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## GEOGRAPHIC ANALYSIS OF PATHOGEN EXPOSURE IN BIGHORN SHEEP (*OVIS CANADENSIS*)

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**ABSTRACT:** Antibody responses were examined among 998 bighorn sheep (*Ovis canadensis*) in California (USA) to determine spatial patterns of pathogen exposure. Using a shifting frame analysis, a specific geographic region was delineated that contained bighorn sheep with higher ( $P < 0.05$ ) levels of multiple exposure (antibodies detected against  $\geq$  two pathogens), as well as higher prevalence values for eight of ten individual pathogens. This region in southwestern California encompassed all of the peninsular populations of bighorn sheep recently proposed for listing as endangered by the U.S. Fish and Wildlife Service.

**Key words:** Bighorn sheep, *Ovis canadensis*, conservation, endangered species, California.

### INTRODUCTION

The introduction of pathogenic microorganisms likely played an important role in reducing the distribution and abundance of bighorn sheep (*Ovis canadensis*) as settlers and their domestic livestock spread westward in the USA during the 1800's (Monson, 1980). Infectious disease continues to be an important cause of morbidity and mortality, and our current understanding of disease processes has been derived primarily from epizootics caused by specific pathogenic organisms (Onderka and Wishart, 1984; Foreyt and Jessup, 1989). Management strategies that address the demographic consequences of disease should be based upon an understanding of the ecological relationships among bighorn sheep and their pathogens. However, in many cases these relationships are unclear or difficult to discern. Our objective in this study was to use a novel shifting frame analysis to identify and delineate spatial patterns of pathogen exposure among bighorn sheep populations in California.

### MATERIALS AND METHODS

Blood samples were collected from 998 bighorn sheep in California between 1978 and 1990. The study sites were located between 32°43'N and 41°38'N of latitude and between 114°39'W

and 120°18'W of longitude. Serological tests were performed on subsets of these samples to detect antibodies against bluetongue virus, bovine herpes virus 1, bovine viral diarrhea-mucosal disease virus, contagious ecthyma virus, epizootic hemorrhagic disease virus, parainfluenza-3 virus, bovine respiratory syncytial virus, *Anaplasma* sp., *Toxoplasma gondii* and *Psoroptes* sp. (Clark et al., 1985, 1993). Results from individual bighorn sheep were scored as positive or negative for each pathogen based on presence or absence of antibodies. Individuals with positive test results were referred to as "exposed," and multiple exposure refers to the presence of antibodies against two or more pathogens.

To delineate spatial effects, we performed a "shifting frame" analysis to determine the percentage of individuals exposed to varying numbers of pathogens over a range of latitudes and longitudes. The latitude and longitude of the center of each of the 50 populations from which samples were obtained was reported previously (Mazet et al., 1992). In the initial step, we grouped all individuals in the most southerly degree of latitude into the first frame and calculated the frequencies of individuals determined to be exposed to varying numbers of pathogens (0, 1, 2, >2). Then we shifted the borders of the frame 0.1 degree of latitude north such that all the individuals from 0.1 degree north of the southernmost location to 1.0 degree north of that point were included in the analysis for the next frame. The frame was then iteratively shifted 0.1 degree north until all individuals in California had been included in at least one frame. We used a similar analysis to evaluate the longitudinal shifts in multiple exposure



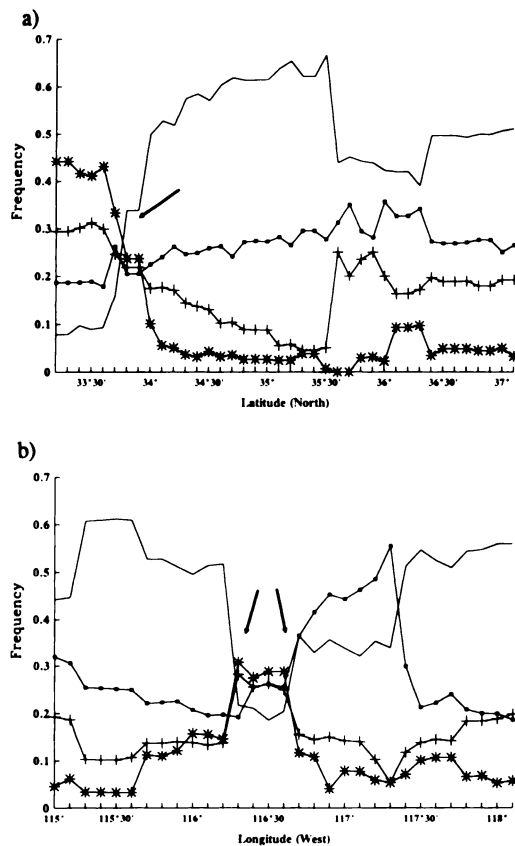


FIGURE 1. Shifting frame analysis over the a) latitudinal and b) longitudinal range of California with frequencies of individuals exposed to 0 (—), 1 (■), 2 (+), or >2 (\*) pathogens presented for the midpoint of each frame. Points of intersection between increasing frequencies of multiple exposure and decreasing frequency of unexposed individuals are shown by arrows.

frequencies. To avoid error due to differences in the number of pathogens for which each individual was tested, only individuals surveyed for greater than seven pathogens were included in the shifting frame analyses.

The shifting frame analysis defined a geographical area containing individuals with apparent increased levels of exposure. These apparent differences were tested by comparing frequencies of multiple exposure and individual pathogen exposure inside and outside of the defined area. We used log-likelihood ratio tests for independence of frequencies (G-tests) for the comparisons except where observed cell frequencies were less than five; in these cases we used Fisher's exact tests (Sokal and Rohlf, 1981).

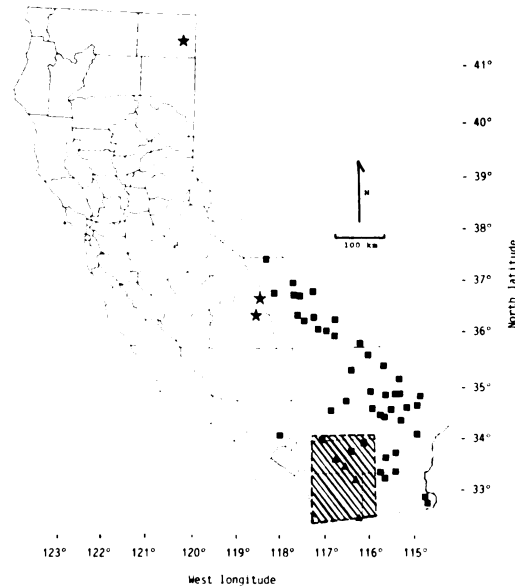


FIGURE 2. Map of sites for which serologic data from bighorn sheep in California were analyzed. Subspecies are indicated as (★) for *O. canadensis californiana*, (■) for *O. canadensis nelsoni*, and (▲) for *O. canadensis cremnobates*. The area of special concern as determined by a shifting frame analysis of multiple exposure is demarcated by hatch marks.

## RESULTS

Based on the shifting frame analysis (Fig. 1), the frequency of individuals exposed to no pathogens was very low for low latitude frames (those below 33°48'N), rose to a maximum at intermediate latitudes (33°48' to 35°36'N), and dropped to moderate levels at higher latitudes (>35°36'N). The reverse of this phenomenon was apparent in the trend of frequencies for individuals exposed to more than one pathogen. Curves representing frequencies of unexposed and multiply-exposed individuals intersected at about 33°48'N. This point of intersection delineated populations of highest exposure, and because this point represents the shifting frame's midpoint, populations of special concern actually lay in latitudes south of a point 0.5 degree north of this intersection point. Based on results of equivalent shifting frame analysis of longitudinal effects, we noted an area of high exposure delineated

by the intersection points at longitudes 116°18'W and 116°36'W. This area also included 0.5 degree either side of those two points because those points represented midpoints of the shifting frame. The geographical area delimited by these longitudinal and latitudinal boundaries defined a set of populations in southwestern California where individual bighorn sheep have been exposed to a greater number of different pathogens (Fig. 2). This association between geographic location and multiple exposure was highly significant ( $G = 118.0$ ;  $P < 0.001$ ). The prevalence of exposure to each pathogen, except *Psoroptes* sp. and *Toxoplasma gondii*, also was significantly higher inside versus outside the defined area (Table 1).

### DISCUSSION

Based on the shifting frame analysis of pathogen exposure frequencies, bighorn sheep populations in a defined region of southwestern California had a higher level of pathogen exposure than populations outside of this region (Table 1, Fig. 1). The area delimited by this analysis included all of the populations currently recognized by the state of California as belonging to subspecies *O. canadensis cremnobates* (Fig. 2). Numbers of *O. canadensis cremnobates* in the peninsular mountain ranges of California have decreased sharply since 1977, and several of the pathogens examined in our study have been isolated from dead and dying bighorn sheep in this area (DeForge et al., 1982). The decline in population numbers coupled with the perceived threat of continuing disease-induced mortality has prompted both state and federal action. The subspecies *O. canadensis cremnobates* has been listed by the state as threatened, and the federal government recently proposed listing the peninsular populations of *O. canadensis* as endangered under the U.S. Endangered Species Act (U.S. Fish and Wildlife Service, 1992).

Our results are compatible with the hypothesis that there is a causal relationship

TABLE 1. Antibody prevalences among bighorn sheep in California located inside and outside of the area determined to be of special concern by shifting frame analysis.  $G$ -statistics and their associated probabilities ( $P$ ) are reported except where cell frequencies were less than five, in which case Fisher's exact probabilities are reported.

Pathogen	Seroprevalence		$G$	$P$
	Outside	Inside		
Ana <sup>a</sup>	0.0	50.0	ND <sup>b</sup>	<0.001
BTV	6.2	46.7	127.62	<0.001
BVD-MDV	4.9	18.0	21.90	<0.001
CEV	20.6	56.2	28.39	<0.001
EHDV	11.1	52.1	91.26	<0.001
BHV1	0.0	2.5	ND	0.003
PI3V	8.1	20.0	15.64	<0.001
Pso	10.6	3.5	ND	0.021
BRSV	6.5	15.4	5.54	0.019
Tox	21.6	25.0	ND	0.775

<sup>a</sup> Pathogens: Ana, *Anaplasma* sp.; BTV, bluetongue virus; BVD-MDV, bovine viral diarrhea-mucosal disease virus; CEV, contagious ecthyma virus; EHDV, epizootic hemorrhagic disease virus; BHV1, bovine herpes virus 1; PI3V, parainfluenza-3 virus; Pso, *Psoroptes* sp.; BRSV, bovine respiratory syncytial virus; and Tox, *Toxoplasma gondii*.

<sup>b</sup> ND = not determined.

between pathogen exposure and the decline of peninsular sheep numbers (DeForge et al., 1982; Clark et al., 1985, 1993; U.S. Fish and Wildlife Service, 1992). However, caution should be exercised in the interpretation of serologic tests in the absence of a demonstrated link to demographic processes. Exposure to a large number of pathogens may have contributed to or even caused the downward trend apparent in these populations. On the other hand, the higher level of exposure to multiple disease agents seen in this study may simply reflect a non-causal association with other factors that are directly responsible for the downward trend. For example, the possibility that dramatic, climate-induced cyclical fluctuations in population size are normal in desert bighorn sheep merits closer scrutiny (Wehausen et al., 1987).

Antibody assays clearly are of limited value in identifying cause and effect relationships. However, we did find a pattern of pathogen exposure in our spatial

analysis of serologic data that may reflect important demographic processes or consequences. Based on these results, there are several questions that should be addressed in light of the apparent decline and proposed listing of peninsular sheep populations. Why do bighorn sheep in southwestern California have higher levels of pathogen exposure? What causal relationship, if any, exists between downward population trend and high pathogen exposure frequencies? Do environmental conditions in this region enhance pathogen exposure? Are peninsular populations of bighorn sheep genetically or immunologically compromised? What role do sympatric domestic and wild ungulates play in pathogen exposure? Answers to these, and other, questions will be needed to develop effective and appropriate management strategies for peninsular bighorn sheep populations in California.

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