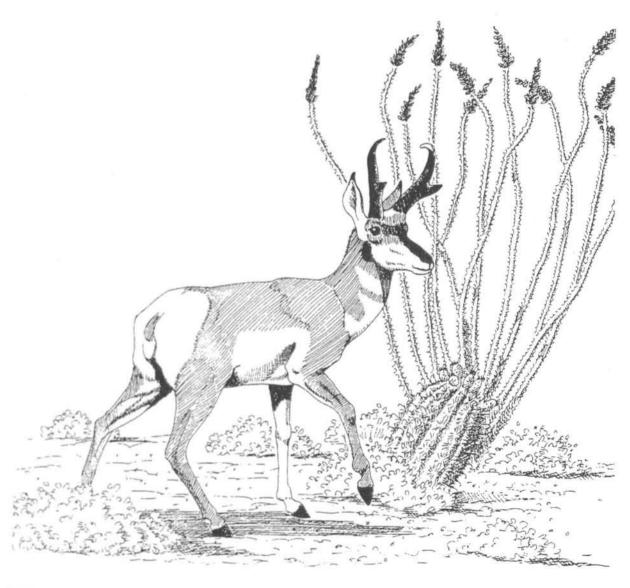
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COPPER/SELENIUM LEVELS AND OCCURRENCE OF BLUETONGUE VIRUS IN ARIZONA PRONGHORN

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Abstract: A total of 288 serum and 330 liver samples were collected from hunter-harvested pronghorn (Antilocapra americana) during fall 1996 hunts throughout Arizona. Of 288 serum samples, 226 (78.5%) were positive for exposure to bluetongue as determined by the Agar Gel Immunodiffusion Test (AGID). Analysis by geographic area revealed no definite pattern of distribution. Serum neutralizing antibody titers ranged from 1:20 to >1:160 for all serotypes. Serotype 11 (BT11) was the most prevalent (77%), followed by BT17 (48.8%). Statewide, 97% (97/100) of liver samples had copper (Cu) levels below 25 ppm, the level reported as minimal for domestic livestock. Average liver Cu value for statewide samples was 8.3 ppm (range = 1.1-36.0 ppm, n = 100). Percent of samples below "adequate" liver Cu values for livestock revealed no apparent age-specific trends. Statewide, 73% (73/100) of liver samples were below 0.25 ppm selenium (Se), which is considered minimal for most domestic livestock. The average liver Se value for statewide samples was 0.19 ppm (range = 0.01-0.81 ppm, n = 100). When summarized by geographic area, samples from southeastern Arizona had the lowest mean liver Se level (0.101 ppm), and northcentral Arizona had the highest mean liver Se value (0.244 ppm).

Key words: Antilocapra americana, bluetongue virus, copper, pronghorn antelope, selenium, serological survey.

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Arizona pronghorn populations occupy a more variable environment, have lower recruitment rates, and are relatively small and isolated when compared to many other pronghorn populations in North America. As a result, these sensitive populations may require more intensive management to remain viable. Managers should strive to learn more about previously neglected sources of mortality, which affect populations spatially or temporally. Disease occurrence and mineral deficiencies in wild ungulates are 2 factors in need of future research. Understanding effects of these factors may result in the development of beneficial management actions and will allow managers to explain population trends to interested constituents.

Several studies have found bluetongue virus (BT) to be enzootic in pronghorn populations. Trainer and Jochim (1969) reported 11.6% (8/96) of pronghorn serum samples from western and central Wyoming and 35.1% (34/97) in Colorado samples were positive for BT in fall of 1969. None of 70 pronghorn killed in Carbon County, Wyoming, were seropositive for BT in late August of 1977 (Thorne et al. 1988).

In 1976, at least 3,200 pronghorn died during a bluetongue epizootic (BT17) in eastern Wyoming (Thorne et al. 1988). In 1984, another BT epizootic killed at least 300 more in the northeastern part of that state. During these disease episodes, fawn recruitment dropped to unusually low levels. Analysis of blood samples collected during the latter half of epizootics

revealed that 81% (1976) and 60% (1984) of animals harvested by hunters in fall were seropositive for BT (Thorne et al. 1988). The 1984 epizootic was estimated to have killed only 4-5% of the pronghorn in that area.

In 1983, a serologic survey in western Nebraska found 19% (16/84) of fawns and yearlings (1.5-years old) and 42.5% (37/87) of 2 to-7-year old animals were seropositive for BT (Johnson et al. 1986).

Selenium deficiencies have been shown to cause "white muscle disease" (nutritional muscular dystrophy) leading to cardiac arrest and may contribute to increased neonatal mortality (Reilly 1996). Sub-lethal effects are manifested by lowered immune response and reductions in feeding efficiency, growth rate, and reproductive rate (Reffett et al. 1988).

Very low levels of Se have been documented in pronghorn antelope (Heffelfinger and Olding 1996a, Stoszek et al. 1980), mountain goat (*Oreamnos americanus*; Hebert and Cowan 1971), mule deer (*Odocoileus hemionus*; Dierenfeld and Jessup 1990, Heffelfinger and Olding 1996b, Oliver et al. 1990), and African bovids (Murry 1967).

Copper deficiencies in domestic ruminants are manifested in reduced fertility, poor/faded pelage, reduced growth rate, diarrhea, cardiovascular disease, unthrifty young, neurologic disease of young animals, skeletal deformities, or even sudden death (Puls 1995). These conditions are readily observable in captive animals under close supervision, but most often go unnoticed in wild populations. Additionally, wild animals frequently seek seclusion when ill, further lowering chances of being observed.

Our objectives were to: 1) document the extent of seropositive response to bluetongue virus, and 2) collect baseline levels of serum/liver copper and selenium in Arizona pronghorn.

STUDY AREA AND METHODS

As part of another project concerning pronghorn genetic differentiation, collection kits were mailed to hunters who obtained permits to hunt pronghorn in Arizona in 1996. Hunters were asked to collect blood in a 50-ml tube and a

small piece of liver in a plastic bag, place the samples on ice, and drop them off at 1 of 8 collection stations located throughout Arizona. Over 700 sample collection kits were mailed in August 1996. Volunteers from the Arizona Antelope Foundation and various universities located throughout Arizona were recruited to help operate collection stations. At collection points, samples were cataloged, and hunter information, harvest location, and estimated age were recorded (Dow and Wright 1962). Blood samples were centrifuged as soon as possible. and serum was collected to minimize negative effects of hemolysis on sample quality. Samples were transported to the Arizona Veterinary Diagnostic Laboratory for AGID screening and mineral analyses. Levels >1:10 are considered indicative of past exposure. Serum samples giving positive reactions with AGID were forwarded to the National Veterinary Services Laboratory (NVSL) for Serum Neutralization (SN) to define titer levels and for serotyping.

Liver samples were analyzed for Cu by atomic absorption spectrometry after digestion in nitric acid (Association of Official Analytic Chemists 1995). Total liver Se was determined as the 2-nitro piazselenol chelate by capillary gas chromatography using electron capture detection (Shimoishi 1976). Data are expressed in terms of wet tissue weight to be directly comparable to values reported by Puls (1995). In 77 of the 100 samples, liver and serum samples were from the same pronghorn, allowing correlation analyses of these 2 measurements.

RESULTS

Bluetongue Virus

A total of 288 serum samples was collected in late September 1996 hunts throughout the state. Of these, 226 (78.5%) were seropositive for exposure to BT as determined by AGID (Table 1).

Seropositive response rates in Game
Management Units (GMUs) with ≥10 samples
ranged from 61.9-100% for units. Summary by
regions revealed no definite pattern of
distribution throughout the state (Table 2). All
11 samples from southeastern Arizona were
positive, while only 5 of 11 samples from
northwestern Arizona were positive.

Table 1. Percent seropositive to bluetongue virus by age class of hunter harvested pronghorn in Arizona, September 1996.

Age	Percent (%) positive for exposure to bluetongue (AGID)	Sample size
1.5	66.7	9
2.5	85.2	27
3.5	74.6	63
4+	78.8	66
Mean	76.3ª	165

Differs from overall statewide average because samples with age data are a subset of the statewide data set.

Table 2. Percent seropositive to bluetongue virus by geographic area of hunter harvested pronghorn in Arizona, September 1996.

Geographic area	GMUs included	Percent (%) positive for exposure to bluetongue (AGID)	Sample size
N. of Grand Canyon	12AB, 13AB	76.5	17
Northwestern	15B, 18AB	45.5	11
Northcentral	6AB, 7, 8, 9, 10, 17A, 19AB, 21	75.2	149
Northeastern	1, 2ABC, 3ABC, 4AB, 5AB	75.0	100
Southeastern	30A, 31, 32, 34B, 35AB	100.0	11

A subsample of 186 AGID-positive samples were sent to the NVSL in Ames, Iowa, for serotyping by SN. Samples were tested for antibodies to bluetongue serotypes 2 (BT2), 10 (BT10), 11 (BT11), 13 (BT13), and 17 (BT17). Twenty-six (13.9%) of these samples were found to be negative for antibodies when analyzed with SN. Of the 160 remaining samples, 21-33 were determined unsuitable for testing among various

serotypes. Titers for the remaining samples ranged from 1:20 to >1:160.

Of samples which were SN antibodypositive for at least 1 serotype, BT11 was the most prevalent (77%, 107/139) serotype, followed by BT17 (48.8%, 62/127), BT13 (22.2%, 30/135), BT10 (20.4%, 28/137), and BT2 (16.9%, 23/136). Some of these positives for individual serotypes may be the result of cross-reaction with other BT serotypes; 71 of 151 (47%) usable samples reacted to more than 1 serotype. Of the 71 samples reacting to ≥2 serotypes, 16 (22.5%) had titers >1:40 for at least 2 serotypes.

Mineral Analysis

Over 330 liver samples were collected during late September 1996. Of these, 100, representing all geographic areas of the state, were selected for Cu and Se analysis. In addition, 288 serum samples were collected and 100 were selected to provide a uniformly distributed sample of serum Cu levels.

Liver Copper--Statewide, 97% (97/100) of the pronghorn samples were below 25 ppm (Table 3). The average liver Cu value statewide samples was 8.3 ppm (n = 100, SE = 5.5, range = 1.1-36.0 ppm).

When summarized by geographic area, samples from units north of the Grand Canyon (12A, 13AB) had the highest average liver Cu level (11.2 ppm), but still had less than half the minimum considered adequate for domestic animals (Table 3). The east-central portion of the state had the lowest average liver Cu value (6.10 ppm, n = 33). The percentage of samples below adequate ranges revealed no apparent geographic trends. Units north of the Grand Canyon had the lowest percentage of samples below the adequate range (8/9). Liver Cu values summarized by age class statewide show no agespecific differences for the 43 samples from known age animals (Table 4).

Liver Selenium--Statewide, 73% (73/100) of pronghorn samples were below 0.25 ppm (\bar{x} = 0.19, SE = 0.11, range = 0.01-0.81 ppm) (Table 3). When summarized by geographic area, samples from southeastern Arizona had the lowest range of values (0.01-0.19 ppm) and lowest average liver Se level (0.101 ppm, n = 18) (Table 3). East-central Arizona had the highest average liver Se value (0.24 ppm, n = 31).

All 18 samples from southeastern Arizona and 81.8% (27/33) of east-central samples were below 0.25 ppm. The northwestern, north-central, and north of the Grand Canyon samples had 55.6-58.1% of the samples below 0.25 ppm (Table 3).

Analysis of liver Se by age class was hampered by small sample size in some age classes, however, a trend of decreasing liver Se levels with increasing age may be indicated (Table 4).

Serum Copper--Of 99 statewide pronghorn samples analyzed, 82 (82.8%) were below 0.70 ppm for serum Cu (Tables 3 and 5). Serum Cu averaged 0.59 ppm throughout the state (SE = 0.19, range = 0.22-1.8 ppm).

Serum copper vs. Liver copper--In 77 of 100 samples, liver and serum samples were from the same individuals allowing a comparison of these parameters. Serum Cu is generally less reliable because levels fluctuate with state of pregnancy, presence of viral/bacterial infections, and dietary intake of protein, molybdenum, Se, and sulphate (Puls 1995). There was no relationship between Cu levels in serum vs. liver (Fig. 1). Less than 1% of liver Cu variation was explained by liver Se values (r² = 0.0016).

DISCUSSION

Bluetongue

BT is a serious disease among pronghorn antelope. Presence of antibody titers (a seropositive reaction) may imply protection for those individuals, since none exhibited clinical signs of disease when harvested. Hoff and Trainer (1972) infected 4 pronghorn with BT8 subcutaneously. Prior to experimental infection, 2 of the pronghorn possessed antibodies (tested positive), 2 did not. Individuals with prior antibodies did not develop clinical signs, become viremic, or succumb. Pronghorn testing negative for exposure (no antibodies) prior to inoculation developed clinical signs and died 7 and 8 days after inoculation.

No trends were apparent when AGID exposure rates were summarized by age class, although yearlings may have lower rates of exposure than older animals (Table 1). Since antibody levels can persist for more than 1 year in black-tailed deer (O. h. columbianus) (Work et al. 1992), this lower exposure may reflect less opportunity to come into contact with disease vectors in younger animals.

Table 3. Serum copper, and Liver copper and selenium values by geographic area for hunter harvested pronghorn in Arizona, September 1996.

		S	Serum Copper (ppm)	(mdd)	ב	Liver Copper (ppm)	(mdc	7	Liver Selenium (ppm)	(mdd) mn
Geographic area	included	× (E)	range	% <0.70ppm	×	range	% <25ppm*	× (ii)	range	% <0.25ppm ^a
Northwestern	15B, 18AB	0.512	0.39-0.63	100.0	8.47	5.0-18.0	100.0	0.194	.0735	55.6
North-central	6AB, 7, 8, 9, 10, 17A, 19AB, 21	0.614	0.38-1.80	88.6	9.50	1.1-36.0	93.5	0.244 (31)	.0581	58.1
N. of Grand Canyon	12AB, 13AB	0.630 (9)	0.33-1.20	77.8	(9)	5.4-28.0	88.9	0.220	.1136	55.6
Northeastern	1,2ABC, 3ABC,4AB, 5AB	0.553 (41)	0.22-0.93	85.4	6.10	2.2-13.5	100.0	0.168	.0436	81.8
Southeastern	30A, 31, 32, 34B, 35AB	0.677 (9)	0.48-0.84	44.4	8.52	5.5-14.0	100.0	0.101 (18)	.01-19	100.0
Statewide	L-35B	0.590	0.22-1.80	82.8%	8.30 (100)	1.1-36.0	%16	0.190	.0181	73%

Normal range reported for domestic goats:

liver copper: 25-150 ppm, liver selenium: 0.25-1.20 ppm, serum copper: 0.70-1.20 ppm.

Table 4. Liver copper and selenium levels by age class in hunter harvested Arizona pronghorn, September, 1996.

		×	Copper		Selenium	
Age Class	n		% <25ppm ^a	₹	% <0.25ppm ⁴	
1.5	4	7.58	100.0	0.21	75.0	
2.5	4	6.85	100.0	0.22	75.0	
3.5	18	9.92	94.4	0.20	66.7	
4+	17	7.53	100.0	0.15	88.2	
Total ^b	43					

^a Normal range reported for domestic goats:

liver copper: 25-150 ppm, liver selenium: 0.25-1.20 ppm.

Table 5. Serum copper levels (ppm) by age class in hunter harvested Arizona pronghorn, September 1996.

Age Class	n	≅	% <0.70ppm ^a	
1.5	5	0.50	80.0	
2.5	8	0.59	75.0	
3.5	23	0.58	87.0	
4+	21	0.64	71.4	
Total ^b	57			

[&]quot;Normal ranges:

0.70-1.20 ppm domestic goats

0.70-2.00 ppm domestic sheep

Exposure to BT seems widespread throughout Arizona. However, actual effects of the disease on our populations remain obscure. BT can affect reproduction because of timing of infections (Thorne et al. 1988). BT, an insect-borne virus, affects animals in late summer (July-Sept), during the peak of vector activity. Because this period coincides with pronghorn breeding seasons, a viremic female may not be in

good reproductive condition or her behavior may be altered enough to disrupt breeding. Bucks which become viremic in pre-rut and rut periods may be unsuccessful in defending breeding territories. In domestic animals, BT has been reported to cause still births, spontaneous abortions, decreased milk production, weight loss, and congenital abnormalities (Leudke et al. 1970).

^b Means reported in Table 3 because age data are a subset of all data.

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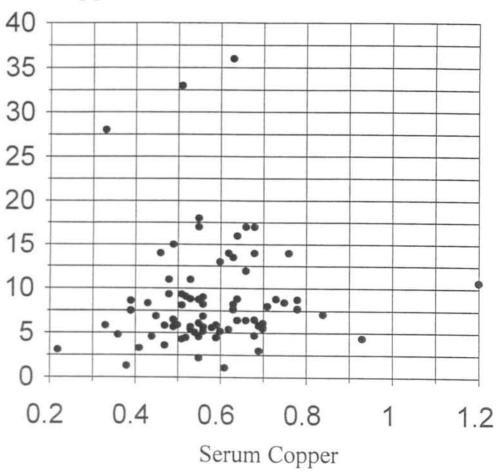


Figure. 1. Relationship between liver copper and serum copper values from individual hunter harvested pronghorn (n = 77) in Arizona, September 1996.

As in the present study, BT11 was the most prevalent serotype in 10 samples collected in a pilot study in southeastern Arizona in 1995. Positives for BT2 are undoubtedly the result of cross-reaction, as this serotype has not been found in the United States in over 16 years and never in the West (G. Gustafson, NVSL, pers. comm.). Some individuals may also have been infected by >1 serotype. Multiple infection are more likely when an animal has high titers (>1:40) for more than 1 serotype.

The percent of seropositive reactions we found seems near the highest level reported for this species, which is remarkable since no mortality has been detected from active disease. This high exposure was unexpected since the principal vector in other areas is reported to be Culicoides variipennis, which thrives in moist areas with standing water. These conditions are not typically found in Arizona pronghorn habitats. To what extent an alternative vector may be involved is unknown. No known epizootics have been reported in recent times in Arizona to account for high exposure rates. If mortality of exposed individuals is high, evidence of exposure should be low because most exposed animals die. If, however, the virus kills only a small percent of those exposed, a higher percent of the population will carry neutralizing antibodies. BT is undoubtedly enzootic in the state's pronghorn populations, constantly cycling but apparently causing little outright mortality of adults, but perhaps affecting them in ways that are less apparent and more significant to the population (i.e., lowered reproduction).

Mineral Analysis

Arizona pronghorn have liver and serum Cu and Se levels far below what is considered normal in domestic ruminants (Puls 1995). Because of variability in Cu and Se values from different geographic areas and diverse taxa, it is unclear if these values pose a substantial problem for Arizona pronghorn. Controlled experiments with captive animals are needed to assess physiological consequences of levels observed in wild animals. It is unknown to what extent wild ungulates use mineral supplementation for domestic livestock. However, this practice has

potential to affect geographic pattern of mineral levels in pronghorn. We currently do not know where supplementation occurs in the state, at what level, or if pronghorn use mineral supplement when available.

Liver Copper--Adequate liver Cu level is 25-150 ppm in domestic goats and 25-100 ppm in domestic sheep and cattle (Puls 1995). Liver Cu levels for pronghorn collected in southeastern Arizona in September 1995 averaged 11.7 ppm (n = 12, range = 4.4-30.8 ppm). Eleven of 12 samples (91.7%) were under 25 ppm (Heffelfinger and Olding 1996a). Of 66 southeastern Arizona mule deer liver samples collected in November 1995, 20 (30.3%) were below adequate Cu values in domestic animals (25 ppm; Heffelfinger and Olding 1996b). The average liver Cu value for these samples was 34.7 ppm (range = 8.3-91.5 ppm). Monitoring trace minerals is very difficult because of complex interrelations and interactions among minerals. For example, absorption of Cu in ruminants is affected by dietary levels of molybdenum, iron, and sulfate. Low levels of Cu found in Arizona pronghorn are cause for concern with respect to historically low recruitment rates. Only through manipulative experiments or evaluation of captive animals will it be possible to determine true effects, if any, of these low levels.

Liver Selenium--Adequate values for liver
Se are reported to be 0.25-1.20 ppm in domestic
goats, 0.25-1.50 ppm in domestic sheep, and
0.25-0.50 ppm in cattle (Puls 1995). Precise
nutritional requirements and tissue
concentrations of Se have not been established in
wild ruminants (Oliver et al. 1990). Liver Se
values for domestic livestock have been reported
to vary widely between 0.04 - 14.7 ppm (Ihnat
1989:147). Large temporal and spatial variation
in liver Se levels makes it difficult to determine
whether a particular species or population is
deficient.

Studies have shown a relationship between Se concentrations in the soil and those in forage available to ruminants (Kubota et al. 1967). Se levels in ruminant tissues is, in turn, related to dietary intake of the element (Brady et al. 1978, Ihnat 1989). Annual variation in climatic conditions also influences Se levels in forage

(Underwood 1977).

Native Arizona grasses from some areas are very low or deficient in Se levels. Several cases of stillborns, white muscle disease, unthriftiness, retained placentas, and diarrhea in domestic animals have been reported in Arizona and may be associated with lack of dietary Se (Frederick 1997).

Analysis of liver Se by age class was hampered by small sample size in some age classes, however, a trend of decreasing liver Se levels with increasing age may be indicated (Table 4). This may also be an artifact of sample size as it conflicts with what is known about pronghorn breeding biology. Dominant pronghorn bucks establish territories where forage quality and quantity are superior during the pre-rut and rut periods, and therefore should have access to a higher nutritional plane. The age class "4+" also contains aged bucks that may not be able to defend optimal habitats.

All samples in this study were from male pronghorn because only bucks are legally harvested by hunters in Arizona. A study involving 151 mule deer in California found no sex-specific differences in whole blood Se (Dierenfeld and Jessup 1990). Sex-specific differences in Se levels are generally not reported (Ihnat 1989:123), however, Oliver et al. (1990) found significant sex-specific differences in Se levels of 1,695 California mule deer.

Stoszek et al. (1980) reported a Se deficiency in Idaho pronghorn populations reported to have pronghorn fawns suffering from "weak fawn syndrome." Se levels in tissue samples collected from these Idaho pronghorn populations which suffered from overall poor fawn recruitment were lower (P < 0.001) than similar samples of Montana pronghorn with no apparent fawn recruitment problems. Se found in the Idaho animals averaged 0.52 ± 0.16 ppm with individual levels as low as 0.35 ppm, whereas Montana pronghorn averaged 1.21 ± 0.20 ppm.

For Arizona pronghorn, Se deficiency may be a real effect because of widely reported deficiencies in cattle and horses in Arizona (Frederick 1997). Mineral supplementation of domestic livestock should be considered as a possible factor effecting trace mineral values in wild ruminants.

Liver Se levels of 12 pronghorn samples collected in southeastern Arizona in September 1995 averaged 0.306 ppm; only 2 (16.7%) were under the 0.25 ppm minimum considered adequate (Heffelfinger and Olding 1996a). Pronghorn in east-central Arizona had the highest average liver Se value (0.24 ppm, n = 31). This runs contrary to the observation by Kubota et al. (1967) that Tertiary volcanic rocks of the east-central area are low in Se.

In October/November 1995, 66 southeastern Arizona mule deer liver samples were analyzed for Se levels (Heffelfinger and Olding 1996b). The average liver Se value was 0.30 ppm, with 19 (28.8%) below 0.25 ppm, however, Odocoileus may have a much lower dietary requirement for Se (Brady et al. 1978). Also, Dierenfeld and Jessup (1990) reported fluctuation over 300% in whole blood Se levels among years during their 8-year study.

Our 1996 sampling effort occurred during an extremely dry year and may have resulted in values lower than during most years. This is supported by higher average values for Cu and Se the previous year (Heffelfinger and Olding 1996a).

Serum Copper--According to reference data, "adequate" ranges for serum Cu are 0.70-1.20 ppm for domestic goats and 0.70-2.0 ppm for domestic sheep (Puls 1995). Nutritional requirements and baseline blood concentrations of Cu have not been established in wild ruminants and are variable temporally, however, available information indicates that adequate levels for domestic livestock are above 0.70 ppm. Summary by age class failed to reveal obvious age-specific differences. Serum Cu levels are not a reliable indicator of Cu status because not all Cu in blood is available to the animal (Puls 1995). In addition, infections or trauma seems to increase serum Cu levels, causing serum levels to be labile. Values are reported here to establish a reference for future sampling limited to blood only (i.e., endangered Sonoran pronghorn [A. a. sonoriensis]).

Cu levels of 7 samples from southeastern Arizona in September 1995 averaged 0.64 ppm. Four (57.1%) were under 0.70 ppm (Heffelfinger and Olding 1996a).

MANAGEMENT IMPLICATIONS

Attempts to correlate precipitation patterns with pronghorn fawn recruitment have been largely unsuccessful (D. E. Brown, Arizona State University, pers. comm.). It seems there are confounding factors that complicate this relationship. Determining effects of enzootic BT infections on fawn survival will be more difficult, but represent an additional factor that may affect fawn recruitment. Research under controlled conditions will be necessary to determine these effects. There may be little that pronghorn managers can do to ameliorate negative effects of BT infection. However, a more thorough understanding of the impacts will allow managers to better explain pronghorn population dynamics to hunters and other interested publics.

Unfortunately, all suppressive factors intensify simultaneously. During drought years, fawns are born with lower birth weights, forage for lactating does is poor, forage for fawns after weaning is poor, hiding cover for fawns is lacking, predators lack abundant alternative foods, and stressed animals are more susceptible to diseases and predation. Separating these factors from effects of viremia during the breeding season would be a daunting task.

Management options for confirmed mineral deficiencies include dietary supplementation with mineral blocks throughout pronghorn habitat. This would provide an opportunity to work with the ranching community to the mutual benefit of both parties. Flueck (1989) reported that supplementation with intraruminal Se pellets in pronghorn increased survival of fawns born to treated does by an overall factor of 2.6.

Investigations are needed to determine if mineral supplementation will enhance immune function and result in better reproduction or survival. Managers should establish a "management experiment" in Se and Cu supplementation. Mineral could be supplied economically, but the livestock operator would have to agree to not supplement in the control area.

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