lyme Disease spirochete, *Borrelia burgdorferi*, in ixodid ticks. Reviews of Infectious Diseases. II. Suppl. 6, s1442-s1450.

Burgess, E.C. 1986a. Experimental infection of dogs with *Borrelia burgdorferi*. Zbl. Bakt. Hyg. A, 263: 49-54.

Burgess, E.C. 1986b. Natural exposure of Wisconsin dogs to the Lyme disease spirochete. Lab Anim Sci. 36: 288-290.

Burgess, E.C., A. Gendron-Fitzpatrick, and W.O. Wright. 1987. Arthritis and systemic disease caused by *Borrelia burgdorferi* infection in a cow. J. Am. Vet. Med. Ass. 191: 1468-1469.

Burgess, E.C. 1988. *Borrelia burgdorferi* infection in Wisconsin horses and cows. Ann. NY Acad. Sci. 539: 235-243.

Burgess, E.C., T.E. Amundson, J.P. Davis, R.A. Kaslow and R. Edelman. 1986. Experimental inoculation of *Peromyscus* spp. with *Borrelia burgdorferi*: evidence of contact transmission. Am. J. Trop. Med. Hyg. 35: 355-359.

Centers for Disease Control. 1985. Update: Lyme disease and cases occurring during pregnancy - United States. Morbid. Mortal. Weekly Rep. 34: 376-384.

Ciesielski, C.A., L.E Markowitz, R. Horsely, A.W. Hightower, H. Russell, and C.V. Broome. 1988. The geographic distribution of Lyme disease in the United States. Ann. NY Acad. Sci. 539: 283-288.

Cohen, D., E.M. Bosler, W. Bernard, D. Meirs II, R. Eisner, and T. Schulze. 1988. Epidemiologic studies of Lyme disease in horses and their public health significance. Ann. NY Acad. Sci. 539: 244-257.

Dammin, G.J. 1989. Erythema migrans: A Chronicle. Rev. Infect. Dis. 2: 142-151.

Dekonenko, E.J., A.C. Steere, V.P. Berardi, and L.N. Kravchuk. 1988. Lyme borreliasis in the Soviet Union: a cooperative US-USSR report. J. Infect. Dis. 158: 748-753.

Fivaz, B.H. and T.T. Petney. 1989. Lyme Disease - A New Disease in Southern Africa? J. S. Afr. Vet. Assoc. 60: 155-158.

Fivaz, B.H., P. Botha, and L.M. Cairns. 1990. A putative outbreak of equine Lyme borreliosis in Natal. J. S. Afr. Vet. Assoc. 61: 128-129.

Godsey, M.S., Jr., T.E. Amundson, E.C. Burgess, W.Schell, J.P. Davis, R. Kaslow, and R. Edelman. 1987. Lyme disease ecology in Wisconsin. Distribution and host preferences of *Ixodes dammini*, and prevalence of antibody to *Borrelia burgdorferi* in small mammals. Am. J. Trop. Med. Hyg. 37: 180-187.

Habicht, G.S., G. Beck, and J.L. Benach. 1988. The role of Interleukin-1 in the pathogenesis of Lyme disease. Ann. NY Acad. Sci. 539: 80-86.

Johnson, R.C., F.W. Hyde, and C.M. Rumpel. 1984a. Taxonomy of the Lyme disease spirochetes. Yale J. Biol. Med. 57: 529-537.

Johnson, R.C., G.P. Schmid, F.W. Hyde, A.G. Steigerwalt and D.J. Brenner. 1984 b. *Borrelia burgdorferi* sp. nov: Etiologic agent of Lyme disease. Int. J. Syst. Bact. 34: 496-497.

Johnson, R.C., C. Kodner, M. Russell, and P.H. Durray. 1988 Experimental infection of the hamster with *Borrelia burgdorferi*. Ann. NY Acad. Scien. 539:258-263.

Kazmierczak, J.J., E.C. Burgess and E.A. Terry. 1988. Susceptibility of the gray wolf (*Canis lupus*) to infection with the Lyme disease agent, *Borrelia burgdorferi*. J. Wild. Dis. 24 (3): 522-527.

Kazmierczak, J.J. and E.C. Burgess. 1989. Antibodies to *Borrelia* spp. in wild foxes and coyotes from Wisconsin and Minnesota. J. Wild. Dis. 25(1): 108-111.

Kelly, R. 1971. Cultivation of *Borrelia hermsii*. Science 173: 443.

Kelly, R. T., 1976. Cultivation and physiology of relapsing fever borreliae. In: R.C. Johnson (ed.). The Biology of Parasitic Spirochetes. Academic Press, New York. pp. 87-94.

Kornblatt, A.N., P.H. Urband, and A.C. Steere. 1985. Arthritis caused by *Borrelia burgdorferi* in dogs. Journal of the Am. Vet. Med. Assoc. 186: 960-964.

Krampitz, H.E. 1986. In vivo isolation and maintenance of some wild strains of European hard tick spirochetes in mammalian and arthropod hosts: a parasitologist's view. Zbl. Bakt. Hyg Hyg [A] 263: 21-28.

Lane, R.S. and W. Burgdorfer. 1987. Transovarial and Transstadial passage of *Borrelia burgdorferi* in the western black-legged tick, *Ixodes pacificus* (Acari:Ixodidae). Am. J. Trop. Med. Hyg. 37(1) pp. 188-192.

Levine, J.F., M.L. Wilson, and A. Spielman. 1985. Mice as reservoirs of Lyme disease spirochete. Am. J. Trop. Med. Hyg. 34: 355-360.

Loken, K.I., C. Wu, R.C.Johnson, and R.F. Bey. 1985. Isolation of the Lyme disease spirochete from mammals in Minnesota. Proc. Soc. Exp. Biol. Med. 179:300-302.

Magnarelli, L.A., 1988. Serologic Diagnosis of Lyme disease. Ann. NY Acad. Sci. 539: 154-159.

Magnarelli, L.A. & J.F. Anderson. 1986. Early detection and persistence of antibodies to *Borrelia burgdorferi* in persons with Lyme disease. Zbl. Bakt. Hyg. A 263:392-399.

Magnarelli, L.A., J.F. Anderson, A.B. Schreier,and C.M. Ficke. 1987. Clinical and serologic studies of canine borreliosis. J. Am. Vet. Med. Assoc., 191: 1089-1094.

Magnarelli, L.A. and Anderson, J.F. 1988. Ticks and biting insects infected with the etiologic agent of Lyme disease, *Borrelia burgdorferi*. J. of Clin. Micro. 26:1482-1486.

Marcelis, L., P. De Marneffe, *et al*. 1987. Horse reservoir for *Borrelia burgdorferi*? Lancet 1(8539):977.

Moffat, C.M., L.H. Sigal, A.C. Steere, D.H. Freeman and J.M. Dwyer. 1984. Cellular immune findings in Lyme disease: Correlation with serum IgM and disease activity. Am. J. Med. 77:625-632.

Mukolwe, S. W., A.A. Kocan and J.H. Wyckoff III. A Lyme disease serological survey in dogs and white-tailed deer in Oklahoma. Submitted to the NY Acad. Sci., April, 1991.

Patrick, C.D. and J.A. Hair. 1975. Laboratory rearing procedures and equipment for multi-host ticks (Acarina:Ixodidae). J. Med. Entomol. 12: 38-390.

Pennell, D., P. Wand, and R. Schell. 1987. Evaluation of a quantitative fluorescence immunoassay (FIAX) for detection of serum antibody to *Borrelia burgdorferi*. Journal of Clin. Microb. 25:2218-2220.

Piesman, J. 1988. Vector competence of ticks in the southeastern United States for *Borrelia burgdorferi*. Ann. NY Acad. Sci. 539: 417-418.

Piesman, J. and R. Sinsky. 1988. Ability of *Ixodes scapularis*, *Dermacentor variabilis*, and *Amblyomma americanum* (Acari:Ixodidae) to acquire, maintain, and transmit Lyme disease spirochetes (*Borrelia burgdorferi*) J. Med. Entomol. 25(5):336-339.

Piesman J. 1989. Personal communication. Society of Vector Ecology meeting, Norman, Oklahoma. Nov. 12-15 1989.

Piesman, J., J.G. Donahue, T.N. Mather, and A. Spielman. 1986. Transovarially acquired Lyme disease spirochetes (*Borrelia burgdorferi*) in field-collected larval *Ixodes dammini* (Acari: Ixodidae). J. Med. Entomol. 23:219.

Piesman, J., J.R. Oliver, and R.J. Sinsky. 1990. Growth Kinetics of the Lyme disease spirochetes (*Borrelia burgdorferi*) in vector ticks (*Ixodes dammini*). Am. J. Trop. Med. Hyg. 42(4):352-357.

Rawlings, J. A. 1986. Lyme disease in Texas. Zbl. Bakt. Hyg. A 263, 483-487.

Reiner, K.L., M.M. Huycke, and S.J.B. McNabb. 1991. The Descriptive Epidemiology of Lyme Disease in Oklahoma. Okla. State Med. Asso. J. (in press).

Rodgers, S.J., R.J. Morton, and C.A. Baldwin. 1989. A serological survey of *Ehrlichia canis, Ehrlichia equi, Rickettsia rickettsii*, and *Borrelia burgdorferi* in dogs in Oklahoma. J. Vet. Diag. Invest. 1:154-159.

Russel, H., J.S. Sampson, G.P. Schmid, H.W. Wilkinson and B. Plikaytis. 1984. Enzyme-linked immunosorbent assay and indirect immunofluorescence assay for Lyme disease. J. Infect. Dis. 149:465-470.

Scrimenti, R.S. 1970. Erythema chronicum migrans. Archives of Dermatology 102: 104-105.

Schwan, T.G. and W. Burgdorfer. 1987. Antigenic changes of *Borrelia burgdorferi* as a result of *in vitro* cultivation. J. Infect. Dis., 156: 852-853.

Schwan, T.G., Burgdorfer, W., Garon, C.F. 1988. Changes in infectivity and plasmid profile of the Lyme disease spirochete, *Borrelia burgdorferi*, as a result of *in vitro* cultivation. Infect. Immun. 56: 1831-1836.

Shrestha, M., R.L. Grodzicki and A.C. Steere. 1985. Diagnosing early Lyme disease. Am. J. Med. 78:235-240.

Schulze, T.L., G.S. Bowen, E.M. Bosler, M.F. Lakat, W.E. Parkin, R. Altman, B.G. Ormiston and J.K. Shisler. 1984. *Amblyomma americanum*: a potential vector for Lyme disease in New Jersey. Science. 224: 601-603.

Spielman, A. 1988. Prospects for suppressing transmission of Lyme disease. Ann NY Acad Sci 539:212-220

Stanek, G., M. Pletschette, H. Flamm., A.M. Hirschl., E. Aberer, W. Kristoferitsch and E. Schmutzhard 1988. European Lyme Borreliosis. Ann NY Acad Sci. 539: 274-282.

Steere, A.C., S.E. Malawista, J.A. Hardin., S. Ruddy, P.W. Askenase, and W.A. Andiman. 1977. Erythema chronicum migrans and Lyme arthritis: the enlarging clinical spectrum. Ann Intern Med 86: 685-698.

Steere, A.C., R.L. Grodzicki, A.N. Kornblatt, J.E. Craft, A.G. Barbour, W. Burgdorfer, G.P. Schmidt, E. Johnson, and S.E., Malawista. 1983a. The spirochetal etiology of Lyme disease. New Engl. J. Med. 308: 733-740.

Steere, A.C., N.H. Bartenhagen, J.E. Craft, G.J. Hutchison, J.H. Newman, D.W. Rahn, L.H. Sigal, P.N. Spieler, P.N, K.S., and S.E. Malawista. 1983b. The early clinical manifestations of Lyme disease. Ann. Intern. Med. 99: 76-82.

Steere, A.C., S.E. Malawista, N.H. Bartenhagen, P.N. Spieler, J.H. Newman, D.W. Rahn, G.J. Hutchinson, J. Green, D.R. Snydman and E. Taylor. 1984. The clinical spectrum and treatment of Lyme disease. Yale J. Biol. Med. 57: 453-461.

Steere, A., D. Snydman, P. Murray, J. Mensch, A. Main, R. Wallis, R. Shope, and S. Malawista. 1986. Historical perspective of Lyme disease. Zentrab. Bakt., Parasiten. infekt. Hyg. A 263: 3-6.

Steere, A.C. 1989. Medical Progress. Lyme Disease. The New England Journal of Medicine. 321: 586-596.

Stoenner, H.G., T. Dodd and C. Larsen. 1982. Antigenic variation of *Borrelia hermsii*. J. Exp. Med. 156 1297-1311.

Wilske, B., Preac-Marsic V., Schierz, G., Kuhbeck, R., Barbour, A.G., Kramer, M. 1988. Antigenic variability of *Borrelia burgdorferi*. Ann. N.Y. Acad. Sci. 539:126-143.

Wilson, M.L., Adler, G.H., and Spielman, A. 1986. Correlation between abundance of deer and that of the deer tick, *Ixodes dammini* (Acari: Ixodidae). Ann. Entomol. Soc. Am. pp 172-176.

2.

VITA

Stanley Wanyangu Mukolwe

Candidate for the Degree of

Doctor of Philosophy

Thesis: EVALUATION OF THE VECTOR POTENTIAL OF AMBLYOMMA AMERICANUM, DERMACENTOR VARIABILIS AND IXODES SCAPULARIS AS VECTORS FOR THE LYME DISEASE AGENT, BORRELIA BURGDORFERI

Major field: Veterinary Parasitology

Bibliography:

Personal Data: Born in Kakamega, Kenya, September 4, 1960, the son of Mr. Herbert Wanyangu and Mrs. Salome Apondi Wanyangu. Married Patience Mkandoe on May 4 1985.

Education: Graduated from Njoro High School, Kenya in December, 1978; received Bachelor of Veterinary Medicine (equivalent to DVM under British Education System) from the University of Nairobi, December, 1984; and Master of Science degree in Veterinary Parasitology from Oklahoma State University, July, 1987; Completed requirements for the Doctor of Philosophy degree at Oklahoma State University in May, 1991.

Professional Experience: Primary School Teacher, Eldoret, Kenya, 1979; capture and transportation of hyenas and a variety of African ungulates, 1982, 1984-85; raising of African buffalo and deer in captivity, Kenya, Oklahoma 1984-90; Veterinary Research Officer, Wildlife Section, Kabete, Kenya, 1984-85; 1987-88; Graduate Teaching Assistant, Department of Veterinary Parasitology, Oklahoma State University, 1986 - 1987, August 1988 - May 1991; trapping ticks and mice from the field, 1989-1990.

Professional Associations: New York Academy of Sciences; Kenya Veterinary Association; Member Sigma Xi, Oklahoma State University Chapter.