EXPERIMENTAL *BRUCELLA OVIS* INFECTION IN WHITE-TAILED DEER (*ODOCOILEUS VIRGINIANUS*)

By

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EXPERIMENTAL *BRUCELLA OVIS* INFECTION IN WHITE-TAILED DEER (*ODOCOILEUS VIRGINIANUS*)

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ACKNOWLEDGMENTS

The stimulus for these studies of Brucella ovis infection in white-tailed deer originated from the unexpected finding of several positive slide agglutination tests performed on sera from free-ranging white-tailed deer in Oklahoma. It had been presumed that this species would not have B. ovis antibodies and that the available stored sera would be a convenient source of antibody-free serum required for developing an ovine B. ovis test system.

In the present project sampling intervals may appear erratic in some of the studies. This occurred due to unavoidable changes in sampling schedules due to a combination of two factors, adverse environmental conditions and inherent characteristics of white-tailed deer. Adult white-tailed deer are nervous and easily excited, and the animals and sometimes the handlers, are put at some risk when immobilization and restraint are required for experimental studies. In some of the studies reported here, more frequent sampling may have yielded more complete data but the dangers of the extra handling of these fractious subjects had to be weighed against the possible value of extra sampling. This was especially so in the buck to doe transmisson study (Chapter V) where the does were immobilized for sampling only once during the trial. Often, inclement, cold winter conditions precluded the immobilization of these does. Immobilization of these deer can be hazardous even when weather conditions are optimal, the deaths of one buck and one doe during these studies being ultimate testimony to this. In the doe study, apart from the possibility

of death of a subject, it was considered that possible pregnancy, which was a primary study factor, would have been jeopardized by more frequent immobilization for sampling.

In addition to many instances of guidance and helpful criticism I am deeply appreciative of the skill, expertise and time freely given by my chief adviser, Dr. Alan Kocan, in maintaining and handling these somewhat unusually hypersensitive "semi-domesticated" experimental animals.

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My thanks and appreciation are sincerely given to Jerri Bullock for her very prompt and skillful execution of typing assignments.

I am deeply indebted to my wife, Margaret, and our two daughters, Catherine and Elizabeth, for their forbearing acceptance of my commitment to a somewhat belated return to scholastic endeavors during the last two years. I have always noted this type of acknowledgment in thesis prefaces but only now can I fully appreciate the sincerity with which they are recorded and I hopefully envision a return to domestic normalcy in the near future.

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CHAPTER I

INTRODUCTION

Literature Review

The isolation of the bacterium Brucella ovis was first recorded in 1953, the organism having been recovered from domestic sheep in New Zealand and Australia.[1, 2] Subsequently, natural infection of sheep with B. ovis has been reported from most sheep-raising countries.[3, 4] All aspects of the disease in sheep have been thoroughly studied and sheep remain the only species reported to sustain natural infection with B. ovis. There are few published results of studies of this organism in other animal species. Attempts to experimentally establish B. ovis infection in rabbits, guinea pigs, and mice were largely unsuccessful, although direct intratesticular inoculation of rats resulted in disease of the testicles analogous to that caused by B. ovis in rams.[4] Male goats experimentally inoculated with B. ovis developed only a transient infection.[5]

The predominant feature of the disease in sheep is infection of the genital tract of rams with resultant lesions in and around the epididymides. Initial epididymitis leads to extravasation of spermatozoa which stimulate a peritubular granulomatous reaction and sometimes adhesions between the overlaying layers of the tunica vaginalis.[6-9] These granulomatous lesions, often containing spermatocoeles, occur predominantly at the tail of the epididymis, less frequently at the

epididymal head and usually develop to a size which is palpable through the scrotal wall.[6-9] The lesions are more commonly unilateral but may be bilateral.[1, 8, 9] Following experimental infection of rams palpable lesions developed in 6 to 7 weeks.[10, 11] Not all infected rams develop palpable lesions.[9, 14]

The infection and resulting lesions lead to a decrease in the number and the motility of spermatozoa, and a majority of those which are produced are morphologically abnormal.[10, 13] Consequently, an infected ram may have markedly lowered fertility or may even be completely infertile. The ultimate and most important effect of Brucella ovis infection in sheep is lowered lambing percentage resulting from impaired ram fertility.

Most semen samples from infected rams contain the B. ovis organisms and many of these rams continue to produce infected semen for up to 4 years.[14]

Investigations of the disease in field flock situations have indicated that natural infection of rams occurs under two sets of circumstances. One mechanism occurs when rams are with ewes during the breeding season and another operates when rams only are kept together. Under natural field conditions, repeated serological testing of rams run with a flock of ewes during the breeding season indicated rapid spread of infection from infected rams to previously uninfected rams.[15] During the breeding season the ewe's vagina may act as a temporary reservoir of infection, clean rams acquiring infection by serving a ewe already mated by an infected ram earlier in the same estrus.[11, 12, 16] Experimentally, the disease has been produced in rams by application of semen from an infected ram to the glans penis of a non-infected ram and also by instillation of B. ovis suspension into the prepuce.[5, 17]

When not with ewes, transmission does occur from infected to uninfected rams in cohabitation.[12, 18, 19] Sodomy among rams has been incriminated as the method of transmission. Typical disease in a ram has also been produced experimentally by rectal instillation of a B. ovis suspension.[5]

In ewes a low incidence of natural infection following mating with infected rams is the typical reported situation.[12, 20] The incidence of ewe infection was also low following experimental inoculation of ewes at mating time or during pregnancy.[21-24] Clinical maifiestations of infection in ewes are various degrees of placentitis with abortion in late pregnancy or birth of weak lambs.[12, 21-24] In some cases, apparently normal lambs may be produced by an infected ewe. In the non-pregnant ewe the infection does not appear to persist.[21, 22]

In epidemiologic studies, B. ovis has been observed to survive for several months in infected material on pasture.[12] Neither clean rams nor clean ewes, however, became infected when grazed in a field recently contaminated by ewes aborting due to B. ovis.[12] This was despite the findings that the fetal membranes, aborted fetuses, uterine discharge, and udder secretions from infected ewes contain many B. ovis organisms and that experimentally, ewes and rams have become infected following either oral or interanasal inoculation.[12, 25]

Additionally, B. ovis has been isolated from the kidneys and urine of infected rams.[6, 12] Therefore infected rams' urine could be a source of B. ovis contamination of the environment.

Experimentally, rams have been infected by many different routes. The following are methods of experimental inoculation using culture suspensions or infected fresh semen which have resulted in typical genital tract infection in rams and which, theoretically, could be routes of

natural transmission: oral dosing,[12] conjunctival instillation,[6, 12] intranasal instillation,[25] preputial instillation,[6, 10] application to the glans penis[17] and rectal instillation.[5] Despite the many possible routes of contamination and transmission of B. ovis among sheep, the natural perpetuation and effects of the organism appear to occur virtually as a venereal disease of rams with transmission either directly from ram to ram by sodomy, or via the ewe's vagina.

Implication of White-Tailed Deer

In 1979, at the Oklahoma State University Wildlife Disease Laboratory, several of a random sampling of frozen-stored sera which had been collected from Oklahoma field-harvested white-tailed deer (Odocoileus virginianus) were positive to a B. ovis slide agglutination test.[a] These test results suggested the possibility of wild white-tailed deer being susceptible to natural infection with Brucella ovis.

If white-tailed deer are susceptible to B. ovis infection the effects and epidemiology of the disease in this species would require evaluation. In some geographical areas, deer share a common range with domestic sheep. Epidemiologic investigation would be desirable in those regions to evaluate the possibility of cross-infection between these two animal species.

Research Objectives

Based upon the known effects and epidemiology of B. ovis infection

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[a]George LW, Carmichael LE: A plate agglutination test for the rapid diagnosis of canine brucellosis. Am J Vet Res, 35:905-909, 1974.

in sheep the following research study plan was formulated.

1. Evaluate the infectivity of Brucella ovis in captive, sexually mature, male white-tailed deer (Odocoileus virginianus). If B. ovis infection is established evaluate:

   a. the duration and effects of the infection;

   b. natural transmission from infected to uninfected male deer in cohabitation;

   c. natural tansmission from an infected buck to uninfected does in cohabitation during the breeding season; and

   d. infectivity in young male deer.

2. Conduct a field survey using hunter-collected samples from wild deer populations to evaluate the possibility of natural infection with B. ovis by:

   a. examination of deer testicles for gross epididymal lesions and if present attempt isolation of B. ovis from the lesions; and

   b. testing serum samples with a B. ovis complement fixation test.

CHAPTER II

MATERIALS AND METHODS

Animals and Facilities

The white-tailed deer (Odocoileus virginianus) used in these studies were part of a captive herd derived from the free-ranging deer population of Oklahoma and maintained for research by the Oklahoma Department of Wildlife Conservation and Oklahoma State University. Each deer was identified with a numbered ear-tag.[a]

The facility consists of two fields, a pen, and six confinement runs adjoining a barn, all within a rectangular area 370 meters by 195 meters bounded by a chain-link fence 2.4 meters high (Fig 1). Dividing fences were also of chain-link wire 2.4 meters high. Each confinement run, three meters by seven meters, opened into its own solid wood lined stall with a galvanized iron roof. The two fields had native prairie cover and the pen and confinement runs had an earth floor with field-tile drainage.

The deer were fed a balanced diet of pellets, grain meal, whole corn, and alfalfa hay in sufficient amounts to ensure growth and development of young animals and to maintain mature animals in healthy body condition. Water was supplied in troughs, the source being a rural community water supply meeting standards for human consumption.

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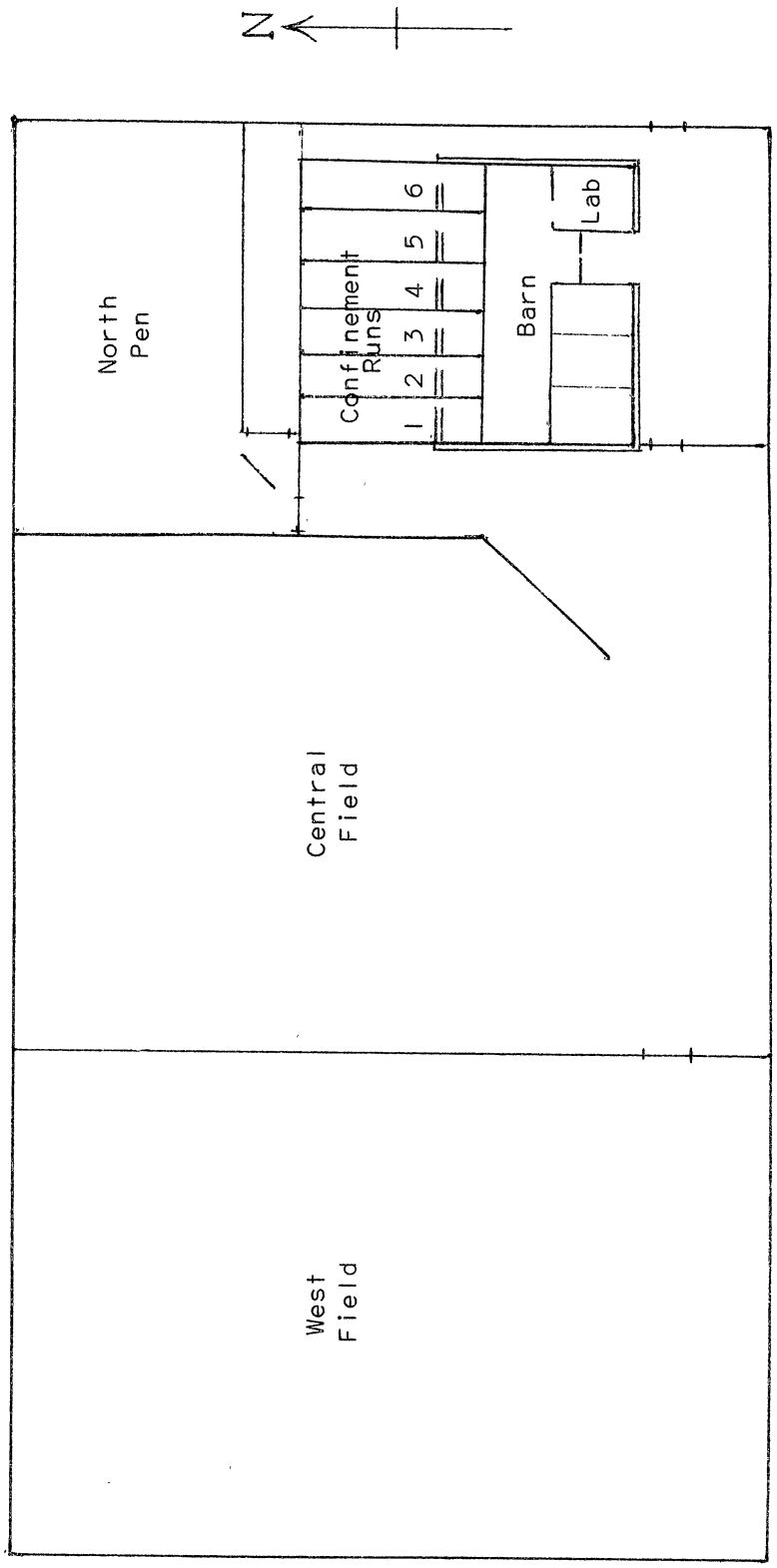
[a]Allflex, Allflex Tag Company, Culver City, California 90230.

Fig 1. Diagram of the Oklahoma State University Deer Research Facility. (Not to Scale)


N
North Pen
Confinement Runs
1
2
3
4
5
6
Barn
Lab
Central Field
West Field

Immobilization and Restraint

Young, tractable deer were immobilized and restrained physically. During physical restraint, the flank of the animal was firmly grasped with one hand and simultaneously the front legs were secured and stretched forward with the other hand as the animal was lifted and drawn firmly against the body of the handler. If necessary a second person would manually immobilze the hind legs in an extended position and the deer could then be placed on its side on the ground and be kept restrained. Chemical immobilization was used for deer considered large enough to jeopardize the safety of both the animal and the handler if physical restraint had been attempted. Succinylcholine chloride dihydrate at a calculated dose of 0.07 mg/kg was administered intramuscularly in powder form delivered via a plastic projectile[b] fired from an air-pistol.[c]

Inoculum

Cultures of Brucella ovis were propagated from frozen stored aliquots of a field isolate originally obtained from semen collected from a naturally infected ram from central Oklahoma. The isolate had been confirmed as Brucella ovis by the National Veterinary Services Laboratories, (N.V.S.L.).[d] As needed, an aliquot was thawed and inoculated on to sheep blood agar plates and incubated at $37^{o}C$ for 48 hours in a 10 percent carbon dioxide atmosphere. Colonies were harvested and diluted with normal saline to a resultant concentration of approximately 5×10^{9} organisims per milliliter.

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[b]Pneu-dart, Pneu-dart Inc., Williamsport, PA 17703.

[c]Pneu-dart Model 177, Pneu-dart Inc., Williamsport, PA 17703.

[d]National Veterinary Services Laboratories, Ames, Iowa 50010

Inoculation

In each case, the deer was immobilized, restrained, and placed on its side; with the eyelids held apart, the eye was repeatedly flooded with inoculum from a syringe during a period of two minutes. The animal was then turned on to its other side and the procedure was repeated with the other eye. Approximately 0.5 ml of inoculum was used for each eye.

Blood Collection

Blood was collected from the jugular vein into 10 ml-draw evacuated clot tubes[e] via a 20 guage 3.75 cm needle. Following thrombus formation the tubes were centrifuged and serum was separated and aliquots were stored frozen.

Complement-Fixation Test

Frozen serum samples were packed in dry ice and shipped to the National Veterinary Services Laboratories for Brucella ovis complement fixation testing.[27] Each serum sample was tested at dilutions of 1:10, 1:20, and 1:50. Less than 75 percent hemolysis (+) of sensitized erythrocytes by a serum dilution of 1:10 or greater was recorded as a positive titer in this study. No hemolysis (++++) at a serum dilution of 1:50 was recorded as a maximum titer.

Semen Collection

Buck deer were immobilized by either of the above described methods and with the animal on its side semen samples were obtained using an

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[e]Vacutainer, Becton, Dickinson and Co., Rutherford, NY 07070.

ovine, rectal probe transistorized electro-ejaculator.[f] The semen was collected directly into sterile tubes which were then placed in ice in an insulated container and transported to the laboratory within two hours of collection.

Physical Examination

Each time a white-tailed buck was restrained the scrotum and its contents were palpated to detect gross abnormalities.

Laboratory Culture

Semen samples were streaked on to sheep blood agar plates and incubated at 37°C in a 10 percent carbon dioxide atmosphere for up to five days. Samples of recovered organisms were sent to N.V.S.L. for Brucella ovis confirmation.

Euthanasia and Necropsy

Deer were immobilized with succinylcholine chloride dihydrate as described above then 0.3 ml/kg of N - [2 - (m - methoxy - phenyl) - 2 ethyl - butyl - (1)] - gamma -hydroxy - butyramide with 4, 4' - methylene - bis (cyclohexyl - trimethyl - ammonium iodide)[g] was administered into the jugular vein.

At necropsy the testicles and epididymides were examined visually and by palpation for gross abnormalities. Sections from the head, body and tail of the epididymis of each testicle were ground separately using mortars and pestles and each sample was cultured for B. ovis using the

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[f] Bailey Ejaculator, Western Instrument Co., Denver, CO 80216.

[g] T61 Euthanasia Solution, Taylor Pharmacal Co., Decatur, IL 62525.

same cultural and identification procedures as described earlier for evaluating semen samples. Sections of the head, body and tail of the epididymides were placed in 10 percent buffered formalin. These tissues were later sectioned and stained with hematoxylin and eosin for histologic examination according to the method described in the "Armed Forces Institute of Pathology Manual."[28]

CHAPTER III

EVALUATION OF INFECTIVITY AND NATURAL TRANSMISSION

Study Procedures

Two male deer, No. 119, 28 months of age and No. 416, 18 months of age, each previously negative to the Brucella ovis complement-fixation (CF) test were immobilized and restrained on October 27, 1980. Blood collection, palpation of the scrotum and its contents and inoculation with B. ovis were performed on each buck. The two bucks were then released into the west field (Fig 1) which already contained two 30-month old female deer, No. 214 and No. 215, and two six month old female deer, No. 404 and No. 411. Each of these females had previously been found negative to the B. ovis CF test.

Male deer No. 119 was found dead on November 4, 1980. The cause of death was not determined.

Periodically the four females were immobilized for blood sampling and buck No. 416 was immobilized for blood and semen sample collection and palpated for testicular lesions.

Two female deer, No. 214 and No. 411, were euthanatized and necropsied on February 4, 1981, and the other two, No. 215 and No. 404, were euthanatized and necropsied on February 9, 1981.

Buck No. 416 was immobilized and moved from the west field and placed in run #2 (Fig 1) on February 4, 1981. On May 5, 1981 a 12-month

old buck No. 401, negative to the B. ovis CF test and without palpable testicular abnormalities was placed in run #2 with buck No. 416. Both bucks were kept together in run #2 until December 29, 1981 when both were euthanatized and necropsied. Blood and semen samples were collected from both deer on October 20, 1981.

Results

Inoculation Infectivity in a Male Deer

The results of tests for B. ovis before and following the October 27, 1980 inoculation of male deer No. 416 are shown in Table 1. The serum collected on November 13, 1980, 17 days following inoculation was the first sample to give a positive B. ovis CF test. Thereafter, each serum sample collected from this animal was CF test positive. B. ovis was cultured from semen collected at 30 days and 73 days post-inoculation (PI). The organism was not recovered from a semen sample collected on October 20, 1981, but B. ovis was isolated from both epididymides when sampled at necropsy, 429 days after inoculation. The cultured organism was confirmed as Brucella ovis by N.V.S.L.

Palpation of the testicles performed on those days that blood samples were collected, revealed no gross abnormalities. At necropsy the testicles and epididymides exhibited no gross lesions (Fig 2), and sections of the epididymides were histologically normal.

Natural Transmission and Infectivity-- Male to Male

The experimentally inoculated male deer No. 416 in run 2 had been

TABLE I

RESULTS OF TESTS FOR THE PRESENCE OR EFFECTS OF *BRUCELLA OVIS* IN EXPERIMENTALLY INOCULATED MALE DEER NO. 416

	1980						1981			
	Oct. 20	Oct. 27	Nov. 13	Nov. 26	Dec. 5	Dec. 29	Jan. 8	Feb. 4	Oct. 20	Dec. 29
Days PI	-7	0	17	30	39	63	73	100	358	429
CF Test	-	-	+	+	+	+	+	+	+	ND
Semen Culture	ND	ND	ND	+	+	ND	+	ND	-	ND Killed Necropsy
Gross Lesions of Epididymides	-	-	-	-	-	-	-	-	-	-
Culture of Epididymides										+
Histopathologic Changes in Epididymides										-

PI Post Inoculation; ND Not Done.

shown to be positive by the B. ovis CF test and by semen culture when the infected male No. 401 was also placed in run 2 on May 5, 1981. On October 20, 1981, the 168th day of cohabitation, buck No. 416 was still CF test positive but B. ovis was not recovered from a semen sample; buck No. 401 was CF test negative and semen-culture negative for B. ovis.

Following an additional 70 days of cohabitation both bucks were euthanatized and necropsied on December 29, 1981. Necropsy findings for deer No. 416 are already described in the previous section.

At necrospy each testicle of deer No. 401 showed the following abnormalities. There was enlargement of the head and the tail of the epididymis (Fig 2) and the overlying tunic was adherent to the head and to the tail of the epididymis. On cross-section both the enlarged head and tail areas of the epididymis exhibited several cavities containing cream-white colored fluid. This fluid varied from cavity to cavity having the consistency of milk in some, and in others that of thick mayonnaise.

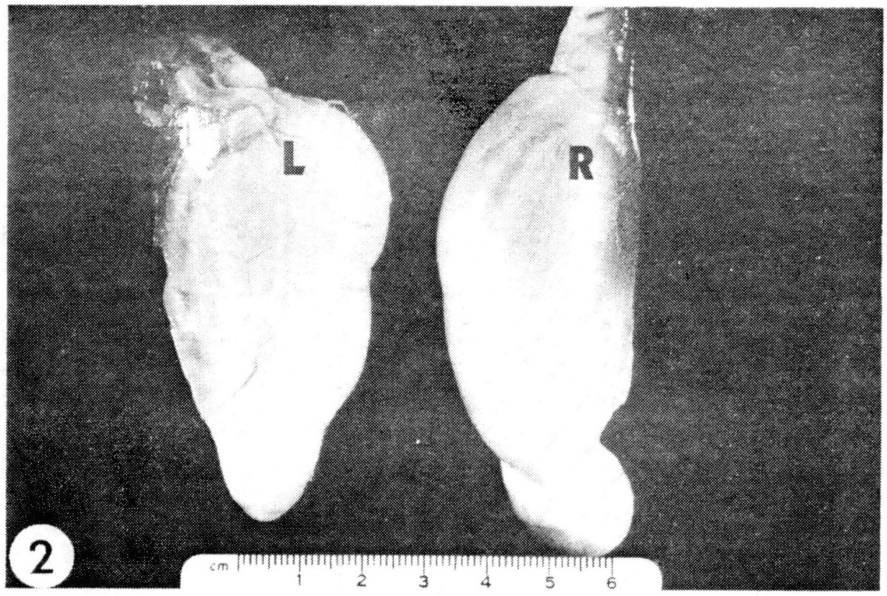
B. ovis was cultured from samples of the head, body, and tail of the epididymis of each testicle from deer No. 401. The cultured organism was confirmed as Brucella ovis by N.V.S.L.

Histologic examination of sections of the epididymides from deer No. 401 (Fig 3) revealed granulomas consisting primarily of macrophages and multinucleated cells and containing some spermatozoa, scattered lymphocytic nodules in the interstitial tissues, and connective tissue proliferation between epididymal tubules. Many tubules showed hyperplasia of the epithelial cells, and in some tubules, cysts or vacuoles were present within areas of epithelial hyperplasia.

Inadvertence is the only appropriate, perhaps somewhat euphemistic description of the failure to collect blood for CF testing from either

Fig 2. Testicle (L) from Deer No. 401 Showing Enlargement of the Head and Tail of the Epididymis. Normal Testicle (R) was from Deer No. 416.

Fig 3. Photomicrograph of the Tail of an Epididymis from Deer No. 401 Showing Vacuoles (Arrows) Within Hyperplastic Epithelium of Tubules. H & E; x 100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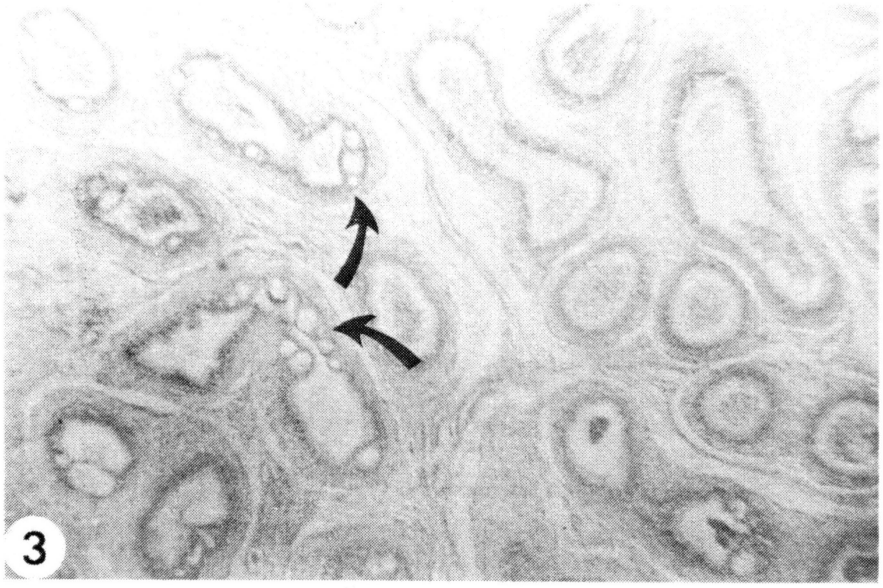
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of the two male deer, Nos. 401 and 416, at the time of euthanasia, December 29, 1981.

Natural Transmission and Infectivity--

Male to Female

The results of B. ovis CF tests performed on sera from the four female deer before and after exposure to the two inoculated male deer are shown in Table II accompanied by the relative data concerning the two introduced male deer.

All serum samples collected from the four female deer were negative to the B. ovis CF test. At necropsy no abnormalities of the reproductive tracts or their contents were observed in any of the four female deer, and no samples were submitted for laboratory culture. The two does No. 214 and No. 215 each contained a grossly normal fetus and membranes and the other two does No. 404 and No. 411 were not pregnant.

Discussion

The findings of this study were:

1. Following inoculation with B. ovis male deer No. 416 developed and maintained the infection and B. ovis was still present in both epididymides at the time of euthanasia 429 days PI. No epididymal lesions were observed.

2. The B. ovis organism was transmitted by some unknown natural method from deer No. 416 to deer No. 401 and the infection in No. 401 resulted in epididymal lesions grossly and histopathologically identical with lesions reported as caused by B. ovis in rams.

3. There were no indications of transmission of infection from

TABLE 11

RESULTS OF TESTS FOR *BRUCELLA OVIS* INFECTION IN INOCULATED MALE DEER AND NON-INOCULATED FEMALE DEER IN COHABITATION DURING THE BREEDING SEASON

	1980										1981		
	Oct 20	Oct 27	Nov 4	Nov 10	Nov 13	Nov 24	Nov 26	Dec 1	Dec 5	Dec 29	Jan 8	Feb 4	Feb 9
Days in Cohabitation	-7	0	8	14	17	28	30	35	39	63	73	100	105
Deer I.D.													
119 (28 mth Male)	-	- *B. ovis* Inoc.	Died										
416 (18 mth Male)	-	- *B. ovis* Inoc.	ND	ND	+	ND	+ SC+	ND	+ SC+	+	+ SC+	+	ND
214 (30 mth Fem.)	-	ND	ND	ND	ND	-	ND	ND	ND	-	ND	- Killed	
215 (30 mth Fem.)	-	ND	ND	-	ND	ND	ND	ND	ND	ND	ND	ND	- Killed
404 (6 mth Fem.)	-	ND	ND	ND	ND	ND	ND	-	ND	ND	ND	ND	- Killed
411 (6 mth Fem.)	-	ND	ND	ND	ND	-	ND	ND	ND	ND	ND	- Killed	

\+ Positive *B. ovis* CF Test; - Negative *B. ovis* CF Test, ND Not Done; SC Semen Culture.

infected buck No. 416 to any of the four does during the breeding season.

Male deer No. 401 did not acquire an apparent infection during the first 168 days of cohabitation with infected deer No. 416 but infection did establish during the latter 70 days of cohabitation, being from October 20 to December 29, 1981. At that time of the year the bucks were in rut and this may have increased the opportunities for transfer of the organism through increased shedding rates and behavioral traits such as increased sexual interest.

Measurements made of the fetuses found in the two does No. 214 and No. 215 indicated that conception had occurred on or about November 13, 1980. At that time (approximately two weeks PI) the inoculated buck No. 416 was probably not yet shedding B. ovis in the semen.

In future studies of transmission from an infected buck to does during the breeding season, the buck should be kept isolated following inoculation until it is ascertained that infection is established and that the organism is being shed in the semen. It would be advantageous if the buck could be fitted with a marker device which would indicate whether or not, and when, mating of does had occurred.

CHAPTER IV

EVALUATION OF INFECTIVITY AND NATURAL TRANSMISSION IN YOUNG MALE DEER

Introduction

Experimental studies of brucellosis caused by B. ovis in sheep have indicated that young rams are susceptible to infection but that the duration of infection is much shorter than if the animal is mature at the time of initial infection.[14] Serologic surveys have confirmed that a similar situation occurs in naturally infected ram flocks as evidenced by a high percentage of young rams which develop serum titers that decline and disappear within four to five months.[26]

Study Plan

The objectives of this study were to evaluate B. ovis infectivity, the duration of infection if established, and whether natural tansmission can occur in young male deer kept in cohabitation.

Study Procedures

Five young male deer, Nos. 501, 505, 522, 523, and 524, each between five and six months old, were restrained, blood sampled, subjected to palpation of the scrotum and its contents, then inoculated with B. ovis. Immediately following these procedures, performed on October 20, 1981, the five deer were placed together in the north pen (Fig 1). Periodically

(Table III) each deer was restrained in order to collect blood and semen samples and to conduct a physical examination. Blood samples were tested by the B. ovis CF test and the semen was cultured for B. ovis.

Two of the deer, No. 501 and No. 522, were euthanatized 77 days PI and the other three were euthanatized 344 days PI. On February 1, 1982, 104 days PI, deer No. 524, having developed and maintained a positive B. ovis titer, was placed in run #2 (Fig 1) along with a serologically negative nine month old male deer, No. 526. After 31 days of cohabitation No. 526 was found dead from a neck injury. The epididymides were cultured for the presence of B. ovis.

On March 5, 1982, a B. ovis CF test-negative 10 month old male deer, No. 527, was placed in run #2 with deer No. 524. These two deer were kept together in the small #2 run for the next 53 days and on April 27, 1982 they were both placed back in the larger north pen with the two B. ovis inoculated deer, No. 505 and No. 523.

Two 12 month old B. ovis CF test-negative male deer, No. 528 and No. 504, were also placed in the north pen on June 3, 1982. The inoculated deer, Nos. 505 and 524, were euthanatized 344 days PI, and NO. 523 was euthanatized 352 days PI. Non-inoculated deer, No. 528, was euthanatized following 118 days in cohabitation, and Nos. 504 and 527 were euthanatized after 179 days and 269 days, respectively, in cohabitation with the inoculated deer. All deer were necropsied and from each deer a composite sample of the head, body, and tail of the epididymis of each testicle was cultured. If any lesions of the testicles or epididymides were observed, sections were collected for histopathological examination.

Results

The results of tests for brucellosis in the deer used in this study are shown in Table III.

The B. ovis CF test titers for the duration of the study are graphically depicted in Fig 4. Three of the five inoculated deer had positive B. ovis CF test titers at the first PI test performed 17 days after inoculation. At 27 days PI each of the five deer had developed test-maximum titers. The two deer, Nos. 501 and 524, still had maximum titers at 55 days PI whereas the titers of the other three deer had declined. Deer No. 524 was the only subject to maintain a maximum titer at 77 days PI and was also the only one of the three surviving deer to show a titer at 90 days PI. Thereafter, No. 524 showed a fluctuating low level until it became negative at 217 days PI and remained negative for all subsequent CF tests.

Of the ejaculate samples obtained at 27 days and at 55 days PI, B. ovis organisms were isolated and their identity confirmed from only one sample, that collected from deer No. 524 on day 27 PI.

The results of examinations and tests of the two deer, No. 501 and No. 522, which were blood sampled, euthanatized, and necropsied on the 77th day PI were as follows: Deer No. 501 had a moderate CF test titer and the tail of each epididymis was slightly enlarged, but B. ovis organisms were not recovered from epididymal cultures. Histopathological examination of the epididymides of this deer revealed hyperplasia of tubular epithelium, in some areas containing vacuoles, and there was periductal smooth muscle hyperplasia (Fig 5). The other deer, No. 522, had a negative B. ovis CF titer and evidenced no gross or histopathologic

TABLE III

RESULTS OF TESTS FOR THE PRESENCE OR EFFECTS OF BRUCELLA OVIS IN INOCULATED AND NON-INOCULATED YOUNG MALE DEER IN COHABITATION

	1981			1982												
Date	Oct 20	Nov 6 16	Dec 1 14	Jan 5 18	February 1 9 16 22	March 1 5 16 29	Apr 27	May 25	June 3 22	Jul 20	Aug 25	Sept 29	Oct 7	Nov 29
Days PI	0	17 27	42 55	77 90	104 112 119 125	132 136 147 160	189	217	226 245	273	309	344	352	405
501	- Inoc	+ + S-	+ + S-	+ H+ C-										
522	- Inoc	- + S-	+ + S-	- H- C+										
505	- Inoc	- + S-	+ + S-	+ -	- ND - ND	- ND - -	-	-	ND -	-	-	- C-		
523	- Inoc	+ + S-	+ + S-	- -	- ND - ND	- ND - -	-	-	ND -	-	-	-	- C-	
524	- Inoc	+ + S+	+ + S-	+ +	+ ND + ND	+ ND ND -	+	-	ND -	-	-	- C-		
526					- ND -									
527						- ND -	-	-	ND -	-	-	ND	ND	- C-
504									- -	-	-	ND	ND	- C-
528									- ND	ND	ND	- C-		

PI Post Inoculation; + Positive B. ovis CF Test, - Negative B. ovis CF Test, C Culture of Epididymides, H Histopathology of Epididymides, S Culture of Semen, ND Not Done.

Fig 4. Graph of *Brucella ovis* Complement Fixation Test Titers Following Inoculation of Five Young Male Deer.

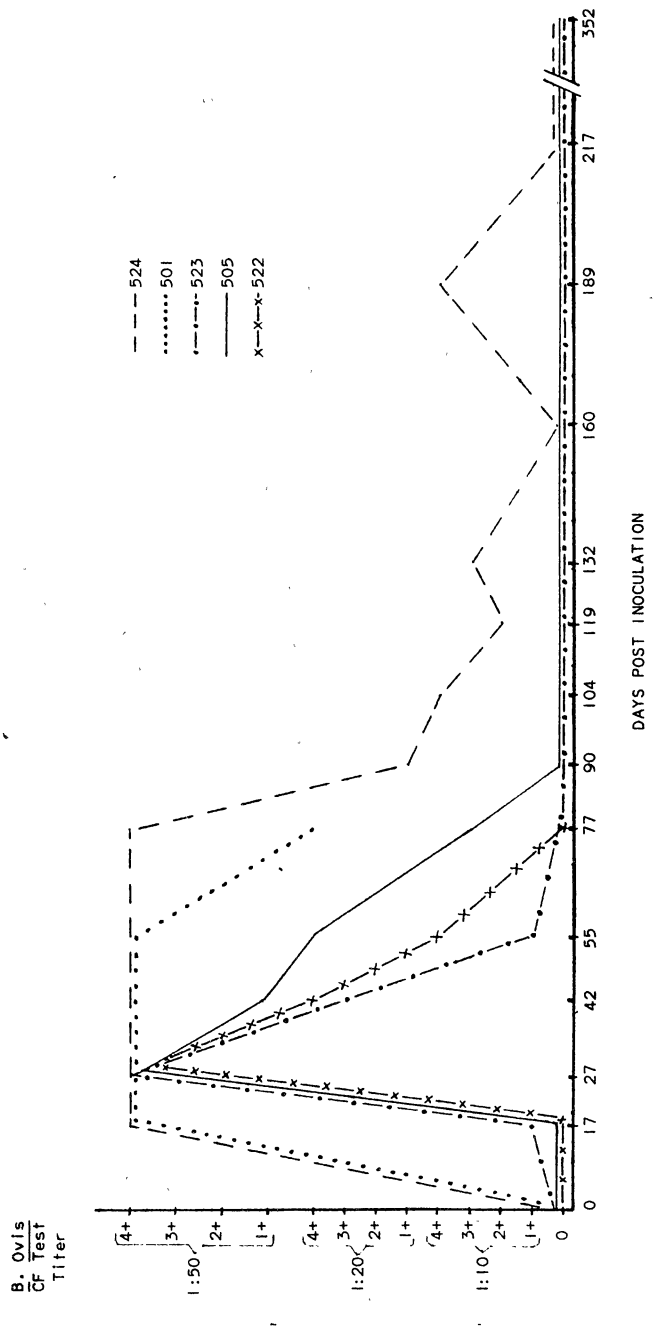
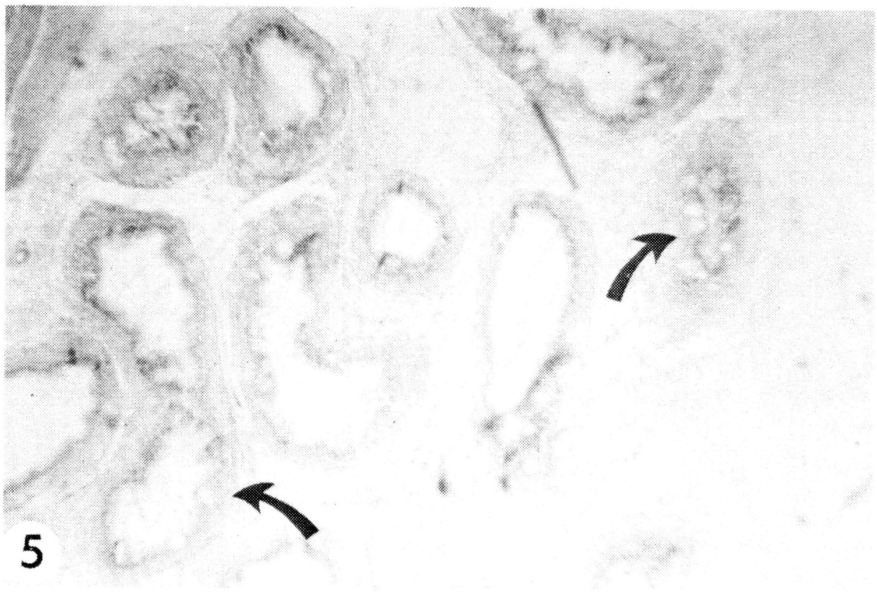

B. Ovis
CF Test
Titer
1:50
1:20
1:10
4+
3+
2+
1+
0
524
501
523
505
522
0
17
27
42
55
77
90
104
119
132
160
189
217
352
DAYS POST INOCULATION

Fig 5. Photomicrograph of the Tail of an Epididymis from Deer No. 501. There is Hyperplasia and Vacuolation (Arrows) of the Epithelium of the Tubules. H & E; x 400.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epididymal changes, but B. ovis organisms were isolated from composite epididymal samples from each testicle.

Following cohabitation with the three inoculated deer, the four uninoculated deer, Nos. 504, 526, 527, and 528, were negative on periodic B. ovis CF tests, had no gross abnormalities of the testes or epididymides, and had no B. ovis cultured from samples of epididymides.

Discussion

The serologic results indicate that following inoculation with B. ovis, young male deer became infected but that the infection was relatively short-lived. Reinforcing this indication of transient infection was the finding of no evidence of infection at necropsy in the three inoculated deer euthanatized at 344 and 352 days PI despite the earlier serological evidence of infection.

The graphic representation of the B. ovis CF titers (Fig 4) of the inoculated deer indicated, apart from the more persistent, fluctuating low titer shown by one deer, No. 524, that the duration of infection in these young male deer was approximately 90 days. These results are similar to the evidence reported from studies in sheep where young rams were found to develop only a transient infection following inoculation with B. ovis.

There were only two periods when non-inoculated deer were in contact with inoculated deer still showing serological evidence of infection. These were when No. 526 was in contact with No. 524 from day 112 through day 132 PI, and when No. 527 was in contact with No. 524 from day 136 through day 189 PI. The probability of natural transmission from inoculated to uninoculated deer would presumably have been greatest during the

first 90 days PI but unfortunately animals were not available to use as monitors during that time.

The histopathology of the epididymides of deer No. 501, euthanatized and necropsied 77 days PI, was further evidence that B. ovis in male white-tailed deer may cause epididymitis typical of that caused in domestic rams, and therefore, it is possible that the resultant infertility which has been shown in rams may also occur in male deer. The other deer, No. 522, euthanatized at 77 days PI had B. ovis bacteria present in the epididymides but there were no tissue changes. This also simulates the situation in rams where B. ovis infection can be present in the epididymides without causing discernible histopathology.[9, 12] The fertility of these individuals may not be markedly affected but they could be a source of infection for other animals.

A problem was encountered in the culturing of the electro-ejaculate samples collected from the deer on days 27 to 55 PI. Overgrowth of culture media by contaminant bacteria occurred and this may have precluded recovery of the relatively slower growing B. ovis organisms. The deer were relatively sexually immature and the penis did not protrude from the prepuce during ejaculate collection. The sample had to be "milked" from the prepuce which was probably the source of the contaminant organisms.

CHAPTER V

EVALUATION OF NATURAL TRANSMISSION FROM AN INFECTED MALE TO FEMALES DURING THE BREEDING SEASON

Introduction

Retrospective examination of the 1980 study of possible transmission from an infected male deer to female deer in cohabitation during the breeding season (Chapter III) revealed the probability that breeding occurred before the male deer was shedding B. ovis in its semen. In that study the male deer was placed with the females immediately following inoculation and at the height of the breeding season. Consequently, this study was designed to evaluate male to female transmission and to ensure that a male deer was infected and shedding B. ovis in semen before being placed with females during the breeding season.

Study Procedures

A 17 month old male deer, No. 12, was immobilized with succinylcholine dihydrate on November 10, 1982, for the purpose of inoculation with B. ovis. Unfortunately, this deer died while immobilized. The only other male deer available on that day was No. 618, six months old. This deer was immobilized, blood sampled, and inoculated with B. ovis on November 10, 1982, and placed in Run #3 (Fig 1). On November 16, 1982, an 18-month old male deer, No. 10, was immobilized, blood

sampled, physically examined, and inoculated with B. ovis, and placed alone in run #4 (Fig 1). Each male deer, No. 10 and No. 618, was immobilized, semen sampled, physically examined, and blood sampled on December 16, 1982. Immediately following these procedures a mating-marker harness[a] was fitted to deer No. 10, which was then placed in the north pen (Fig 1) with six female deer. These female deer, Nos. 5, 6, 7, 609, 619, and 14, all between six and seven months of age were aslo blood sampled on December 16, 1982.

The forelegs of the male deer became entangled in the mating-marker harness on December 19, 1982, and the harness was removed.

Blood and semen samples were collected from male deer No. 10 on the 44th and 107th days PI. On the 105th day of cohabitation with the six female deer, male deer No. 10 was blood sampled, then euthanatized and necropsied. The epididymides were examined and histopathologic and bacteriologic tests were performed.

On the 76th and 77th days of cohabitation with the infected male deer, No. 10, the six female deer were immobilized, blood sampled, and pregnancy tested by manual methods and by ultrasound.[b] One deer, No. 7, died as a result of immobilization. The remaining five female deer were blood sampled, euthanatized, and necropsied on August 11, 1983, the 238th day from introduction of the inoculated male deer, No. 10.

The other inoculated male deer, No. 618, was kept isolated in run #3 as a reserve animal in case of incapacitation of male deer No. 10. Blood and semen samples were collected from No. 618 at 36 days PI and a

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[a]Sire-sine, Mid-States Woolgrowers Coop. Assn., South Hutchinson, Kansas 67505.

[b]Pregnosticator, Animark Inc., Aurora, Colorado 80011.

blood sample was collected on the 49th day PI. Eighty-three days after inoculation, male deer No. 618 was euthanatized, the testicles and epididymides were examined and epididymal samples were cultured for the presence of B. ovis.

Results

The results of this study are shown in Table IV. On December 16, 1982, 30 days PI, male deer No. 10 had a B. ovis CF test maximum titer and B. ovis was cultured from the semen. On this day, No. 10 was placed with the six female deer each having no titer on the B. ovis CF test.

At 44 days and again at 77 days after being placed with the females, buck No. 10 had maintained a B. ovis CF test maximum titer and B. ovis was cultured from semen samples. At 135 days PI, and after 105 days of cohabitation with the females, male deer No. 10 was blood sampled and euthanatized. This deer still had a B. ovis CF test maximum titer but B. ovis was not isolated from samples of either epididymis. The tail of the left epdidymis was approximately twice the size of the apparently normal right side epididymal tail (Fig 6). The overlying tunic was adhered to the enlarged left epididymal tail. Microscopic examination of stained sections of the epididymis revealed proliferation and vacuolation of tubular epithelium and various sized granulomas, some with a necrotic center surrounded by macrophages and lymphocytes. There was also hyperplasia of peritubular smooth muscle (Fig 7).

On the 76th and 77th days of cohabitation, of the six female deer, two, Nos. 5 and 619, were diagnosed as pregnant. No. 619 had a B. ovis CF test maximum titer and No. 14 had a moderate B. ovis CF test titer.

Deer No. 7 which died as a result of immobilization, was necropsied and the reproductive tract was examined. The uterus was immature and

TABLE IV

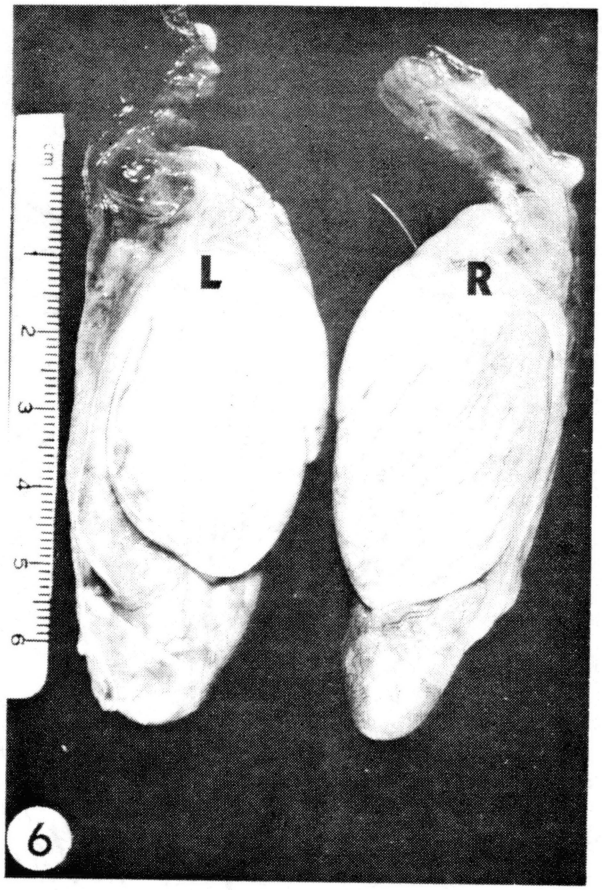
RESULTS OF TESTS FOR THE PRESENCE OR EFFECTS OF *BRUCELLA OVIS* IN DEER USED IN A NATURAL TRANSMISSION STUDY

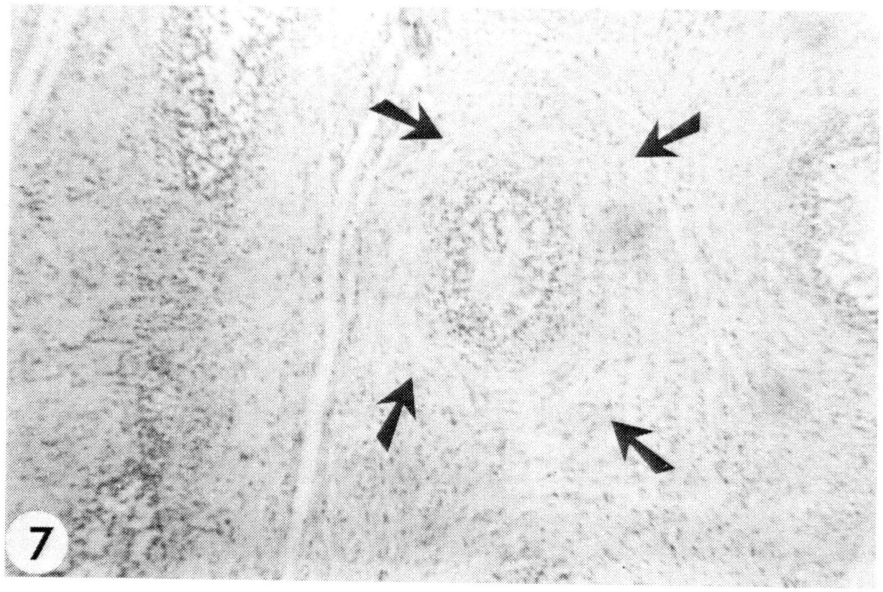
	1982					1983					
Deer I.D.	Nov. 10	Nov. 16	Dec. 16	Dec. 29	Dec. 30	Feb. 1	Mar. 2	Mar. 3	Mar. 31	July 17	Aug. 11
12 (17 mth Male)	Inoc. Died										
Days PI		0	30		44			107	135		
10 (18 mth Male)		– Inoc.	+ SC+		+ SC+			+ SC+	+ Ep. C–, H+		
Days in Cohabitation			0		14		76	77	105	213	238
5 (Female)			–				– PT+			Small	–
6 (Female)			–					– PT–		Weak Fawn	–
7 (Female)			–				– PT–Died			Born Necropsy:	
14 (Female)			–				+ PT–			Lung Liver	– Ut–
609 (Female)			–				– PT–			Spleen Neg. for	–
619 (Female)			–				+ PT+			*B. ovis*	– Ut–
Days PI	0		36	49		83					
618 (16 mth Male)	– Inoc.		+ SC–	–		Ep. C+					

PI Post Inoculation; + Positive *B. ovis* CF Test; – Negative *B. ovis* CF Test; Ep. C Culture of Epididymides; Ep. H Histopathology of Epididymides; PT Pregnancy Test; SC Semen Culture; Ut Uterus Culture.

Fig 6. Testicles from Deer No. 10. The Epididymis of the Left Testicle (L) is Markedly Enlarged. The Right (R) has no Gross Abnormalities.

Fig 7. Photomicrograph of the Left Epididymis of Deer No. 10. There is Vacuolated Hyperplastic Epithelium of the Tubules and Periductal Smooth Muscle Hyperplasia (Arrows). H & E; x 100.


L
R
6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the ovaries were very small with no visible corpora lutea.

On July 17, 1983, 213 days after introduction of the infected male deer, a small, weak fawn weighing 1.4 kg was found in the north pen with the remaining five female deer. None of these females exhibited any vulvar or mammary gland indications of having produced the fawn. The fawn was euthanatized on July 18, 1983, and a necropsy was performed. No gross abnormalities were found, and B. ovis was not isolated from samples of the fawn's lungs, liver, and spleen.

There were no more fawns born and the remaining five female deer, Nos. 5, 6, 609, 619, and 14 were all B. ovis CF test negative on August 11, 1983, at which time they were euthanatized and necropsied. The genital tract of each doe was examined. No adnormalities were observed in Nos. 5, 6, or 609. The lumena of both horns of the uterus of each doe, No. 619 and No. 14, contained approximately 2 ml of thick, yellowish-white fluid. Culture of the uterine contents of each of these two does yielded no bacteria.

The inoculated male deer, No. 618, kept in isolation as a reserve, had a high B. ovis CF titer but B. ovis was not isolated from a semen sample at 36 days after inoculation. The blood sample collected at 49 days PI was B. ovis CF test negative. B. ovis was cultured from composite samples of each epididymis following euthanasia on the 83rd day after inoculation although there were no gross abnormalities of the testicles or epididymides.

Discussion

In contrast with the earlier pilot study (Chapter III) in which, immediately following inoculation, male deer no. 416, was placed with

female deer, the male deer, No. 10, in this study was kept in isolation for 30 days following inoculation before being placed with females for breeding.

On the day of introduction to the females the male deer, No. 10, had a test-maximum B. ovis titer and was shedding B. ovis in its semen. Tests at 14 days and again at 77 days after having been placed with the females showed that this deer had maintained a test-maximum B. ovis CF test titer and that the organisms were being shed in its semen. When euthanatized 105 days after being introduced to the six females, male deer No. 10 still had a maximum CF test titer and typical B. ovis-induced gross lesions (Fig 6) and histopathologic changes were present in the left epididymis (Fig 7). These findings indicated that there had been optimal opportunity of transmission of organisms at any matings that may have occurred.

females were the B. ovis CF test titers shown by the two females No. 619 and No. 14 at 76 days after introduction of the infected male deer. These two females had no B. ovis CF test titer 162 days later when they were euthanatized, but, although no organisms were isolated, these were the only two does from the five tested to show any uterine abnormality.

It is possible that the B. ovis infection decreased the fertility of the male deer consequently causing the low reproductive rate in this group of animals.

CHAPTER VI

EVALUATION OF *B. OVIS* INCIDENCE IN FREE-RANGING DEER

Introduction

This study was designed to determine the prevalence of *B. ovis* in wild white-tailed deer from Oklahoma. The experimental procedure involved testing of deer testicles and blood samples collected by hunters. Sera from blood samples were subjected to the *B. ovis* CF test. Deer testicles were examined visually and by palpation for any abnormalities. Testicles showing evidence of *B. ovis* infection were sampled for bacteriologic and histopathologic examinations.

Study Procedure

Personnel of the Oklahoma Department of Wildlife Conservation issued equipment and instructions for collection of samples to deer hunters at the Atoka, Bartlesville, Fort Sill, Pushmataha, and Spavinaw Wildlife Management Areas during November and December, 1981. Hunters were requested to attempt to collect blood from any deer bagged and to excise the scrotum and contained testicles flush with the abdominal wall from any buck bagged.

At hunter check stations the blood tubes and testicles were placed in styrofoam insulated boxes containing ice. Within 24 hours the blood

samples were centrifuged, serum was collected and frozen and each scrotum and its contents was examined.

On February 16, 1982, aliquots of frozen serum were sent for B. ovis CF testing at N.V.S.L.

Results

Serum suitable for testing was obtained from 17 male and nine female white-tailed deer. Each of these 26 sera was negative to the B. ovis CF test.

The scrotum and testicles from 68 male deer were examined. Only two abnormalities were observed. The left testicle of one deer was affected as follows: A 2 mm diameter by 1 mm thick yellowish-white lesion was present on the tunic of the testicle in the region of the junction of the tail of the epididymis and the testicle. This lesion was directly underneath the point on the scrotal skin where a female Ixodes tick was attached. Bacterial culture of a swab of the lesion yielded only Micrococcus spp.

A pinpoint adhesion between the scrotal skin and the underlying testiclular tunic was observed at the longitudinal mid-point of the anterior aspect of the left testicle of one other deer. Many of the deer scrotums examined were infested with lice, keds, or ticks and circumstantial evidence suggested that the adhesion just described resulted from skin penetration by a feeding tick. No lesions suggestive of B. ovis infection were observed and consequently no samples were submitted for culture or histopathologic examination.

Discussion

No evidence of B. ovis infection was found from any of the hunter-collected white-tailed deer samples in this study. These findings indicate that natural B. ovis infection is not present in the population of Oklahoma deer sampled.

There were few sheep in the areas where the deer were sampled. Assuming that B. ovis infected sheep would be the most likely source of natural infection in deer, future studies should sample deer in areas where there is sharing of habitat by deer and sheep and where B. ovis has been diagnosed in the latter.

CHAPTER VII

SUMMARY AND CONCLUSIONS

Summary

Experimental inoculation of male white-tailed deer with Brucella ovis isolated from a naturally infected domestic ram generated the following findings.

Ten male white-tailed deer were experimentally inoculated. One deer died while immobilized for inoculation and one other deer died of an undetermined cause eight days PI. Infection was established in each of the other eight experimentally inoculated male deer.

A mature non-infected buck, placed in confinement with an experimentally infected mature buck, acquired infection and developed epididymal lesions during the latter two months of a seven months period of cohabitation. The route of this transmission is unknown.

The only indication of transmission from an infected buck to does during the breeding season was the findings of two does with B. ovis CF titers in sera collected 76 days after introduction of the infected buck.

Screening tests of hunter-collected samples from free-ranging white-tailed deer gave no indication of natural B. ovis infection in the populations sampled. However, the areas sampled were only sparsely populated with sheep and were not areas of recorded incidence of B. ovis in sheep.

Indications from the limited number of animals available for these studies were that infection was transient in young males but remained

established in bucks that were mature when inoculated. Young bucks inoculated between four and five months of age developed infection but typically did not maintain the infection for more than three months. This is analagous to the situation in sheep in which young rams develop infection following experimental B. ovis inoculation but the disease does not persist as long as infection initially induced in older rams.

The mature buck No. 416, inoculated when 17 months old, still had B. ovis organisms present in both epididymides when euthanatized at 14 months PI although no resultant lesions were caused by the infection. The buck No. 10, 18 months old when inoculated, had maintained infection and developed lesions and histopathological changes typical of B. ovis-induced ram epididymitis, when killed 135 days after inoculation.

Conclusions

The results of these experiments indicate that white-tailed deer are susceptible to Brucella ovis infection and develop pathological manifestations virtually identical with those observed in sheep infected with this organism.

Although deer have been shown to be susceptible to experimental infection, and buck to buck transmission was demonstrated, the social and breeding behavior patterns which are important in the spread of the disease in sheep are different for the white-tailed deer. The habits of white-tailed deer may preclude widespread dissemination of the infection in this species. Nevertheless, where unexplained low reproductive rates are encountered in white-tailed deer populations, the possibility of B. ovis infection should be investigated.

Within the state of Oklahoma and in other areas of the United States

there are locations where white-tailed deer share habitat with sheep which have been infected with Brucella ovis. Evaluation of deer would be desirable in these areas to ascertain if natural infection of deer has occurred. The possible incidence of natural B. ovis infection in deer in these areas should be evaluated.

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VITA 2

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