PREDICTING ADULT AND FAWN WHITE-TAILED DEER MORTALITY USING INDICIES OF NUTRITIONAL AND IMMUNOLOGICAL CONDITION

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PREFACE

Fort Sill Military Reservation (FSMR), a United States Army Artillery and Missle Training Center, encompasses 94,302.20 acres in Comanche County in southwestern Oklahoma. The area includes diverse habitats, ranging from relatively flat prairie in the east to steep granite hills in the west. Three artillery impact areas, with a multitude of firing positions, observation posts, and surveyed target locations lie within FSMR. White-tailed deer is the only native big game animal present on the area in significant numbers. FSMR has three ranges, East, West, and Quanaha each with a independently managed, heavily hunted deer herd. Each range is distinct in deer numbers as well as in soil and habitat types. The purpose of this study was to determine survival and cause-specific mortality rates for white-tailed deer on West Range of FSMR; to compare habitat quality of East and West Range using postmortem morphologic, physiologic, and dietary indices from fall-harvested deer and seasonal fecal indices of dietary quality.

This thesis is comprised of three manuscripts formatted for submission to the Journal of Wildlife Diseases. The manuscripts (Chapters I, II, III) are complete as written and do not need supporting material.

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CHAPTER I

USE OF CONDITION INDICATORS TO EVALUATE HABITAT QUALITY FOR TWO ADJACENT WHITE-TAILED DEER HERDS IN SOUTHWESTERN OKLAHOMA

ABSTRACT: We examined the usefulness of condition profiles, incorporating postmortem morphologic, physiologic, and dietary indices from fall-harvested deer and seasonal fecal indices of dietary quality, for evaluating differences in habitat quality between adjacent populations of white-tailed deer (Odocoileus virginianus). This study was conducted on East Range (4.5% of 12,000 ha cultivated) and West Range (0.8% of 18,000 ha cultivated), Fort Sill Military Reservation, southwestern Oklahoma, from November 1987 to August 1989. Serum concentrations of urea nitrogen, creatinine, triglycerides, glucose, alkaline phosphatase, total bilirubin, lactic dehydrogenase, alanine aminotransferase, and gamma-glutamyl transpeptidase did not differ between postmortem samples obtained from harvested deer and live-caught deer. An evaluation of postmortem samples revealed that the East Range deer herd had higher concentrations of serum urea nitrogen, urea/creatinine ratios, nitrogen in rumen contents, and nitrogen in feces, but lower concentrations of creatinine, acid detergent fiber, neutral detergent fiber, and insoluble nitrogen in rumen contents, and acid detergent fiber and insoluble nitrogen in fecal samples compared to the West Range deer herd. Seasonal fecal indices also indicated an overall higher concentration of nitrogen and lower acid detergent fiber levels in diets of deer from East Range compared to West Range. Morphological indices were not sensitive to the apparent differences in habitat quality between the two ranges. However,

physiological and dietary indices may provide wildlife managers a practical and sensitive means for early detection of changes in habitat quality.

Key words: white-tailed deer, Odocoileus virginianus, condition indices, morphology, physiology, diet quality, fecal indices, blood chemistry, postmortem changes.

INTRODUCTION

Nutritional deficiencies, from marginal single-nutrient deficiencies to complete starvation, are undoubtedly common among many herbivore populations. Reproductive physiology of individuals and recruitment into populations are sensitive to nutritional conditions (Teer et al., 1965; White, 1978). More importantly, it is becoming increasingly clear that marginal deficiencies of a wide range of nutrients can alter immunocompetence of an animal, resulting in increased morbidity and mortality (Gershwin et al., 1985). Because of these relationships, diagnostic systems for monitoring the nutrition of animals are regarded as important aspects of game management programs (Kie, 1988).

Numerous techniques for directly assessing the condition of white-tailed deer (Odocoileus virginianus) and indirectly assessing the nutritional quality of their range have been developed through controlled research (Brown, 1984). Morphological measurements of condition which have been suggested for use include visual estimation (Riney, 1955; Kistner et al., 1980), weights of selected organs (Ozoga and

Verme, 1978), assessment of fat reserves (Cheatum, 1949; Finger et al., 1981), body size (Severinghaus, 1955; Kopf et al., 1984), and antler characteristics (Kie et al., 1983). Physiological indices of condition also have been examined and include analyses of metabolic and endocrine constituents of blood serum (Seal et al., 1978b; Warren et al., 1981), urine (DelGiudice et al., 1987, 1988), and vitreous humor (DeLiberto et al., 1989), as well as a variety of hematological parameters (Rosen and Bischoff, 1952). Indices for assessing recent nutritional history of deer include analyses of nutrient or plant cellular components of rumen contents (Klein, 1962; Kirkpatrick et al., 1969) and feces (Kie et al., 1984; Leslie and Starkey, 1985).

Although a wealth of indices have been suggested for use in assessing condition of white-tailed deer, few examples of their practical application in the management of wild populations exist in the literature. Kie et al. (1984) found that selected morphological indices were useful in comparing condition of two black-tailed deer (O. hemionus columbianus) herds in California. Seal et al. (1978a) and Kie et al. (1983) demonstrated that physiological profiles could be used to detect differences in habitat quality of white-tailed deer herds in northern Minnesota and south Texas, respectively. Similarly, metabolic profiles were shown to be useful for comparing habitat quality of three pronghorn (Antilocapra americana) populations in Idaho (Seal and Hoskinson, 1978).

This study was initiated to test the hypothesis that

condition profiles of white-tailed deer that incorporate corollary comparisons of morphologic, physiologic, and nutritional indices (Waid and Warren, 1984) are sensitive to differences in habitat quality. We tested this hypothesis by comparing two adjacent white-tailed deer herds in southwestern Oklahoma using fall-harvested animals and seasonal changes in fecal constituents. It was also our objective to supplement the limited physiological and nutritional information on deer herds in this geographic region.

MATERIALS AND METHODS

Study area

This study was conducted on Fort Sill Military Reservation (FSMR) which is located in the Central Rolling Red Plains and Central Rolling Red Prairies Land Resource Areas (Gray and Galloway, 1969) of southwestern Oklahoma (Appendix A). The area includes diverse habitats, ranging from relatively flat prairie in the east to steep granite hills in the west. Monthly mean temperatures range from 4.8 C in January to 28.7 C in August. Most rainfall occurs in the spring which accounts for 34% of total annual precipitation ($\bar{x}$ = 77 cm) (Appendix B). Livestock grazing has been excluded for 30 years on FSMR, and major disturbances are primarily limited to annual prescribed burning and wildfires which result from military training exercises.

Vegetation on FSMR is dominated by tall-grasses such as big bluestem (Andropogon gerardi), little bluestem (Andropogon scoparius), sand bluestem (Andropogon hallii), switchgrass (Panicum virgatum), and Indiangrass (Sorghastrum nutans) on moist prairie sites. Mid- and short-grasses such as blue grama (Bouteloua gracilis) and sideoats grama (Bouteloua curtipendula) occupy the more drought-natured sites and slickspot soils (Comanche County, SCS 1970). Wooded areas are primarily restricted to riparian zones and isolated rocky upland sites and include American elm (Ulmus americana), pecan (Carya illinoensis), western hackberry (Celtis occidentalis), red oak (Quercus sp.), blackjack oak (Quercus marylandica), post oak (Quercus stellata), bur oak (Quercus macropcarpa), and chinquapin oak (Quercus muhlen bergii).

We used condition profiles to compare habitat quality between two adjacent white-tailed deer populations on East Range and West Range, FSMR (Appendix A). There is no apparent transitional movements by deer between ranges (Dinkines, 1990; Pilcher and Wampler, 1982). East Range (12,000 ha) is dominated by rolling tall-grass prairie with one well-developed stream bottom. West Range (18,000 ha) has a more diverse landscape ranging from rugged granite outcrops to tall-grass prairie with intermittent stream bottoms of various sizes. Soil types also differ between East Range (Zaneis and Lucien-Zaneis-Vernon series) and West Range (Foard and Granite series). Approximately 4.5% of the total

area is cultivated on East Range (fields average 9.0 ha), compared with 0.8% on West Range (7.5 ha). Cultivated fields are uniformly distributed on East Range, but are concentrated along east and south boundaries on West Range. Agricultural crops produced include alfalfa (Medicago sativa), milo (Sorghum bicolor), and winter wheat (Triticum aestivum).

Density of deer on East Range was estimated at 3.33 deer/100 ha in 1987 and 3.75 deer/100 ha in 1988 compared to 2.50 deer/100 ha in both 1987 and 1988 on West Range (Stout, 1989). Ratios of fawns per doe ranged from 0.66 (1987) to 0.68 (1988) on East Range and 0.64 (1987) to 0.61 (1988) on West Range. The mean fawn:doe ratio for the period 1982-1988 was 0.78 on East Range and 0.60 on West Range. An intensive predator control program was initiated in 1977 on both East and West Range (Stout, 1982). A total of 35 and 51 coyotes were removed from East and West Range, respectively, from 1987 to 1988.

Morphological indices

Check station records for 515 white-tailed deer harvested from East Range and West Range in November and December from 1984 to 1988 were used to evaluate differences in gross morphology between the two populations. Age class of each animal was determined by tooth eruption and wear (Severinghaus, 1949). Live weights for deer that were not eviscerated in the field and eviscerated carcass weights for all deer were recorded to the nearest 0.5 kg using a standard spring scale. Antler characteristics were also recorded,

including total number of points > 2.54 cm long and main beam circumference measured 2.54 cm above the burr. Weights (to the nearest 0.1 g) of the cervical thymus gland and spleen were recorded for deer harvested in 1987 and 1988.

Physiological indices

Hunters were instructed not to eviscerate harvested deer in the field, but return them to the check station as soon as possible after death (determined to be < 2 hr) during the 1987 and 1988 harvest. Blood samples were obtained from a total of 65 white-tailed deer in the 0.5 to 6.5 year age-class. Blood samples were obtained from the superior vena cava using 10-ml evacuated serum separation tubes (Becton Dickenson, Rutherford, New Jersey 07070, USA), stored on ice for < 1 hr, and centrifuged for 15 min at 1500 xg. Collected serum was stored frozen at -20 C until analyses could be performed as a batch following each harvest.

For obvious reasons, postmortem serum samples are not appropriate for the analysis of many serum constituents (Blankenship and Varner, 1977). As a result, only a few selected blood constituents that remain reasonably stable soon after death were of interest in this study. To help ascertain which of those constituents to use, we statistically compared blood serum profiles of live-caught deer with postmortem samples. Blood serum was obtained from a total of 44 live-caught white-tailed deer on West Range in December 1987 and 1988 in conjunction with a radio-telemetry study (Dinkines, 1990). A helicopter-net gun system

(DeYoung, 1988) was used to capture animals. Mean concentrations of selected serum chemistries for live-caught and postmortem blood samples were compared using one-way analysis of variance.

The degree of hemolysis of each serum sample was determined by ocular estimation using criteria of Frank et al. (1978). Serum samples that were severely hemolyzed were discarded from all analyses because of the potential for altered serum chemistries (Blankenship and Varner, 1977; Frank et al., 1978; Dorner et al., 1981). Serum samples were submitted to the Fort Sill Medical Diagnostic Laboratory for analyses which were performed on an Ektachem-700 Analyzer (Eastman Kodak Co., Rochester, New York 27705, USA), according to procedures specified by the manufacturer. Constituents analyzed in serum included urea nitrogen (BUN), creatinine, glucose, uric acid, sodium, chloride, phosphorous, calcium, total protein, albumin, total bilirubin, cholesterol, triglycerides, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGT), creatine phosphokinase (CPK), and lactic dehydrogenase (LD). A serum urea nitrogen/creatinine ratio (U/C) was also calculated. Only postmortem BUN, creatinine, U/C, and triglycerides were used to assess physiological condition of harvested deer from East Range and West Range.

Diet quality indices

Differences in the quality of vegetation in the diet of

white-tailed deer from East Range and West Range were evaluated using both fall-harvested animals and seasonal collections of feces. A minimum of 15 fresh fecal pellet groups were collected from random locations on both East Range and West Range in fall (October), winter (February), spring (May), and summer (August), 1987 to 1989. Additionally, postmortem samples of feces from the rectum and contents of the rumen were obtained from eviscerated deer harvested in the fall from each study area. All collected samples were stored frozen at -20 C for later analysis.

In the laboratory, fecal and rumen samples were oven-dried at 50 C and blended in a high-speed blender. Subsamples of each individual fecal group or rumen sample were ground in a Wiley mill (Thomas Scientific, Swedesboro, New Jersey 08085, USA) through a 1-mm mesh screen prior to analysis. Samples were analyzed for concentration of nitrogen using the Kjeldahl method (Williams, 1984) on duplicate 0.25 g samples. If > 5% error occurred between duplicates, they were discarded and nitrogen was determined from two new samples. Concentrations of soluble contents of cells (neutral detergent fiber) and cell wall fractions (acid detergent fiber) in fecal and rumen samples were determined following the procedure of Goering and Van Soest (1970) using a Fibertec System (Tecator Inc., Herndon, Virginia 22070, USA). Concentration of nitrogen in the acid detergent fiber fraction of fecal and rumen samples was also determined as an index of ınsoluble nitrogen content (Wofford et al., 1985).

Data analysis

To account for the effects of age on morphological variables, we used analysis of covariance with age class (for antler measurements and body weights) or eviscerated carcass weight (for organ weights) treated as a covariate to test the main and interactive effects of sex and habitat (East Range vs. West Range) for adult deer ($\geq$ 1.5 yr age class). Differences in morphological indices of fawns were tested for main factor effects of sex and habitat using two-way analysis of variance. For dependent dietary and physiological variables, the main and interactive effects of season, year, and habitat were examined using three-way analysis of variance. Protected multiple comparisons (LSD) were used when significant differences ($P < 0.05$) were present. Pearson correlation coefficients were used to examine relationships among physiological and dietary variables. The Statistical Analysis System (SAS) was used for all data analyses (SAS Institute Inc., 1982).

RESULTS AND DISCUSSION

Morphological indices

Body weight and antler characteristics were recorded for a total of 515 white-tailed deer harvested from East Range (108 females, 149 males) and West Range (61 females, 197 males) from 1984 to 1988 (Table 1). Males were heavier than females, and all deer continued to increase in both carcass weight ($r = 0.77$) and eviscerated carcass weight ($r = 0.77$)

with age ($P < 0.01$). Carcass weights ($\bar{x} = 50.55 \pm 1.23$ (SE) kg) and eviscerated carcass weights (37.57 ± 0.48) did not differ ($P > 0.10$) between deer herds on East Range and West Range. Although body weights have been suggested as a useful measure of deer condition (Severinghaus, 1955), they tend to be highly variable with respect to season, age, and sex (Kie, 1988). Interfering factors probably include voluntary weight loss during the rut (Ozoga and Verme, 1970) and lactational stresses among does. Body weights also are less sensitive to acute dietary changes compared to long-term nutritional restriction (Holter and Hayes, 1977; Warren et al., 1981). Body weight indices would be more sensitive for assessing condition among fawns because of their relative growth demands (Seal et al., 1978b; Holter and Hayes, 1977). However, no differences ($P > 0.10$) in carcass weights or eviscerated carcass weights were detected between ranges for male fawns ($P > 0.45$) or female fawns ($P > 0.16$).

Spleen and thymus gland are important organs of the immune system which have been shown to respond to changes in nutritional status of fawns (Ozoga and Verme, 1978; Verme and Ozoga, 1980). We observed no differences ($P > 0.10$) in spleen (281.99 ± 9.29 g) or thymus gland (8.40 ± 1.12 g) weights between deer herds on East Range and West Range (Table 1). Because thymic atrophy occurs with increasing age, we reexamined these data for only the yearling age class, but found no difference ($P > 0.10$) between herds.

Number of antler points (r = 0.57) and main beam circumference (r = 0.40) both increased significantly ($P < 0.01$) with age (Table 1). Number of antler points tended ($P < 0.07$) to be greater for bucks harvested from West Range (6.5 ± 0.2) compared to those on East Range (5.7 ± 0.3). This difference could have been due to restrictions placed on hunters regarding type of weapons used, and not a reflection of habitat quality. Hunters on East Range were predominately limited to use of shotguns, whereas those on West Range used rifles. This could have led to some bias in the size of antlered bucks harvested. Long et al. (1959) reported that short-term feed restrictions had no apparent effect on the number of antler points in white-tailed deer, although chronic malnutrition reduced not only the number of antler points, but also main beam diameters (Cowan and Long, 1962). We found no significant difference ($P > 0.10$) in main beam circumference between deer herds.

Physiological indices

Serum concentrations of BUN, creatinine, triglycerides, glucose, ALP, total bilirubin, LD, ALT, GGT, and U/C ratio did not differ between postmortem samples obtained from harvested deer and live-caught deer (Table 2). Most electrolytes and several enzymes were greatly altered in our postmortem serum samples. The fact that several enzymes did not differ between our live-caught and harvested deer illustrates the impact that capture stress can have on blood chemistry. Previous studies have demonstrated that serum

enzyme concentrations are greatly altered by stress (Wesson et al., 1979) and sampling time after death (Coe, 1974; Schoning and Strafuss, 1980). Blood is not always suitable for some postmortem chemical analysis due to contamination by bacteria, autolytic cellular disintegration, and postmortem metabolism of serum chemicals (McLaughlin and McLaughlin, 1988). In contrast, BUN and creatinine concentrations have been found to be relatively stable in postmortem blood samples. Wesson et al. (1979) found no effects of time (max 30 min) of blood sampling after death on BUN concentrations of white-tailed deer. Schoning and Strafuss (1981) reported postmortem BUN concentrations in blood remained stable for 12 hours at 20 C, and 6 hours at 37 C. Likewise, Schoning and Strafuss (1980) reported creatinine values to be similar in postmortem (max 6 hrs) and antemortem blood samples.

We limited comparisons of physiological indices between East Range and West Range to BUN, creatinine, triglyceride, and U/C ratio which appeared normal in postmortem samples. No significant ($P > 0.10$) age or year differences were demonstrated in concentrations of these constituents. We observed significant differences in concentrations of BUN, creatinine, and the U/C ratio between deer herds (Figure 1). Overall, concentration of BUN averaged 25.0 ± 1.5 for East Range and 16.6 ± 1.4 mg/dl for West Range, and was not influenced by sex. Creatinine levels also differed significantly between areas and there was a significant interaction with sex. Concentrations of creatinine were

lower in males harvested from East Range (1.68 ± 0.14) than those from West Range (2.52 ± 0.22 mg/dl). There were no apparent differences among females. The U/C ratio of deer was greater on East Range (16.51 ± 1.82) than West Range (7.11 ± 0.55) and also differed with respect to sex ($P < 0.05$). No significant difference ($P > 0.10$) in the concentration of triglycerides was observed between East Range and West Range deer herds.

Serum chemistry parameters are attractive indices for assessing condition because they reflect recent nutritional history (Seal, 1978). Serum concentrations of BUN in our study were similar to values recently reported for deer from south-central Oklahoma in the fall (DeLiberto et al., 1989). Postmortem blood profiles of deer on FSMR suggested that nutritional quality of habitat on East Range was better than on West Range. Positive relationships between recent protein intake and concentration of BUN or the U/C ratio have been demonstrated by several investigators (Seal et al., 1978a; Bahnak et al., 1979; Warren et al., 1981). Seal et al. (1978a) found that BUN values were useful in detecting differences among habitats of white-tailed deer in Minnesota. Caution must be used interpreting BUN values because a negative energy balance can often elevate levels (Kirkpatrick et al., 1975). For example, Kie et al. (1980) observed higher BUN concentrations among deer from a high density herd in Texas, despite their poorer condition, than those from a low density herd. However, differences in habitat quality

between deer herds on FSMR are probably more related to protein than energy availability as suggested by serum chemistry profiles that reflected significant differences in BUN, but similar triglyceride (an indicator of energy status) levels.

Diet quality indices

We obtained postmortem rumen contents and fecal samples from 36 harvested deer (14 females, 22 males) on East Range and 38 (9 females, 29 males) from West Range during fall 1987 and 1988. No significant ($P > 0.10$) year effects were observed for concentrations of nitrogen, neutral detergent fiber, and acid detergent fiber in either postmortem rumen contents or fecal samples. Concentrations of nitrogen and acid detergent fiber in rumen and fecal samples, and neutral detergent fiber in rumen samples, differed between East Range and West Range deer herds (Table 3). Concentrations of nitrogen in feces and rumen contents were 18 and 33% greater on East Range than West Range, respectively. Conversely, acid detergent fiber levels in feces and rumen contents were greater among deer harvested on West Range than East Range. Concentration of neutral detergent fiber in rumen contents was greater on West Range than East Range; there was no difference for fecal samples. We also found higher levels of insoluble nitrogen in feces from West Range than East Range, but no difference for rumen contents (Table 3).

There were no relationships ($P > 0.10$) between age class and any postmortem dietary index measured in this study. We

observed that concentration of nitrogen in feces correlated inversely with acid detergent fiber levels in feces ($P < 0.01$, $r = -0.62$) and positively with nitrogen ($P < 0.01$, $r = 0.35$) and insoluble nitrogen ($P < 0.03$, $r = 0.28$) levels in rumen contents. Acid detergent fiber concentration of feces correlated inversely with nitrogen ($P < 0.01$, $r = -0.32$) and positively with acid detergent fiber ($P < 0.01$, $r = 0.43$) concentrations in rumen contents. Nitrogen concentration of rumen contents was correlated inversely with acid detergent fiber content in rumen contents ($P < 0.01$, $r = -0.40$), but not feces ($r = 0.06$).

Nitrogen concentration of rumen contents has been used to estimate dietary crude protein intake in deer (Klein, 1962; Kirkpatrick et al., 1969), despite the fact that levels are often higher than in the diet (Klein, 1962) as a result of endogenous sources. Several investigators have demonstrated that rumen crude protein levels fluctuate seasonally in response to changes in forage quality (Kopf et al., 1984; Waid and Warren, 1984). The inverse relationship observed between nitrogen and acid detergent fiber in rumen contents suggests that forage digestibility increases with increased crude protein. This is supported by Kopf et al. (1984) who noted that in vitro digestible dry matter and crude protein levels in rumen contents of deer from south Texas showed similar seasonal fluctuations. The positive relationship between feces and rumen contents for nitrogen is in agreement with several studies demonstrating a

strong relationship between diet and fecal nitrogen levels (Klein, 1962; Holechek et al., 1982; Kirkpatrick et al., 1969). Insoluble nitrogen content determined in this study is thought to reflect the amount of fiber-bound, cell-wall protein (Mould and Robbins, 1981). Wofford et al. (1985) reported that soluble phenolic problems are indicated when fiber-bound nitrogen concentration exceeds 1.00%. Since the insoluble nitrogen concentration was below 1.00% for both East Range and West Range, we suspect that the concentration of nitrogen in feces reflects a difference in diet quality and not a problem with insoluble phenolics.

We measured BUN concentrations and U/C ratios to provide sensitive indices of recent protein nutritional history of deer. However, BUN concentration was only weakly related to rumen nitrogen concentration of deer on FSMR. Waid and Warren (1984) reported a similar relationship ($\underline{r} = 0.24$) for deer in central Texas, but reported a stronger correlation between the urinary U/C ratio and rumen crude protein content. We found that the serum U/C ratio was significantly correlated with rumen nitrogen concentration ($\underline{P} < 0.01$, $\underline{r} = 0.65$). Because obtaining rumen contents requires the sacrifice of animals, the use of feces to index seasonal changes in dietary quality has received considerable attention from biologists. Concentration of nitrogen in feces has been shown to be related to both digestibility (Holloway et al., 1981) and protein content (Leslie and Starkey, 1985) of the diet. However, few reported studies

have attempted to use fecal indices to determine differences in habitat quality among white-tailed deer populations (Jenks et al., 1989).

Differences in seasonal fecal indices between deer herds on FSMR were similar to those observed for postmortem serum and diet quality profiles. Concentration of nitrogen in fecal pellet groups showed significant ($P < 0.01$) seasonal and annual fluctuations (Figure 2). Mean seasonal concentrations of nitrogen for the 2 years combined varied from a low of 1.99 $\pm$ 0.05 in winter to 2.70 $\pm$ 0.09% in spring. Multiple range tests indicated that concentrations of nitrogen in feces were not different ($P > 0.05$) between summer and spring; all other comparisons were significant ($P < 0.05$). Annual mean concentration of fecal nitrogen was greater in 1988 (2.50 $\pm$ 0.05) than 1987 (2.25 $\pm$ 0.06%). Fecal nitrogen concentrations averaged about 18% greater ($P < 0.01$) on East Range than West Range, and there was a significant ($P < 0.05$) interaction with season. Differences in nitrogen concentration of feces between deer herds were most pronounced in summer and fall.

Concentration of acid detergent fiber in feces also showed significant ($P < 0.01$) seasonal and annual effects (Figure 2). Acid detergent fiber levels were lower ($P < 0.05$) in fall (42.09 $\pm$ 0.9) compared with winter (49.7 $\pm$ 0.8), spring (49.9 $\pm$ 1.2), and summer (46.9 $\pm$ 0.7), and greater in 1988 (47.9 $\pm$ 0.9) than 1987 (46.6 $\pm$ 0.7%). There also was a significant ($P < 0.01$) difference between deer

herds and deer herd by season interaction ($P < 0.05$). Acid detergent fiber concentrations were greater on West Range (overall, 49.1 ± 0.7) than East Range (45.2 ± 0.8%) for all seasons except winter. Acid detergent fiber concentrations were inversely correlated ($P < 0.01$, $r = -0.41$) with nitrogen in feces, and showed a slight correlation ($P < 0.01$, $r = 0.23$) with the insoluble nitrogen component.

Concentration of insoluble nitrogen in feces showed significant ($P < 0.01$) seasonal and annual fluctuations (Figure 2). Insoluble nitrogen levels were highest ($P < 0.02$) in spring (1.0 ± 0.03) compared with summer (0.9 ± 0.03), fall (0.5 ± 0.03), and winter (0.5 ± 0.02%). Annual mean concentration of insoluble nitrogen was greater ($P < 0.01$) in 1988 (0.81 ± 0.02) than 1987 (0.69 ± 0.02%). The concentration of nitrogen in fecal samples was correlated ($P < 0.01$, $r = 0.48$) with the insoluble nitrogen component. Differences between East Range and West Range were similar to those observed for nitrogen; insoluble nitrogen was greater ($P < 0.01$) on East Range (0.74 ± 0.03) than West Range (0.69 ± 0.03%).

CONCLUSIONS

Results from our study on FSMR demonstrate the feasibility and utility of using condition profiles incorporating morphologic, physiologic, and dietary quality indices for assessing or comparing habitat quality among white-tailed deer herds. Small sample sizes usually make

interpretation of a single index of habitat quality difficult and risky (Kie, 1988). A myriad of intrinsic and extrinsic variables unrelated to habitat quality can potentially mask differences in an index that might otherwise be attributable to habitat quality. As a result, single variable models for assessing habitat quality should be avoided. Condition profiles for assessing habitat quality that are based on a variety of indices, and are not confined to a single aspect such as physiology, should provide more sensitivity in the presence of interfering intrinsic and extrinsic variables.

Our study also suggests that useful physiologic and dietary quality information can be derived postmortem from hunter-harvested white-tailed deer. In particular, serum BUN levels which have been widely used to assess protein intake in deer remain stable up to 2 hours after death. Rumen contents and fecal samples from hunter-harvested deer can also provide useful information on diet quality for the fall season. Changes in diet quality during other seasons can be easily monitored by periodic profiling of nutrient and cellular constituents in deposited feces, at relatively low cost (Jenks et al., 1989).

Both physiologic and diet quality indices supported our conclusion that the nutritional quality of habitat on East Range was higher than on West Range. A plausible explanation for this difference is the greater amount and distribution of cultivated fields available on East Range compared to West Range. Alfalfa, a major crop in these

fields, is a high protein forage readily consumed by white-tailed deer. It is important to note that although differences in diet quality were detected between deer herds, no major differences were observed in morphology. This apparent disparity is most likely an indication that even though East Range is providing deer a higher quality diet than West Range, the differences are not significant. East Range and West Range are both providing deer adequate nutrients for morphologic maintenance and development. Morphology undoubtedly better reflects long-term conditions of the habitat (Kie, 1988). Differences in density and fawn:doe ratios between deer herds on FSMR suggest that nutritional conditions of the habitat influenced population dynamics (nutrient-demanding recruitment) but not morphology (given our sample size) in the fall.

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Table 1. Mean body weights (kgs), organ weights (gms), and antler measurements (cms) for hunter-harvested white-tailed deer from East and West Ranges Fort Sill Military Reservation, Oklahoma, USA.

	East Range						West Range						P	
	Males			Females			Males			Females				
Index	n	$\bar{x}$	SE	n	$\bar{x}$	SE	n	$\bar{x}$	SE	n	$\bar{x}$	SE	Sex effect	Range effect
Weights														
Carcass														
Fawns	10	29.86	1.1	3	27.27	2.0	2	32.27	5.0	5	30.18	0.8	0.01	0.09
Adults	24	58.41	2.1	15	48.82	2.4	33	57.52	1.5	7	48.77	2.6	0.15	0.49
Eviscerated carcass														
Fawns	39	22.59	0.5	34	20.86	0.5	21	23.66	0.9	18	22.07	0.8	0.22	0.17
Adults	109	15.29	0.9	69	39.96	0.6	174	43.09	0.6	42	35.83	0.7	0.37	0.88
Spleen														
Fawns	6	212.54	12.5	2	122.10	7.5	1	242.28		5	246.49	16.5	0.88	0.94
Adults	16	331.95	20.2	9	252.69	32.8	25	293.69	10.3	4	313.34	10.9	0.98	0.12
Thymus gland														
Fawns	5	12.63	3.5	2	17.06	5.6	1	10.21		1	25.07		0.19	0.68
Adults	8	4.65	0.9	5	6.40	1.0	9	5.04	1.9	4	11.76	2.6	0.67	0.44
Antlers														
Main beam circumference	106	9.06	0.4				170	9.35	0.4					0.17
Total number of points	108	5.76	0.3				173	6.50	0.2					0.09

Table 2. Mean concentrations of selected serum constituents for hunter-harvested (n = 36) and live-caught (n = 44) white-tailed deer collected from West Range on Fort Sill Military Reservation, Oklahoma, in November and December 1987 and 1988.

Blood serum constituent	Hunter-harvested		Live-caught		
	$\bar{x}$	SE	$\bar{x}$	SE	P
Urea nitrogen (mg/dL)	16.57	1.44	16.85	0.65	0.50
Creatinine (mg/dL)	2.29	0.18	1.99	0.10	0.30
Urea nitrogen/ creatinine	7.11	0.55	9.28	0.58	0.13
Triglycerides (mg/dL)	117.44	14.50	131.73	9.97	0.42
Alkaline phosphatase (U/L)	231.26	51.28	127.88	13.77	0.18
Total bilirubin (mg/dL)	1.40	0.48	0.55	0.06	0.80
Glucose (mg/dL)	168.21	22.84	236.63	9.65	0.11
Lactic dehydrogenase (U/L)	1210.00	70.01	1094.33	161.46	0.43
Alanine amino-transferase (U/L)	83.95	3.17	136.85	24.81	0.63
Gamma-glutamyl transpeptidase (U/L)	188.78	17.63	153.04	5.82	0.62

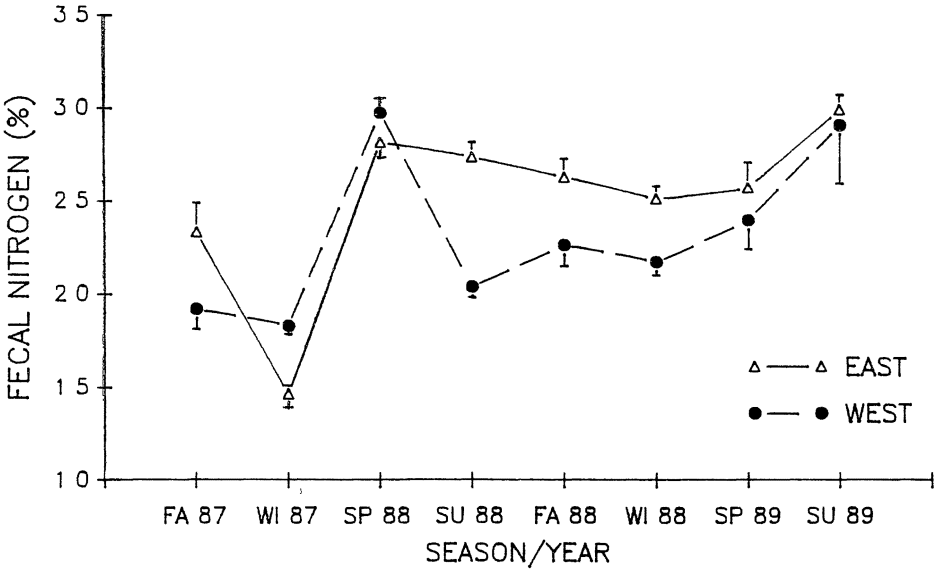
Table 2. Continued.

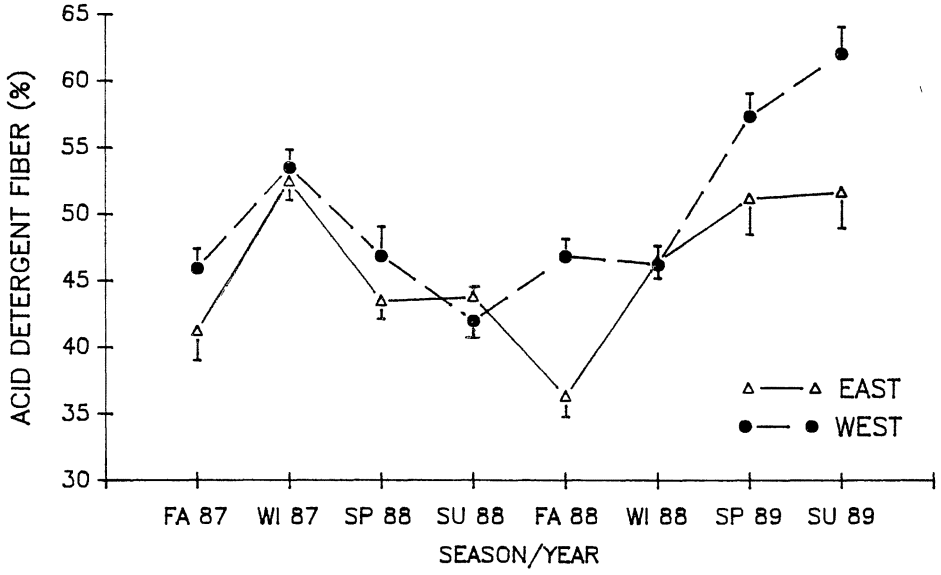
Blood serum constituent	Hunter-harvested $\bar{x}$	Hunter-harvested SE	Live-caught $\bar{x}$	Live-caught SE	P
Uric acid (mg/dL)	0.53	0.09	0.21	0.06	0.01
Sodium (mmol/L)	136.65	1.66	159.68	1.35	0.01
Potassium (mmol/L)	12.18	0.52	5.86	0.17	0.01
Chloride (mmol/L)	104.58	1.72	118.56	1.50	0.05
Phosphorous (mg/dl)	14.81	0.85	10.16	0.43	0.01
Calcium (mg/dL)	9.53	0.27	10.40	0.15	0.05
Total protein (g/dL)	6.43	0.25	7.55	0.13	0.01
Albumin (g/dL)	3.73	0.17	4.26	0.08	0.01
Cholesterol (mg/dL)	47.28	0.81	67.85	1.00	0.01
Aspartate amino-transferase (U/L)	164.54	11.21	418.88	85.29	0.01
Creatine phosphokinase (U/L)	347.53	51.79	1239.29	236.94	0.02

Table 3. Diet quality indices for hunter-harvested white-tailed deer from East (n = 36) and West Range (n = 36), Fort Sill Military Reservation, Oklahoma, in November and December 1987 and 1988.

Index (% dry wt.)	East Range $\bar{x}$	East Range SE	West Range $\bar{x}$	West Range SE	P
Feces					
Nitrogen	2.49	0.09	2.11	0.72	0.01
Acid detergent fiber	38.95	1.30	46.59	1.01	0.01
Insoluble nitrogen	0.50	0.02	0.61	0.03	0.04
Rumen					
Nitrogen	3.73	0.17	2.80	0.09	0.01
Neutral detergent fiber	56.63	1.14	64.56	1.39	0.01
Acid detergent fiber	33.16	0.96	41.65	1.43	0.01
Insoluble nitrogen	0.43	0.03	0.50	0.03	0.13

Figure 1. Mean (± SE) concentrations of urea nitrogen, creatinine, and urea/creatinine ratios in blood serum obtained from white-tailed deer harvested from East Range and West Range, Fort Sill Military Reservation, Oklahoma, November to December 1987 and 1988.


FECAL NITROGEN (%)
35
30
25
20
15
10
EAST
WEST
FA 87
WI 87
SP 88
SU 88
FA 88
WI 88
SP 89
SU 89
SEASON/YEAR


ACID DETERGENT FIBER (%)
65
60
55
50
45
40
35
30
EAST
WEST
FA 87
WI 87
SP 88
SU 88
FA 88
WI 88
SP 89
SU 89
SEASON/YEAR

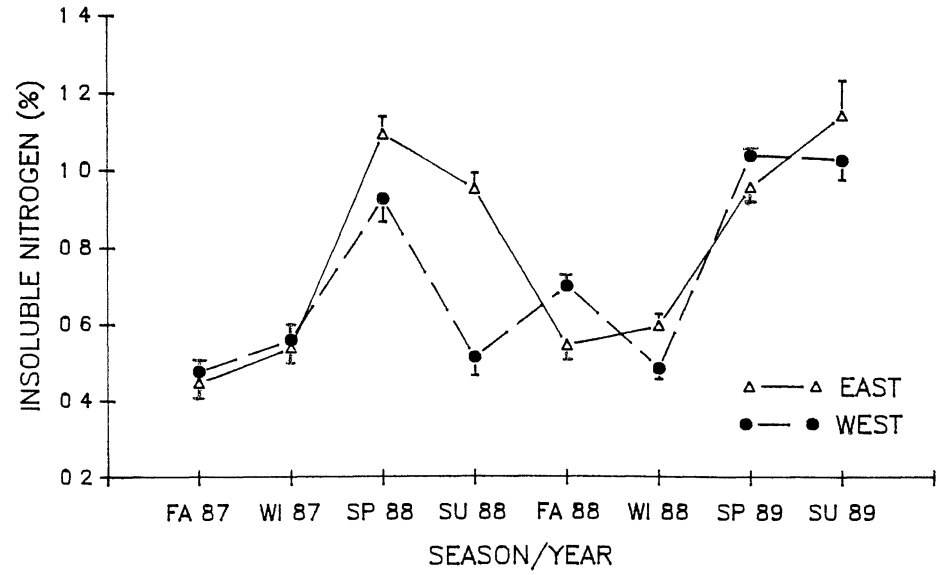
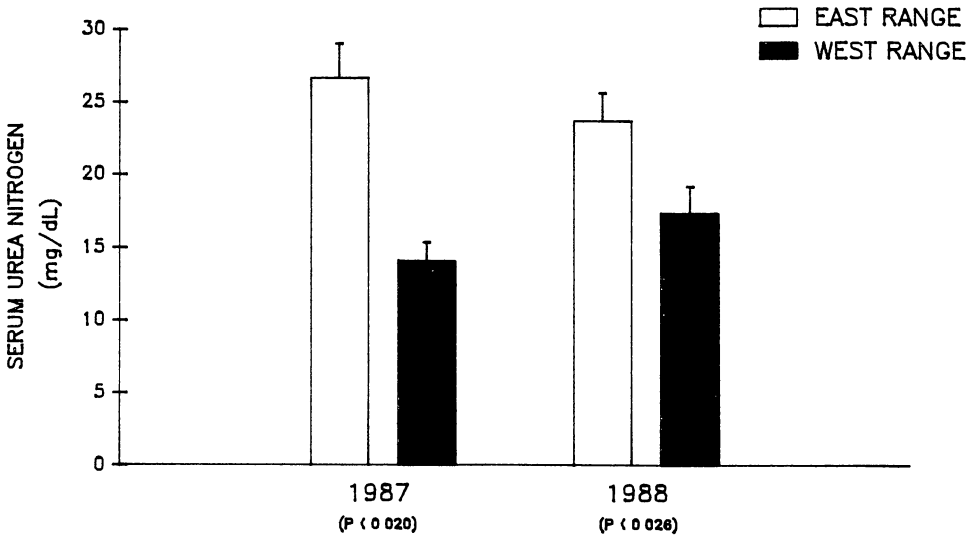
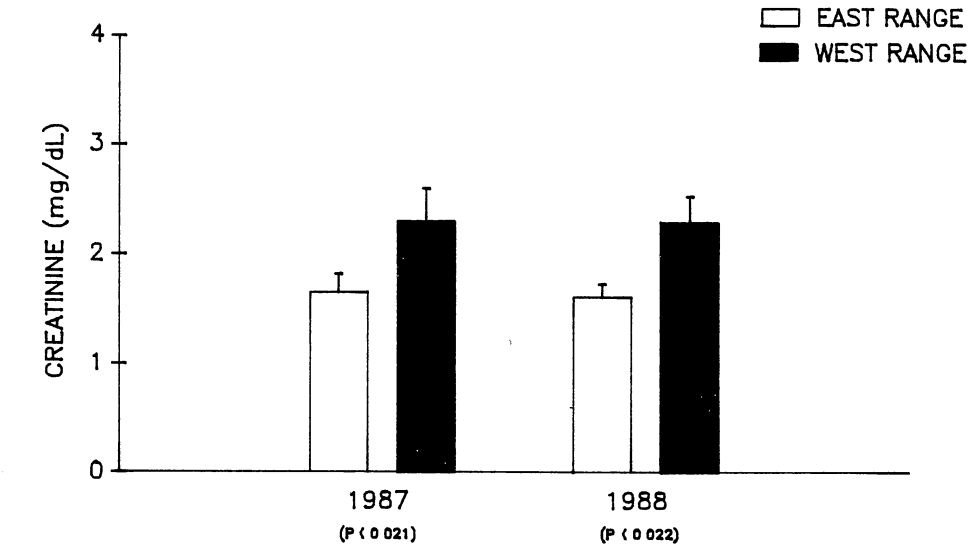
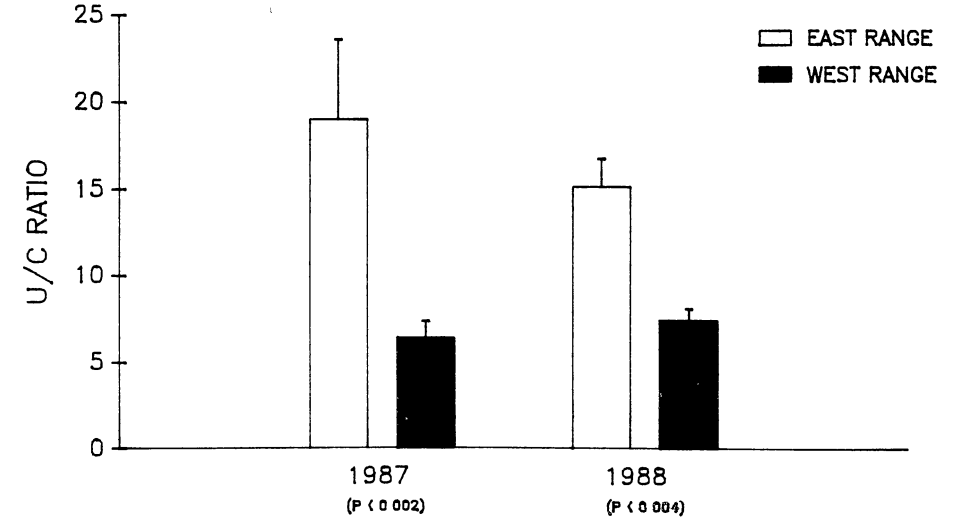

INSOLUBLE NITROGEN (%)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
EAST
WEST
FA 87
WI 87
SP 88
SU 88
FA 88
WI 88
SP 89
SU 89
SEASON/YEAR

Figure 2. Seasonal variations in concentrations (%) of nitrogen, acid detergent fiber, and insoluble nitrogen in fecal samples collected from East Range and West Range, Fort Sill Military Reservation, Oklahoma, from fall 1987 to summer 1989. Values represent mean $\pm$ standard error.


EAST RANGE
WEST RANGE
SERUM UREA NITROGEN (mg/dL)
30
25
20
15
10
5
0
1987
(P < 0 020)
1988
(P < 0 026)


EAST RANGE
WEST RANGE
CREATININE (mg/dL)
4
3
2
1
0
1987
(P < 0 021)
1988
(P < 0 022)


EAST RANGE
WEST RANGE
U/C RATIO
25
20
15
10
5
0
1987
(P < 0 002)
1988
(P < 0 004)

CHAPTER II

EFFECTS OF MILITARY TRAINING ON MOVEMENTS AND MORTALITY OF WHITE-TAILED DEER IN SOUTHWESTERN OKLAHOMA

ABSTRACT: Radio-telemetry was used to monitor movements and mortality of white-tailed deer (Odocoileus virginianus) in response to intensive military training on West Range (18,000 ha) of Fort Sill Military Reservation, Oklahoma. Cause-specific mortality was determined for a total of 56 radio-collared deer, including adults (≥ 2.0 years old), yearlings (0.6-2.0 years old), and fawns (≤ 7 days old) from 1987 to 1989. Winter home ranges of deer were largely confined to a 14,411 ha artillery impact area centrally located on West Range. Movements of adult does from winter to summer home ranges outside the artillery impact area were associated with prepartum behavior. The mean annual mortality rate was 0.50 for adults and yearlings combined. Fifty-percent of all adult and yearling mortality was attributed to military training, 28% to hunting, 16% to collisions with automobiles, and 6% to unknown causes. The mean monthly mortality rate was 0.61 for neonatal fawns. Predation and malnutrition accounted for 75% and 25% of all fawn mortality, respectively. Serum concentrations of gamma globulin and alkaline phosphatase were higher in fawns that died compared to those that survived. All captured deer ≥ 2.6 years old, 82% 1.6 years old, 10% 0.6 years old, and all deer ≤ 7 days old were seropositive for bluetongue virus (BTV). BTV serotypes 10 (36% reactors), 11 (69%), 13 (91%), 17 (67%), and epizootic hemorrhagic disease virus (EHDV) serotypes 1 (87%) and 2 (89%) were detected using a virus-neutralization test.

Key words: white-tailed deer, Odocoileus virginianus, mortality, movements, military training, bluetongue virus, epizootic hemorrhagic disease virus, serology.

INTRODUCTION

In addition to estimates of legal harvest mortality, an understanding of other man-induced and natural mortality agents is a prerequisite to a successful white-tailed deer (Odocoileus virginianus) management program (Halls, 1984). Overexploitation of intensively hunted deer herds remains a possibility when adequate survival and cause-specific mortality information is unavailable (Nelson and Mech, 1986). Several investigations in Texas (Carroll and Brown, 1977; Cook et al., 1971) and Oklahoma (Bolte et al., 1970; Bartush, 1978; Garner et al., 1976; Logan, 1973) have demonstrated the importance of fawn mortality in determining recruitment into the huntable population. However, studies elucidating nonhunting causes of mortality among adult deer in the south-central United States are limited (Kie and White, 1985; DeYoung, 1989) and nonexistent for Oklahoma.

Previous research in Oklahoma has shown that coyote (Canis latrans) predation and parasites are major nonhunting causes of mortality in white-tailed deer. Fawn mortality in the Wichita Mountains, Oklahoma, has been reported as 87 to 89% with coyotes responsible for 88 to 96% of the mortality (Garner, 1976; Garner et al., 1976; Bartush, 1978). Predation has also been shown to be a significant cause of

adult deer mortality in Texas (DeYoung, 1989). Ectoparasite infestations are a leading cause of fawn mortality in high density deer herds in eastern Oklahoma (Bolte et al., 1970).

We used radio-telemetry to determine survival and cause-specific mortality rates for fawn, yearling, and adult white-tailed deer on Fort Sill Military Reservation (FSMR), Oklahoma. The FSMR deer herd has an eruptive history and fawn mortality from predation has been implicated as a major dampening agent to herd recruitment (Steele, 1969). Despite an intensive predator control program that was initiated in 1977, the deer herd on FSMR appeared to be maintained below carrying capacity of the habitat even though net fawn production as measured by fawn/doe counts increased by an estimated 154% (Stout, 1982). As a result, we suspected an undetermined mortality agent was limiting herd recovery. Because a sharp decline in the FSMR deer herd in 1979 was attributed to hemorrhagic disease (Pfister, 1985), we also report evaluations of sera obtained from captured deer for antibodies to several hemorrhagic disease serotypes.

MATERIALS AND METHODS

Study Area

Our study was conducted on FSMR, located in the Central Rolling Red Plains and Central Rolling Red Prairies Land Resource Areas (Gray and Galloway, 1969) of southwestern Oklahoma (Appendix A). The area includes diverse habitats, ranging from relatively flat prairie in the east to steep

granite hills in the west. Monthly mean temperatures range from 4.8 C in January to 28.7 C in August. Most rainfall occurs in the spring which accounts for 34% of total annual precipitation ($\bar{x}$ = 77 cm) (Appendix B). Livestock grazing has been excluded for 30 years on FSMR, and major vegetative disturbances are primarily annual prescribed burning in selected areas, wildfires resulting from military training exercises, and degradation due to artillery impact and vehicular traffic.

Vegetation on FSMR is dominated by tall-grasses such as big bluestem (Andropogon gerardi), little bluestem (Anropogon scoparius), sand bluestem (Andropogon hallii), switchgrass (Panicum virgatum), and Indiangrass (Sorghastrum nutans) on most prairie sites. Mid- and short-grasses such as blue grama (Bouteloua gracilis) and sideoats grama (Bouteloua curtipendula) occupy more droughty sites and slickspot soils (Comanche County SCS, 1970). Wooded areas are primarily restricted to riparian zones and isolated rocky upland sites and include American elm (Ulmus americana), pecan (Carya illinoensis), western hackberry (Celtis occidentalis), red oak (Quercus sp.), blackjack oak (Quercus marylandica), post oak (Quercus stellata), bur oak (Quercus macrocarpa), and chinquapin oak (Quercus muhlen bergii).

We used radio-telemetry to monitor mortality of white-tailed deer on West Range (18,000 ha), FSMR. West Range is used primarily for intensive field artillery training by the United States Army. The impact area (Appendix C) encompasses

14,411 ha of West Range and is subject to year-round munitions bombardment and small arms fire in conjunction with military training. Vegetation is maintained in early successional stages as a result of wildfires. Density of the deer population on West Range was estimated at 2.50/100 ha in both 1987 and 1988 (Stout, 1989). The deer herd consisted of 0.45 bucks and 0.64 fawns per doe in 1987, and 0.51 bucks and 0.61 fawns per doe in 1988. An intensive predator control program was initiated in February 1977 on West Range and a total of 51 coyotes were removed in 1987 and 1988.

Radio-telemetry

Twenty-two adult ($\geq$ 2.0 years old) and 21 yearling (0.6-2.0 years old) white-tailed deer were captured within the artillery impact area of West Range in December 1987 (n = 25) and 1988 (n = 18) using helicopters and a hand-held net-gun (Coda Enterprises, Inc., Mesa, Arizona 85203, USA). Age class of each animal was determined by tooth eruption and wear (Severinghaus, 1949). Each animal was equipped with a collar-mounted transmitter containing a mortality sensor (Advanced Telemetry Systems, Inc., Isanti, Minnesota 55040, USA), samples of blood and feces obtained for physiologic and dietary profiling (Dinkines, 1990), and released at the capture site.

Nine neonatal fawns of radio-collared does and 4 fawns of uncollared does were captured and fitted with radio transmitters during the summers of 1988 and 1989. Fawns ($\leq$ 7 days old) were captured using methods described by Bartush

and Lewis (1978), Garner et al. (1976), and White et al. (1972). Radio-collared does were relocated and observed daily during the fawning season in order to increase our chances of locating newborn fawns. Fawns observed nursing or following a doe were approached quickly to elicit the "drop" response (Nelson and Woolf, 1987). Age was determined by measuring new hoof growth (Haugen and Speake, 1958) and live weight measured to the nearest 0.5 kg using a hand-held spring-scale. The general condition of fawns was subjectively assessed based on behavior, presence or absence of inflammation of the umbilicus and anus, and level of ectoparasite infestation. Fawns were marked with numbered aluminum ear tags with 2.5 x 7.5 cm strips of saflag material (Safety Flag Company of America, Pawtuckett, Rhode Island 02860, USA) attached as described by Downing and McGinnes (1969). We used AVM SMI Solar-L-Modules (AVM Instrument Company, Dublin, California 94568, USA) affixed to white, expandable elastic collars to monitor movements and survival of individual fawns. Fawns were released at the exact location of capture to reduce the chances of abandonment by the doe.

Radio-telemetry equipment was used to relocate collared deer and to aid in determining their livelihood. An AVM model LA12 portable receiver (AVM Instrument Company, Dublin, California 94568, USA) and 3-element hand-held yagi antenna (AVM Instrument Company, Dublin, California 94568, USA) were used to monitor transmitter signals. Radio-collared

deer were monitored as often as possible throughout the year, sometimes daily, but no less than twice a month. Marked does were relocated daily during the fawning season (26 May to 12 June) and observed without disturbance. Fawn locations were triangulated twice daily to 15 August, or until they moved into the impact area and transmitter signals could not be detected. All locations of radio-collared deer were recorded by grid on standardized forms.

We determined approximate size of home ranges using the minimum-area method of Mohr (1947). Home ranges were delineated into summer and winter ranges, with the beginning of the winter season defined as when > 50% of the marked animals left their summer range and ceased transient movements to winter ranges (Tierson et al., 1985). When reporting sample sizes, radio-collared deer were counted as different "individuals" if they progressed from the yearling cohort to the adult cohort (DeYoung, 1989).

Mortality

When a mortality occurred, cause of death was determined by field necropsy and detailed inspection of the surrounding area for signs of the mortality agent. Adult and yearling carcasses were usually several days to several weeks old when found because of their location in the impact area which was inaccessible during daily military training exercises. Military training was considered to be the probable cause of death when fresh artillery craters surrounded the immediate vicinity of a carcass or its remains. We differentiated

between predator-killed carcasses and predator-scavenged carcasses using criteria of Garner (1976), White (1973), Smith (1945), and Cook et al. (1971). One intact and one partially consumed fawn carcass were stored on wet ice and shipped to Oklahoma State University College of Veterinary Medicine for necropsy.

Clinical evaluations

Serum samples were submitted to the Fort Sill Medical Diagnostic Laboratory for standard chemical profiling (Dinkines, 1990) on an Ektachem-700 Analyzer (Eastman Kodak Co., Rochester, New York 27705, USA) according to procedures specified by the manufacturer. The passive transfer of colostral immunoglobulins to fawns was indirectly assessed by measuring concentrations of serum proteins. Total serum protein, albumin, gamma globulin, alpha globulin, and beta globulin concentrations were determined using Helena zip-zone electrophoresis on Titan III cellulose acetate plates following procedures of manufacturer (Helena Laboratories, Beaumont, Texas 77704, USA).

Serum samples from captured deer were tested for antibodies to bluetongue virus (BTV) by personnel of the Department of Veterinary Clinical Pathology, Stillwater, Oklahoma, using an immunodiffusion test (IDT) for group-specific antibodies (Fulton et al., 1989). Sera positive by IDT were tested for serotype-specific antibodies against five BTV serotypes (2, 10, 11, 13, and 17) and two epizootic hemorrhagic disease virus (EHDV) serotypes (1 and 2) using a

microtitration virus-neutralization test (VNT) in 96-well cell cultures following modified procedures of Fulton et al. (1989) using Madin-Darby bovine kidney cells for cell cultures. An antibody titer $\geq$ 1:40 was considered to be a positive result.

Sterile cotton rectal swabs and fecal smears were obtained from each fawn at the time of capture for Salmonella bacteria culture. Fecal samples and swabs were directly inoculated in culture media and placed in enrichment broth for reinoculation following procedures of Carter (1986). All isolates were serotyped by the National Veterinary Service Laboratory, Ames, Iowa.

Data analysis

Differences in winter and summer home range sizes were tested for main factor effects of sex and age using two-way analysis of variance. Physiologic, dietary, and immunologic variables were tested for differences between deer that died and those that survived using one-way analysis of variance. Protected multiple comparisons (LSD) were used when significant differences ($P < 0.05$) were present. Pearson correlation coefficients were used to examine relationships between age and morphometrics of fawns and titers for BTV/EHDV serotypes between radio-collared does and their fawns. The Statistical Analysis System (SAS) was used for all data analyses (SAS Institute Inc., 1982).

Annual survival and cause-specific mortality rates were calculated using the microcomputer program MICROMORT (Heisey

and Fuller, 1985). Annual and seasonal (September-February, March-August) survival and mortality rates were determined for two adult age groups (0.6-2.0 and ≥ 2.0 years old); monthly survival and mortality rates were determined for newborn fawns (≤ 75 days old; all ages and sexes combined). Mortality attributed to military training included artillery ordinance and machine gun fire; hunting mortality included legal hunting, poaching, and wounding loss. Program MICROMORT was used to compute confidence intervals and variances.

Selected survival and mortality rates were tested for differences by Z-tests (Heisey, 1985). For annual rates, we compared survival and mortality rates between 0.6-2.0 and ≥ 2.0 year age groups. Seasonal (September-February vs. March-August) differences in survival and mortality rates were tested with both age groups combined (yearlings and adults). Summer survival and cause-specific mortality rates for fawns were also compared for differences. Differences in mortality and survival rates were considered significant at $P \leq 0.10$; all other comparisons were considered significant at $P \leq 0.05$ level.

RESULTS

Seasonal home ranges

A total of 39 female and 4 male white-tailed deer were radio-collared and collectively relocated on 3,540 occasions from December 1987 to December 1989. There were no

differences ($P > 0.05$) in sizes of winter home ranges due to sex for yearlings or adults (Table 1). Winter home ranges of adult females were larger ($P < 0.01$) than those in summer. Sizes of summer home ranges were larger ($P < 0.03$) for adult males than adult females. Distance between summer and winter home ranges averaged 2.93 $\pm$ 1.01 for adult males and 2.31 $\pm$ 0.28 km for adult females. Adult does maintained summer home ranges outside the artillery impact area, whereas winter home ranges generally were located within this area.

Mortality rates

Twenty-two of 56 radio-collared deer died from various causes during the two-year study (Table 2). Thirty-nine percent of all adult and yearling mortalities were attributed to artillery ordinance, 16% to collisions with automobiles, 11% to machine gun fire, 11% to legal hunting, 11% to wounding loss, 6% to poachers, and 6% to unknown causes. The annual mortality rate for deer pooled across ages (adults and yearlings), sexes, and causes was 0.50 (95% CI = 0.31-0.63). Survival rates did not differ ($P > 0.69$) between adult and yearling age classes (Table 3) and mortality rates of both age classes were similar for military training ($P > 0.85$), hunting ($P > 0.65$), and collisions with automobiles ($P > 0.92$).

Eight male and 5 female neonatal fawns ranging from 3 to 5 days old ($\bar{x} = 4.30 \pm 0.23$ SE) were captured and radio-collared. Fawn weights were positively correlated ($P < 0.01$, $r = 0.74$) with estimated age at capture. Physical

condition of all captured fawns was judged to be excellent. Fawns were relocated on 1,560 occasions during the summers of 1988 and 1989, yielding 674 deer days of observation and 4 mortalities (Table 3). Monthly mortality rate of fawns pooled across ages, sexes, and causes was 0.61 (95% CI = 0.02-0.85). Mortality rate attributed to predation by coyotes (Canis latrans) and bobcats (Lynx rufus) was significantly higher ($P < 0.09$) than nonpredator causes of mortality.

The seasonal survival rate of adults and yearlings combined was significantly higher ($P < 0.01$) during March-August than September-February (Table 4). Mortality rates due to military training and collisions with automobiles were similar ($P > 0.36$) for both seasons, whereas hunting mortality rates were significantly lower ($P < 0.05$) than all nonhunting mortality rates combined. Seasonal survival rate of adults from September-February (0.58) was significantly lower ($P < 0.01$) than from March-August (0.90). Seasonal survival rates of yearlings for September-February (0.57) and March-August (0.78) were similar ($P > 0.32$). There were no differences in mortality rates between adult and yearling deer within a season due to military training ($P > 0.24$), hunting ($P > 0.57$), or collisions with automobiles ($P > 0.31$).

Physiological and dietary indices

Physiologic and nutritional profiles of radio-collared deer were evaluated to determine their condition at time of

capture. All deer grossly appeared to be in good to excellent condition at the time of capture. No differences ($P > 0.05$) were detected in any blood or fecal condition index of adult and yearling deer that died compared to those that survived during our study (Dinkines, 1990).

Results of electrophoresis provided information on physiologic status of newborn fawns captured from radio-collared does (Table 5). Fawns that died during our study had significantly higher ($P < 0.01$) concentrations at time of capture for serum total protein and gamma globulins (6.40 $\pm$ 0.35, 2.78 $\pm$ 0.49 mg/ml) than those that survived (5.44 $\pm$ 0.11, 1.46 $\pm$ 0.15 mg/ml, respectively). Serum concentrations of alkaline phosphatase also were higher ($P < 0.05$) in fawns that died (1545 $\pm$ 38.20 U/L) compared to those that survived (1071 $\pm$ 108.14 U/L). We observed no differences ($P > 0.05$) in serum concentrations of albumin, alpha globulin, and beta globulin between fawns that survived and those that died.

Of 49 deer evaluated by the IDT, 100% ($n = 7$) of deer $\leq$ 7 days old, 10% ($n = 1$) of deer 0.6 years old, 82% ($n = 9$) of deer 1.6 years old, and 100% ($n = 21$) of deer $\geq$ 2.6 years old had antibodies to BTV. Results of the VNT (Table 6) for deer 0.6 years old revealed 100% reactors to BTV serotype 10 ($n = 1$), 100% to serotype 11 ($n = 1$), 100% to serotype 13 ($n = 1$), 100% to serotype 17 ($n = 1$), 100% to EHDV serotype 1 ($n = 1$), and 0% to serotype 2 ($n = 1$); for deer 1.6 years old 11% reactors to BTV serotype 10 ($n = 11$), 100% to serotype 11 ($n = 9$), 100% to serotype 13 ($n = 9$), 44% to serotype 17 ($n =$

4), 100% to EHDV serotype 1 (n = 9), and 89% to serotype 2 (n = 8); for deer ≥ 2.6 years old 33% reactors to BTV serotype 10 (n = 7), 76% to serotype 11 (n = 16), 90% to serotype 13 (n = 19), 71% to serotype 17 (n = 15), 81% to EHDV serotype 1 (n = 17), and 95% to serotype 2 (n = 20). None of the radio-collared deer were seropositive for BTV-2 on the basis of VNT. Results of the VNT also revealed that neonatal fawns (≤ 7 days old) captured from seropositive does were subsequently seropositive for similar BTV and EHDV serotypes (Table 6). Rectal swabs obtained from 13 newborn fawns yielded one positive culture for Salmonella arizona representing an incidence of 8%. However, no mortalities or clinical signs due to salmonellosis were detected in any of the radio-collared fawns.

DISCUSSION

Individual radio-collared deer showed consistency in terms of occupying seasonal home ranges with similar locations, sizes, and configurations from year to year. This is in agreement with Tierson et al. (1985) who reported similar behaviors for white-tailed deer in the Adirondacks. Radio-collared deer on West Range of FSMR maintained winter home ranges within the artillery impact area, but most adult does shifted their home ranges to areas outside of the impact area in spring. We believe this is a prepartum response of does actively seeking isolated grassy-dominated prairies away from conspecifics and disturbances from military training

(Bartush and Lewis, 1978; Townsend and Bailey, 1975).

We observed 75% of all adult and yearling mortality occurred between September and February. Lower September-February survival rates were partially attributed to hunting-related mortality which occurred from October-December. However, the lower September-February survival rate was also indicative of the deer shifting their summer home ranges to winter home ranges inside the impact area, which predisposes them to mortality factors related to military training. DeYoung (1989) reported that natural mortality of white-tailed deer in south Texas from December-March was twice the April-November rate. Similarly, Gavin et al. (1984) found most natural mortality occurred during November-January in white-tailed deer, possibly a result of rutting stress.

Annual and seasonal survival rates of deer on FSMR generally were similar between yearlings and adults. This is in agreement with Nelson and Mech (1986) who reported little difference in mortality rates between yearling and older age classes of deer in Minnesota. In contrast to McCullough (1979) who noted that hunting accounted for all mortality of adult males on Michigan's George Reserve, mortality attributed to natural causes exceeded hunting losses in south Texas (DeYoung, 1989). Reported annual mortality rates for adult males in southwestern Washington (0.40; Gavin et al., 1984) and northeastern Minnesota (0.53; Nelson and Mech, 1986) were similar to our estimate for adults on FSMR.

We observed that all mortality of radio-collared newborn

fawns on FSMR occurred during the first 17 days of age. Similar observations have been previously reported in Oklahoma (Bartush and Lewis, 1978) and Texas (Cook et al., 1971). The predator-involved mortality rate of fawns in our study (75%) was lower than that reported by Garner et al. (1976) for southwestern Oklahoma. This disparity is thought to be the result of the annual coyote control program on FSMR that was initiated after 1976 (Stout, 1982).

It has been suggested that fawns may be predisposed to predators due to malnutrition, infections, or other diseases (Cook et al., 1971; Logan, 1973). Logan (1973) suggested that nutritional stress can decrease fawn vigor and increase their susceptibility to secondary infection from tick infestations in eastern Oklahoma. Failure to obtain sufficient colostral immunoglobulins has been shown to be a major mechanism of lamb (McGuire et al., 1983) and calf (McGuire et al., 1976) mortality. Placentation in deer dictates that the principal avenue of absorption of immunoglobulins is via colostrum (Harrison and Hamilton, 1952) and fawns failing to nurse have no detectable gamma globulin concentrations (Hartsook et al., 1975). Since gamma globulins and antibodies to specific BTV/EHDV serotypes were present in serum of fawns that both survived and died during our study, we concluded that the initial passive transfer of immunity from does to fawns was not a predisposing factor to mortality.

For reasons that are not entirely clear, differences in

concentrations of gamma globulin and alkaline phosphatase at time of capture were observed for fawns that died and survived. Admittedly, our sample sizes were unavoidably low. However, elevated concentrations of these constituents in fawns that subsequently died suggests that an infectious agent was a contributing factor. Microbial infections of the mammary gland probably occur in all species of mammals, although little is known about their etiology in wild animals (Newbould, 1984). Mastitic milk can contain as much as 10-fold increases in immunoglobulins and 5-fold increases in alkaline phosphatase concentrations (Walstra and Jennes, 1984; MacKenzie and Lascelles, 1968). Similarly, elevated immunoglobulin levels in newborns due to intrauterine antigenic stimulation has been reported (Fahey and Morris, 1978; Tizard, 1977).

Stout (1982) speculated that apparent benefits of increased neonatal survival through predator control on FSMR may be offset by significant mortality of younger does due to density independent diseases. Serologic surveys have become a standard method of establishing baseline data on the possible prevalence of various infectious diseases in wild animal populations (Kocan, 1983). The 5 serotypes of BTV (2, 10, 11, 13, and 17) and 2 serotypes of EHDV (1 and 2) are recognized in the United States (Barber and Jochim, 1975; Gibbs et al., 1983). To our knowledge, only BTV serotypes 11, 13, and 17 (Kocan et al., 1987) and no EHDV serotypes have been reported for white-tailed deer in Oklahoma. Our

survey of deer on FSMR revealed a higher percentage (94%) of seropositive deer (≥ 1.6 years old) than a previously reported survey (57%; by Kocan et al., 1987) of white-tailed deer in southwestern Oklahoma. The high percentage (100%) of seropositive neonatal fawns (≤ 7 days old) also suggests that newborns are receiving early protection against various BTV serotypes via colostrum. However, the subsequently low incidence of seropositive (10%) deer 0.6 years old and high incidence of seropositive (94%) deer ≥ 1.6 years old suggests a loss of maternal antibody and evidence of subsequent natural infection. Incidence of antibodies to BTV and EHDV in some deer populations can approach 100% with no observable clinical disease (Trainer and Jochim, 1969; Kocan et al., 1987). Mortality due to a specific etiologic agent in free-ranging deer on FSMR has never been documented and was not a contributing factor in this study. The high incidence of BTV/EHDV seropositive deer suggests that deer on FSMR may be approaching endemic stability.

CONCLUSIONS

The lack of evidence supporting mortality in deer on FSMR due to disease agents suggests that other factors are affecting the population density. Data collected from our radio-telemetry suggests that military training is the unaccountable mechanism directly (observed mortality) and indirectly (habitat loss) responsible for maintaining the deer density on West Range of FSMR below their potential. This

high of "natural" mortality coupled with hunter-induced mortality and losses in the annual fawn crop due to predation could lead to a severe unbalance in population dynamics if hunters are allowed to remove too many animals. Our study provides evidence that nonhunting causes of mortality are significant and should become an integral part of deer population management on military installations. Further research is needed to determine if discrepancies in physiologic profiles between fawns that died and those that survived is an artifact of our low sample size. Research should also clarify the cause of these differences and determine if they are linked to factors predisposing neonatal fawns to mortality.

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Table 1. Seasonal changes in home-range size (ha) of radio-collared white-tailed deer on Fort Sill Military Reservation, Oklahoma, from 1987 to 1989.

Age and sex at capture	Winter			Summer		
	n	$\bar{x}$	SE	n	$\bar{x}$	SE
Yearlings						
Males	4	54.30	19.41			
Females	6	66.31	14.99			
Adults						
Males	1	59.98		4	45.96	30.59
Females	47	52.11	5.58	48	16.98	2.99

Table 2. Causes of death among radio-collared white-tailed deer on West Range of Fort Sill Military Reservation, Oklahoma, from 1987 to 1989.

Deer number	Sex	Age at capture (mos.)	Age at death (mos.)	Cause of death
2	F	6	30	Artillery ordinance
4	F	18	36	Artillery ordinance
5	F	30	40	Wounding loss
6	M	6	17	Poacher
8	M	6	13	Machine gun fire
13	M	6	17	Hunter
14	M	6	30	Artillery ordinance
17	F	6	15	Deer-vehicle collision
19	F	18	20	Artillery ordinance
20	F	30	40	Deer-vehicle collision
21	F	30	41	Wounding loss
22	F	30	40	Hunter
23	F	18	29	Unknown
24	F	18	39	Machine gun fire
32	F	54	60	Deer-vehicle collision
36	F	18	20	Artillery ordinance
38	F	42	43	Artillery ordinance
40	F	42	53	Artillery ordinance

Table 2. Continued.

Deer number	Sex	Age at capture (mos.)	Age at death (mos.)	Cause of death
88[a]	M	5	17	Bobcat predation
93[a]	F	4	6	Starvation
97[a]	M	5	16	Probable coyote predation
99[a]	F	4	17	Coyote predation

[a]Age at capture and death in days.

Table 3. Annual (yearlings and adults) and monthly (fawns) survival and cause-specific mortality rates for radio-collared white-tailed deer on West Range, Fort Sill Military Reservation, Oklahoma, from 1987 to 1989.

	Age class		
Variable	Fawns (≤ 75 days)	Yearlings (0.6-2.0 yrs)	Adults (≥ 2.0 yrs)
No. deer	13	21	22
Deer days	674	5628	137815
No. deaths	4	6	12
Annual survival[a]	0.39	0.45	0.52
95% CI	0.15-0.98	0.24-0.85	0.36-0.76
Mortality rates			
Military training[b]	0.00	0.27	0.24
Hunting[c]	0.00	0.18	0.12
Automobile collision	0.00	0.09	0.08
Predation	0.46	0.00	0.00
Malnutrition	0.15	0.00	0.00
Unknown	0.00	0.00	0.04

[a]Rates and confidence intervals according to Heisey and Fuller (1985).

[b]Artillery ordinance and machine gun fire

[c]Legal harvest, wounding loss, and poaching.

Table 4. Seasonal survival and cause-specific mortality rates of radio-collared white-tailed deer (yearlings and adults combined) on West Range of Fort Sill Military Reservation, Oklahoma, from 1987 to 1989.

Variable	September-February	March-August
No. deer	25	27
Deer days	9508	9901
No. deaths	14	4
Seasonal survival[a]	0.58	0.86
95% CI	0.43-0.77	0.74-0.99
Mortality rates		
Military training[b]	0.18	0.10
Hunting[c]	0.15	0.00
Collisions with automobiles	0.06	0.04
Unknown	0.03	0.00

[a]Rates and confidence intervals according to Heisey and Fuller (1985).

[b]Artillery ordinance and machine gun fire.

[c]Legal harvest, wounding loss, and poaching.

Table 5. Serum protein concentrations of doe (n = 8) and fawn (n = 12) white-tailed deer captured on West Range of Fort Sill Military Reservation, Oklahoma, from 1987 to 1989.

	Doe	Fawn
Serum Constituent	$\bar{x} \pm$ SE	$\bar{x} \pm$ SE
Total protein (gm/dL)	7.45 ± 0.27	5.76 ± 0.19
Albumin (gm/dL)	4.34 ± 0.09	2.71 ± 0.09
Alpha 1-globulin (gm/dL)	0.53 ± 0.09	0.13 ± 0.03
Alpha 2-globulin (gm/dL)	0.36 ± 0.06	0.23 ± 0.01
Beta-globulin (gm/dL)	0.94 ± 0.03	0.86 ± 0.05
Gamma-globulin (gm/dL)	1.30 ± 0.05	1.86 ± 0.27
Alkaline phosphatase (U/L)	85.00 ± 8.56	1229.50 ± 98.27

Table 6. Virus-neutralizing antibody titers of white-tailed deer to bluetongue virus (BTV) and epizootic hemorrhagic disease virus (EHDV) serotypes from West Range of Fort Sill Military Reservation, Oklahoma, in December 1987 and 1988. Age of deer (yrs) when tested is in parentheses.

Deer	Antibody titer to BTV and EHDV serotypes						
No.[a]	BTV-2	BTV-10	BTV-11	BTV-13	BTV-17	EHDV-1	EHDV-2
42 (0.6)	0	80	80	80	80	40	0
16 (1.6)	0	0	40	320	40	321	≥ 1280
19 (1.6)	0	0	80	320	40	80	80
24 (1.6)	0	0	0	80	0	160	0
27 (1.6)	0	0	0	≥ 1280	40	40	640
30 (1.6)	0	0	40	≥ 1280	0	40	160
33 (1.6)	0	0	0	≥ 1280	0	80	160
35 (1.6)	0	40	80	80	640	160	≥ 1280
36 (1.6)	0	0	0	≥ 1280	0	40	≥ 1280
43 (1.6)	0	0	≥ 1280	320	80	40	320
1 (2.6)	0	0	0	320	0	≥ 1280	320
5 (2.6)	0	0	≥ 1280	40	160	0	320
9 (2.6)	0	0	40	≥ 1280	40	40	640
15 (2.6)	0	0	0	0	0	640	160
18 (2.6)	0	0	≥ 1280	≥ 1280	80	40	320
22 (2.6)	0	0	≥ 1280	0	40	160	320
26 (2.6)	0	0	80	≥ 1280	320	80	160
29 (2.6)	0	0	0	≥ 1280	0	80	80
31 (2.6)	0	0	0	320	0	0	320
11 (3.6)	0	40	≥ 1280	320	40	160	640
25 (3.6)	0	40	≥ 1280	≥ 1280	80	160	640
28 (3.6)	0	40	320	320	80	160	640
37 (3.6)	0	160	≥ 1280	160	160	160	640
38 (3.6)	0	40	320	80	320	80	320
40 (3.6)	0	40	≥ 1280	640	40	80	160
41 (3.6)	0	0	≥ 1280	160	160	40	0
3 (4.6)	0	0	≥ 1280	320	40	≥ 1280	320
7 (4.6)	0	40	≥ 1280	≥ 1280	640	160	80
32 (4.6)	0	0	40	≥ 1280	0	0	160
34 (4.6)	0	0	0	≥ 1280	0	0	640
44 (4.6)	0	0	40	640	80	80	320

[a]Deer positive to BTV antibodies by immunodiffusion test

Table 7. Corresponding virus-neutralizing antibody titers in does (≥ 1.6 yrs old when sampled) and their neonatal fawns (≤ 7 days old when sampled) to bluetongue virus (BTV) and epizootic hemorrhagic disease virus (EHDV) serotypes from West Range of Fort Sill Military Reservation, Oklahoma, 1987 to 1989.

Doe[a] and fawn	Antibody titer to BTV and EHDV serotypes						
	BTV-2	BTV-10	BTV-11	BTV-13	BTV-17	EHDV-1	EHDV-2
7	0	40	≥ 1280	≥ 1280	640	160	80
97	0	40	640	160	160	160	160
5	0	0	≥ 1280	40	160	0	320
96	0	0	≥ 1280	0	40	0	640
25	0	40	≥ 1280	≥ 1280	80	160	640
95	0	40	640	160	160	160	640
37	0	160	≥ 1280	160	160	160	640
94	0	40	160	320	80	160	640
25	0	40	≥ 1280	≥ 1280	80	160	640
93	0	40	640	≥ 1280	80	80	640
43	0	0	≥ 1280	320	80	40	320
92	0	40	≥ 1280	≥ 1280	160	40	320
44	0	0	40	640	80	80	320
88	0	0	40	≥ 1280	160	80	320

[a]Each pair is a doe followed by her fawn.

CHAPTER III

MEASUREMENT OF ANTIBODY TO CLOSTRIDIUM SORDELLI IN WHITE-TAILED DEER BY ENZYME-LINKED IMMUNOSORBENT AND PASSIVE HEMAGGLUTINATION ASSAYS

Abstract: An indirect enzyme-linked immunosorbent assay (ELISA) and passive hemagglutination assay (PHA) were developed for quantitating antibodies to Clostridium sordelli in sera obtained from free-ranging and captive white-tailed deer (Odocoileus virginianus). Even though both techniques detected antibody to C. sordelli in preimmunization sera, the ELISA was more sensitive and detected a much higher titer than the PHA. Titers detected by ELISA ranged from 400 to $\geq$ 25600 compared to 8 and 128 for the PHA. Slightly elevated titers were detected by ELISA in sera obtained 6 weeks postimmunization from captive adult does. Titers measured by PHA failed to reflect any differences. Sera obtained from newborn fawns of immunized free-ranging and captive does had titers to C. sordelli based on results of ELISA and PHA. In view of this, it appears that white-tailed deer are naturally exposed to and produce antibodies for C. sordelli, which are capable of being passed via colostrum to their offspring. Owing to its specificity, sensitivity, and reproducibility, the ELISA is a favorable alternative to other conventional assays for detecting low antibody titers.

Key words: Clostridium sordelli, titer, indirect enzyme-linked immunosorbent assay, ELISA, passive hemagglutination assay, PHA, antibody, white-tailed deer, Odocoileus virginianus.

INTRODUCTION

Immediate immunologic protection in the neonatal ruminant is provided through the passive transfer of maternal colostral immunoglobulins (Osburn et al., 1974; Redman, 1979; Tizard, 1977). Failure to obtain sufficient colostral immunoglobulins has been shown to be a major mechanism of lamb (McGuire et al., 1983) and calf (McGuire et al., 1976) mortality. Parkinson et al. (1982) reported similar findings for captive mule deer fawns (Odocoileus hemionus), but no studies to date have been attempted with free-ranging white-tailed deer (Odocoileus virginianus).

The adequacy of passive immunoglobulin transfer is routinely assessed by measuring serum immunoglobulin levels in the newborn animal after it has nursed. A variety of procedures have been developed to quantitate total immunoglobulins (Pfeiffer et al., 1977), individual immunoglobulin isotypes (Logan and Gibson, 1975), or the transfer of antigen-specific antibodies (VanZaane et al., 1986).

The various procedures for identifying and quantitating antibodies vary in their sensitivity. Trindle et al. (1979) used an immunodiffusion test to detect antibodies to a 7-way Clostridium toxoid vaccine in experimentally vaccinated mule deer fawns. However, they reported that the immunodiffusion test could only detect antibodies in sera at relatively high titers. It has been shown that the enzyme-linked immunosorbent assay (ELISA) and passive-hemagglutination

assay (PHA) are more sensitive than immunodiffusion tests for detecting low titers to clostridial enterotoxins (Nilo and Cho, 1984). The ELISA is becoming widely used for determining antibody titers to a variety of pathogens (Barton and Campbell, 1988). Advantages of the ELISA include speed, sensitivity, specificity, safety, potential for automation, broad applicability, and potential for field use (Charan and Gautam, 1984). Elimination of operator subjectivity in evaluating results and adaptability of the assay design are obvious advantages which favor the ELISA over conventional immunological tests implying all-or-none responses (Malvano et al., 1982).

In the present paper, we describe the development of an indirect enzyme-linked immmunosorbent assay and a passive hemagglutination assay for quantitating antibodies to Clostridium sordelli in white-tailed deer. We also report our incidental findings of natural titers to C. sordelli in free-ranging and captive adult does and their fawns.

MATERIALS AND METHODS

Serum samples

Sera from 7 free-ranging adult females were obtained from blood samples taken as part of a radio-telemetry study (Dinkines, 1990). Adult does were vaccinated intramuscularly at time of capture (December) with 3 cc of a Clostridium chauvoei, C. septicum, C. novyi, C. sordelli bacterin-toxoid (Convac-CSNS, Affiliated Laboratories, Bristol, Tennessee

37620, USA). Blood samples were collected within 1 week postpartum (June) from 8 fawns of these does and 4 fawns captured from unvaccinated does.

Blood was also collected for serum from 6 captive adult does maintained at the Camp Redland Deer Research Facility near Stillwater, Oklahoma. Four of the animals received a single, 3 cc intramuscular injection of the Clostridium bacterin-toxoid while the fifth doe received a second injection five weeks later. The sixth doe, which was not vaccinated, died early in the experiment due to an injury. Each animal was bled prior to immunization (January), six weeks later (February), and one week postpartum (June). A total of 5 fawns from these does were bled within a week postpartum (June).

Passive hemagglutination test

The PHA test was a modification of the procedure of Johnson et al. (1968). Thirty ml of blood were collected from an anesthetized New Zealand White rabbit via heart puncture into a syringe containing an equal volume of Alsever's solution. Rabbit red blood cells (RRBC) were packed by centrifugation for 10 min at 1000 xg. The supernatant was removed by aspiration and the cells were washed by resuspending them in 30 ml of Alsever's solution followed by centrifugation for 10 min at 1000 xg. The pellet was resuspended and the wash procedure repeated twice with phosphate buffered saline (PBS) pH 7.2. After the final wash, sufficient PBS was added to the packed cells to give a

50% (V/V) suspension. The RRBC were sensitized by the addition of 1.0 ml of a 50% cell suspension to a solution of 20 ml of PBS containing 1 mg/ml of C. sordelli bacterin-toxoid protein (graciously provided by Dr. Dale Bort, Beecham Laboratories, Whitehall, Illinois 62092, USA) and 5 ml of 1-ethyl-3-(3-dimethylamino-propyl) carbodiimide. The mixture was incubated 1 hr at room temperature while periodically resuspending the cells by gentle mixing. The cells were then washed twice in PBS, once in Alsever's solution and stored as a 0.5% suspension in Alsever's at 4 C. Prior to use, RRBC were resuspended and the desired volume of a 0.5% suspension removed and packed for 5 min at 1000 xg. The cells were washed once in PBS and the resulting pellet resuspended to make a 0.5% suspension in PBS containing 1% chicken egg albumin (CEA).

Ten percent normal (nonsensitized) RRBC in PBS were centrifuged for 10 min at 1000 xg. The supernatant was removed and a volume of heated (30 min at 56 C) deer serum equal to the packed RRBC volume was added. The RRBC were suspended in heated serum by lightly vortexing and incubated for 1 hr at room temperature. The cells were centrifuged for 10 min at 1000 xg and the serum was removed for testing. We verified that all natural RRBC agglutinins had been removed from the heat-inactivated sera using direct hemagglutination at a 1:2 dilution of the absorbed sera (Johnson et al., 1968).

Serial two-fold dilutions of serum were made in duplicate in PBS-1% CEA in 96-well v-bottom microtiter plates

(Linbro Scientific Company, Hamden, Conneticut 06517, USA). An equal volume of sensitized RRBC in PBS-1% CEA was added to each well. Each plate contained positive and negative controls (known positive serum control or PBS-CEA) substituted for the test serum. The plates were covered and incubated 24 hr at room temperature. The titer was recorded as the reciprocal of the greatest serum dilution in which the RRBC did not form a compact button on the bottom of the well.

Enzyme-linked immunosorbent assay

The technique used was a modification of the method described by Nilo and Cho (1984). Optimal dilutions of antigen and enzyme labelled antibody were determined by checkerboard titrations (Voller et al., 1979). Polystyrene 96-well flat bottom microtiter plates (A/S Nunc, Roskilde, Denmark 4000, USA) were coated overnight at 4 C with 200 ul/well of a solution of 10 ug/ml C. sordelli bacterin-toxoid protein in 0.5 M carbonate buffer, pH 9.6. Plates were washed once with phosphate buffered saline pH 7.2 containing 0.1% Tween 20 (PBS-T). Excess binding sites were blocked by the addition of 200 ul/well of PBS containing 1% normal rabbit serum (NRS) for 1 hr at 37 C. Serial two-fold dilutions of sera were made in PBS-T containing 0.1% NRS. The blocking solution was decanted and 50 ul aliquots of the serum dilutions were added to the appropriate wells. Positive and negative controls (known positive serum or PBS-T only) were included. The plates were covered, incubated 1 hr at room temperature on a rocker platform (Bellco

Biotechnology, Vineland, New Jersey 08360, USA), decanted, and washed 5 times with PBS-T using a Nunc-Immuno Wash 12 (A/S Nunc, Roskilde, Denmark 4000, USA). Plates were decanted and 50 ul of a 1:400 dilution of peroxidase labelled rabbit anti-deer IgG (Kirkegaarde and Perry Laboratories, Gaithersburg, Maryland 20879, USA) in PBS-T containing 0.1% NRS was added to each well. The plates were incubated at room temperature for 30 min on the rocker platform, washed 5 times with PBS-T (as previously described) and 100 ul of freshly prepared substrate (25 ml .15 M citrate buffer, pH 5.0, 10 mg O-phenylenediamine, 5 ul 30% hydrogen peroxide) was added. The plates were covered and allowed to stand for 30 min at room temperature in the dark. The absorbance of the enzyme reaction product was measured at 490 nm on a microplate reader (Vmax Kinetic Microplate Reader, Molecular Devices Corporation, Palo Alto, California 94304, USA), with the readings corrected internally for background caused by nonspecific binding. Mean absorbance readings were calculated from duplicates and were used to estimate titers. Reactions with an absorbance greater than 0.52 (mean optical density value of the negative control plus 2 standard deviation units) were considered positive.

Data analysis

Pearson correlation coefficients were used to examine relationships among doe and fawn titers and results obtained from ELISA and PHA. The Statistical Analysis System (SAS) was used for data analyses (SAS Institute Inc., 1982).

RESULTS AND DISCUSSION

The ELISA methodology we developed was approximately 3 to 11 fold more sensitive than the PHA for detecting C. sordelli antibody. This is similar to the results of Nilo and Cho (1984) who reported ELISA results 4 to 12 fold more sensitive than PHA for C. perfringens enterotoxin antibody. Based on results of the ELISA and PHA test, all does and fawns (100%) tested were seropositive for C. sordelli antibody. Titers detected by ELISA ranged from a minimum of 400 to $\geq$ 25600 compared to 8 and 128 for PHA. C. sordelli antibodies were detected in sera from captive does taken before immunization, 6 wks postimmunization, after parturition, and in their newborn fawns (Table 1). Results ot the ELISA showed sera collected from captive does 6 wks postimmunization had slightly higher titers than preimmunization sera. Conversely, no differences were detected by the PHA. By week 13, these elevated titers had dropped to their initial preimmunization levels based on results of the ELISA. Preimmunization sera obtained from free-ranging does also had detectable levels of antibody to C. sordelli (Table 2). Fawns captured from free-ranging and captive does had similar but slightly elevated antibody titers at time of capture compared to those found in their does. Similarly, both assays detected titers in the 4 fawns captured from unimmunized free-ranging does. Results of the PHA showed a significant correlation between titers in free-ranging does and their fawns ($P < 0.05$, $r = 0.66$) and

captive does and their fawns ($P < 0.05$, $r = 0.71$). This suggests that maternally acquired antibodies to C. sordelli are readily passed via colostrum to newborn fawns (Grimstad et al., 1987; Parkinson et al., 1982). However, no correlations were observed between doe and fawn titers detected by ELISA. As well, no correlation between titers from the ELISA compared to PHA were found. The sensitivity of the ELISA coupled with serum samples containing low antibody titers may have caused the descrepancies (Barton and Campbell, 1988).

Based on the results obtained, the ELISA method appears to be superior to the more conventional PHA test for measuring low concentrations of C. sordelli antibody. The serious limitation of the ELISA is that it requires a meticulous standardization for each component of the system (Charan and Gautam, 1984). The optimal timings for each step and the other requirements such as reagent concentrations, incubation temperature, and pH have to be ascertained precisely to obtain consistent and reproducible results. Nonetheless, the data presented in this study demonstrate that the ELISA test results in a rapid, highly sensitive, reproducible assay suitable for use in serological studies of wildlife sera.

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Table 1. Comparison of antibody titers to *Clostridium sordelli* detected by ELISA and PHA in captive white-tailed deer from Camp Redland Deer Research Facility, Stillwater, Oklahoma. Titers are expressed as the reciprocal of dilution.

Doe and fawn no.[a]	ELISA titer			PHA titer		
	1 Jan.	15 Feb.	1 June	1 Jan.	15 Feb.	1 June
201	1600	3200	1600	64	64	128
1201			12800			256
202	800	6400	1600	32	64	64
1203			6400			128
1005	6400	6400	3200	128	128	128
1204			3200			128
817	1600	3200	3200	64	32	32
1206			6400			64
205	800	3200	1600	32	32	32
1208			1600			64

[a]Each pair is a doe followed by her fawn.

Table 2. Comparison of antibody titers to Clostridium sordelli as detected by ELISA and PHA in free-ranging white-tailed deer from West Range, Fort Sill Military Reservation, Oklahoma, 1987 to 1990. Titers are expressed as reciprocal of dilution.

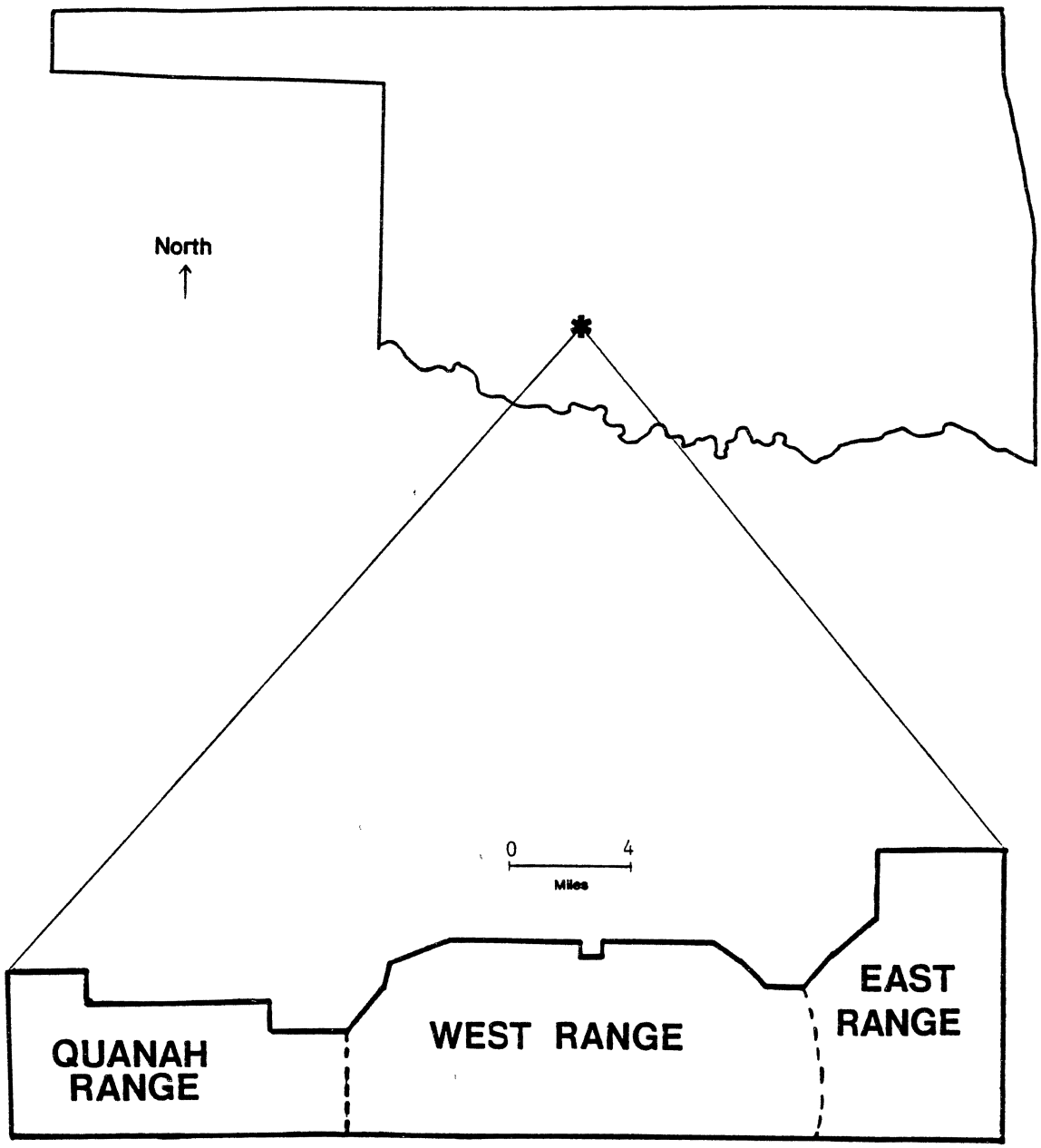
Doe and fawn no.[a]	ELISA		PHA	
	20 Dec.	1 June	20 Dec.	1 June
20	800		8	
98		6400		16
21	800		16	
99		1600		64
25	800		32	
95		400		32
93		1600		64
05	1600		32	
96		6400		32
37	≥25600		32	
94		1600		32
43	400		128	
92		1600		128
44	1600		64	
88		400		64
91[b]		800		8
89[b]		1600		128
87[b]		1600		32
86[b]		3200		64

[a]Each pair is a doe followed by her fawn(s).
[b]Fawns from unvaccinated does.

APPENDIXES

APPENDIX A

LOCATION OF EAST (12000 ha) AND WEST (18000 ha) RANGE STUDY AREAS ON FORT SILL MILITARY RESERVATION, COMANCHE COUNTY, OKLAHOMA.



FORT SILL MILITARY RESERVATION

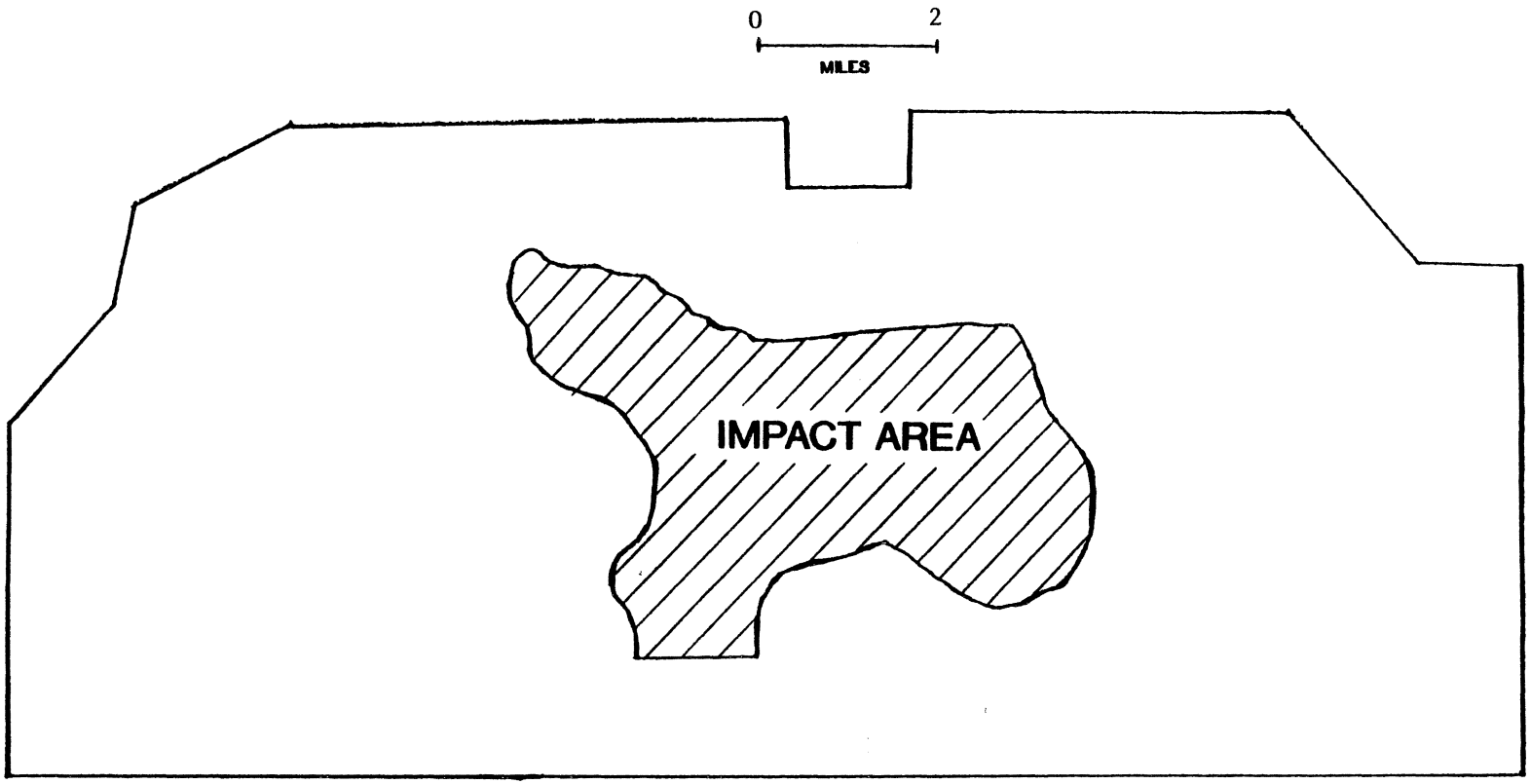
APPENDIX B

AVERAGE MONTHLY RAINFALL ON FORT SILL MILITARY RESERVATION, OKLAHOMA, 1985 to 1989.

	1985		1986		1987		1988		1989	
	cm	Running Total	cm	Running Total	cm	Running Total	cm	Running Total	cm	Running Total
Jan	2.26	2.26	0	0	4.88	4.88	3.99	3.99	3.61	3.61
Feb	8.86	11.13	3.10	3.10	10.36	15.24	0.04	4.03	6.45	10.06
Mar	17.42	28.55	3.30	6.40	3.48	18.72	9.45	13.48	5.56	15.62
April	10.03	38.58	8.97	15.37	0.20	18.92	5.64	19.12	0.15	15.77
May	8.48	47.07	12.88	28.24	27.81	46.73	0.09	19.21	17.80	78.57
June	19.18	66.24	10.49	39.74	13.41	60.14	2.92	22.13	20.98	49.55
July	3.71	69.95	0.86	39.60	3.58	63.72	3.35	25.48	2.01	51.56
Aug	7.24	77.19	3.25	42.85	3.56	67.28	3.56	79.04	5.46	57.02
Sept	15.72	90.37	17.30	60.15	9.93	77.21	15.49	44.53	12.85	69.87
Oct	15.24	108.15	24.74	84.89	4.88	82.09	7.51	47.04	1.32	71.19
Nov	5.21	113.36	10.26	95.15	1.93	84.02	1.96	49.0	0.00	71.19
Dec	0.51	113.87	3.10	98.25	7.06	91.08	2.77	51.77	0.15	71.34

APPENDIX C

LOCATION AND RELATIVE SIZE OF THE IMPACT AREA WITHIN WEST RANGE, FORT SILL MILITARY RESERVATION, COMANCHE COUNTY, OKLAHOMA



WEST RANGE FORT SILL MILITARY RESERVATION

VITA

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