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SEROLOGIC SURVEY FOR BRUCELLOSIS IN FERAL SWINE, WILD RUMINANTS, AND BLACK BEAR OF CALIFORNIA, 1977 TO 1989

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ABSTRACT: A retrospective analysis of brucellosis serologic testing results in eight wildlife species in California from 1977 to 1989 was done. Samples were collected from 5,398 live-captured or hunter-killed animals and tested by combinations of up to six serologic tests for antibodies to *Brucella* spp.. Twenty-three of 611 (3.8%) feral swine (*Sus scrofa*), one of 180 (0.6%) black bear (*Ursus americanus*), one of 355 (0.3%) California mule deer (*Odocoileus hemionus californicus*), and one of 1,613 (0.06%) blacktail deer (*Odocoileus hemionus columbianus*) samples were considered reactors. Suspect serologic reactions occurred in three of 619 (0.5%) desert bighorn sheep (*Ovis canadensis nelsoni*) and one of 355 (0.3%) California mule deer samples. Brucellosis is not considered an important wildlife health problem in California except in feral swine.

Key words: Brucellosis, serologic survey, *Brucella* spp., black bear, elk, bighorn sheep, mule deer, blacktail deer, pronghorn antelope, feral swine, prevalence.

INTRODUCTION

Brucellosis is an infectious, zoonotic disease caused by five known species of the genus *Brucella*. The United States Department of Agriculture began a cooperative testing and eradication program for *B. abortus* in domestic cattle in 1934 and for *B. suis* in domestic swine in 1973. California was declared certified free of brucellosis in cattle in 1969 and gained Class A status in 1982. In 1981, California was declared a swine brucellosis validated free state. As the goal of complete eradication of the disease in domestic animals nears completion, the consideration of wildlife as reservoirs of brucellosis will become important.

Serologic surveys are commonly used to determine the prevalence of brucellosis in wildlife populations. Complete herd testing of free-ranging wildlife is impossible, but extensive serologic surveys and literature reviews have shown a generally low to moderate prevalence of brucellosis in ruminants (Moore and Schnurrenberger, 1981; McCorquodale and DiGiacomo, 1985; Tessaro, 1986). However, some populations of bison (*Bison bison*) and Rocky Mountain elk (*Cervus elaphus nelsoni*) are infected with *B. abortus* (Thorne et al., 1978; Moore and Schnurrenberger, 1981; McCorquodale and DiGiacomo, 1985; Tessaro, 1986). *Brucella suis* biotype 4 in caribou and reindeer (*Rangifer tarandus*) (Huntley et al., 1963; Broughton et al., 1970; Rausch and Huntley, 1978; Tessaro, 1986) and *B. suis* biotype 1 (Wood et al., 1976; Becker et al., 1978; Zygmont et al., 1982; Corn et al., 1986) and *B. suis* biotype 3 (Clark et al., 1983) in feral swine (*Sus scrofa*) have also been identified.

Serologic surveys for brucellosis in California wildlife have been conducted previously (Hoq, 1978; Riemann et al., 1979; Ruppanner et al., 1982; Clark et al., 1983). The prevalence of brucellosis has been low; however, these surveys were very localized or restricted in numbers or species of animals compared to the present survey.

Surveillance of wildlife for evidence of disease is conducted cooperatively by the California Department of Fish and Game, the California Department of Food and Agriculture, and the California Veterinary Diagnostic Laboratory System. Serologic testing of wildlife sampled from 1977 to

TABLE 1. Age and sex of wildlife species tested for antibodies to *Brucella* spp. in California, 1977 to 1989.

Species	Sex			Age			Total
	Male	Female	Unknown	Juvenile	Adult	Unknown	
Black bear	103	68	9	15	101	64	180
Elk	323	387	53	86	627	50	763
Bighorn sheep	265	465	53	55	675	53	783
Mule deer	444	588	14	113	862	71	1,046
Blacktail deer	715	819	79	126	1387	100	1,613
Pronghorn	293	93	16	50	291	61	402
Feral swine	317	270	24	181	346	83	611

1989 provided the opportunity for a retrospective evaluation of the importance and prevalence of brucellosis in feral swine, four species of wild ruminants, and black bear.

MATERIAL AND METHODS

The California Department of Fish and Game routinely obtains blood and tissue samples from wildlife to monitor the prevalence of various diseases. In this survey, 5,398 samples were tested for antibodies to *Brucella* spp. which included 611 feral swine (*Sus scrofa*); 180 black bear (*Ursus americanus*); 763 elk including 728 tule elk (*Cervus elaphus nannodes*) and 35 Roosevelt elk (*C. elaphus roosevelti*); 783 bighorn sheep including 619 desert bighorn sheep (*Ovis canadensis nelsoni*), 113 California bighorn sheep (*O. canadensis californiana*), and 51 peninsular bighorn sheep (*O. canadensis cremnobates*); 1,613 black-tailed deer (*Odocoileus hemionus columbianus*); 1,046 mule deer including 658 Rocky Mountain mule deer (*O. hemionus hemionus*), 355 California mule deer (*O. hemionus californicus*), 25 Inyo deer (*O. hemionus inyoensis*), and 8 burro deer (*O. hemionus eremicus*); and 402 pronghorn antelope (*Antilocapra americana*). Age and sex ratios of these sample sets are shown in Table 1. For this retrospective survey, animals were grouped as either juveniles (<1 yr) or adults using information and records available. Feral swine were grouped into juvenile (<1-yr-old), subadult (1- to 2-yr-old), and adult (>2-yr-old) age groups. The numbers of animals and locations of populations sampled are shown in Table 2 and Figures 1 to 7.

Blood samples were collected from animals during routine capture operations conducted by the California Department of Fish and Game (Rancho Cordova, California 95670, USA) and from hunter-killed animals. Blood was collected via venipuncture in all live-captured animals and directly from the heart or jugular veins during field dressing of hunter-killed animals. Blood samples were allowed to clot and centrifuged within 24 hr of collection. Sera were removed, frozen at −20 C and stored until tested.

Sera were tested at the California Department of Food and Agriculture (Sacramento, California 95842, USA) the Thurman California Veterinary Diagnostic Laboratory (Davis, California 95616, USA) or the National Animal Disease Laboratory, U.S. Department of Agriculture (Ames, Iowa 50010, USA). Standard tests utilized by the U.S. Department of Agriculture for testing cattle and swine were used (Anonymous, not dated). The buffered acidified plate agglutination test (BAPA) and/or the standard plate agglutination test (SPT) were used to screen all ruminant and bear samples for antibodies to *Brucella* spp. Samples with positive reactions were tested with rivanol agglutination and/or card tests to separate true positive from nonspecific and false positive reactions. One hundred thirty bighorn sheep sera were tested for *B. ovis* antibodies using an ELISA test (Walker et al., 1985).

Feral swine samples were tested using a variety of test batteries. Three hundred fifty one samples were screened with a BAPA. Seventy samples were tested with both a BAPA and a standard tube test (STT). Fifteen samples were tested with BAPA, STT, and complement fixation tests (CF). Eleven samples were tested with BAPA, STT, card, and rivanol tests. Fifty-nine samples were tested with BAPA, STT, SPT, card, and rivanol tests. One hundred five samples were tested with BAPA, STT, SPT, card, rivanol, and CF tests.

Serologic reactions were classified using standard criteria of the U.S. Department of Agriculture Cooperative Brucellosis Eradication Program for cattle, bison, and swine (Anonymous, 1979). Ruminants and bears were considered reactors if they had complete agglutination on an SPT at ≥1:100 and/or a positive BAPA on initial screening followed by positive supplementary tests (a positive card test or a positive rivanol test at ≥1:25). Ruminants and bears

FIGURE 1. Distribution of black bears (▨) and location of animals sampled for antibodies to *Brucella* spp. (■) in California by county, 1977 to 1989. ●, counties with reactor animals.

FIGURE 3. Distribution of bighorn sheep (▨) and location of animals sampled for antibodies to *Brucella* spp. (■) in California by county, 1977 to 1989. ●, counties with reactor animals.

were classified as suspects if they exhibited partial to complete agglutination on an SPT at any dilution or a positive BAPA on initial screening with no positive supplementary tests. Because the feral swine samples were from an incomplete herd test and with the variation in test batteries used, feral swine were considered reactors if they exhibited complete agglutination

FIGURE 2. Distribution of elk (▨) and location of animals sampled for antibodies to *Brucella* spp. (■) in California by county, 1977 to 1989. ●, counties with reactor animals.

FIGURE 4. Distribution of mule deer (▨) and location of animals sampled for antibodies to *Brucella* spp. (■) in California by county, 1977 to 1989. ●, counties with reactor animals.

TABLE 2. Numbers of animals sampled for antibodies to *Brucella* spp., by species and county, in California, 1977 to 1989.

County	Black bear	Elk	Bighorn sheep	Blacktail deer	Mule deer	Prong-horn	Feral swine
Alameda		29					5
Alpine					66		
Amador				25			
Butte							
Calaveras				9	26		
Colusa		7		13			
Contra Costa		4					
Del Norte							
El Dorado	48			30	1		
Fresno					17		
Glenn		3					
Humboldt	8	25		116			
Imperial			25				
Inyo		348	172		142	3	
Kern		114		1	186		
Kings							
Lake		10		15			
Lassen					64	15	
Los Angeles							
Madera				3			
Marin		12		153[c]			6
Mariposa							
Mendocino		18		120			
Merced		75		1			17
Modoc			6		70	227	
Mono			20		289		
Monterey		25		207			81 (18)[a]
Napa				13			
Nevada				9			
Orange							
Placer	20			22			
Plumas	1			36	6		
Riverside			60		8		
Sacramento				28	1		
San Benito				36			
San Bernardino			496 (3)[b]				
San Diego			4	24			49[d]
San Francisco				2			
San Joaquin							
San Luis Obispo				173	181 (1)[a] (1)[b]	3	27 (3)[a]
San Mateo							
Santa Barbara							1
Santa Clara		2		320			300
Santa Cruz							
Shasta				70		69	
Sierra				16	37		
Siskiyou		8		101	6	83	
Solano		83					
Sonoma				4			55
Stanislaus				2 (1)[a]			
Sutter							
Tehama				90			59 (2)[a]
Trinity	54			73			
Tulare	49 (1)[a]				50		

TABLE 2. Continued.

County	Black bear	Elk	Bighorn sheep	Blacktail deer	Mule deer	Prong-horn	Feral swine
Tuolumne					12		
Ventura				2	47		
Yolo							
Yuba							

[a] reactor; [b] suspect; [c] Angel Island; [d] San Clemente Island.

on an STT at ≥1:25, a positive BAPA and a positive card test, a CF test reaction of 1+ at ≥1:10, or a positive rivanol test at ≥1.25. No suspect category was used for feral swine samples.

RESULTS

One black bear, one blacktail deer, and one California mule deer were considered reactors (Table 3). Three desert bighorn sheep and one California mule deer were considered suspects (Table 3). Six of the reactor and suspect bears and ruminants were adults, one of the suspect bighorn sheep was a juvenile. Both mule deer and one bighorn sheep were females. Prevalence of reactors in black bear, California mule deer, and blacktail deer was 0.6%, 0.3%, and 0.06%, respectively. The prevalence of suspects in desert bighorn sheep and California mule deer was 0.5% and 0.3%, respectively. All bighorn sheep sera tested by ELISA for antibodies to *B. ovis* were negative. No reactor or suspect pronghorn antelope or elk were detected. The locations of counties with reactor animals are shown in Figures 1–6.

Twenty-three of 611 (3.8%) feral swine were considered reactors (Table 4). Subadult animals comprised 40% of the sample population, but accounted for 78% of the reactors. Subadult males comprised 52% of the reactors. Incomplete agglutination reactions to a rivanol, STT, or SPT

FIGURE 5. Distribution of blacktail deer (▨) and location of animals sampled for antibodies to *Brucella* spp. (■) in California by county, 1977 to 1989. ●, counties with reactor animals.

FIGURE 6. Distribution of pronghorn antelope (▨) and location of animals sampled for antibodies to *Brucella* spp. (■) in California by county, 1977 to 1989. ●, counties with reactor animals.

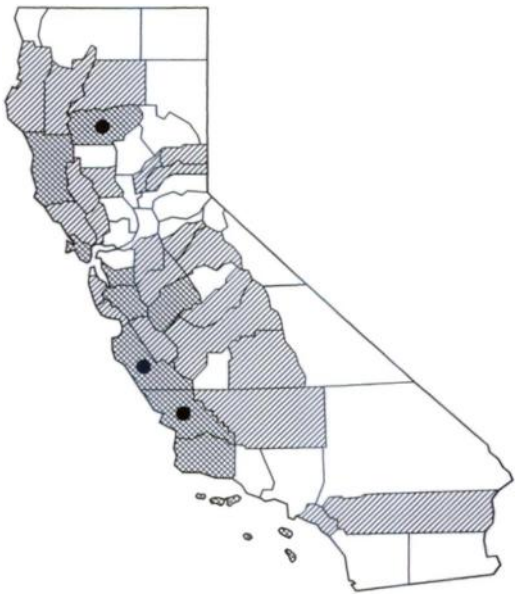

FIGURE 7. Distribution of feral swine (⚀) and location of animals sampled for antibodies to *Brucella* spp. (■) in California by county, 1977 to 1989. ●, counties with reactor animals.

occurred in 56 of 611 (9.2%) samples tested and in 13 of 23 (56.5%) reactors. Over 90% of the reactor swine were from two counties in west central California (Fig. 7).

DISCUSSION

Serologic surveys have been commonly used to determine the prevalence of brucellosis in free-ranging ruminants in North America. Free-ranging ruminants, with the exception of a few bison and elk populations, are considered free of *Brucella abortus* and unimportant in the epidemiology of brucellosis in domestic livestock (Moore and Schnurrenberger, 1981; McCorquodale and DiGiacomo, 1985; Tessaro, 1986). The presence of *B. abortus* in a few free-ranging elk and bison populations has led to land use and animal health conflicts which are still unresolved.

The serologic testing for *Brucella* spp. antibodies reported here utilized standard testing procedures and interpretations for domestic cattle and swine. Antibodies to *B. abortus*, *B. suis*, and *B. melitensis* can be found using standard livestock tests. Antibodies to *B. ovis* do not react on standard tests for *B. abortus*, but can be detected using an ELISA test (Walker et al., 1985). Interpretation of serologic reactions in brucellosis testing in wildlife is unclear due to the possibility of false positive, false negative, and cross reactions from other Gram negative organisms. The immunologic basis for serologic reactions to *Brucella* spp. in cattle (Morgan, 1969) and swine (Deyoe, 1972) are assumed to be applicable to wildlife species although further study is needed. A liberal but stringent interpretation of serologic reactions, such as was used in this survey, would facilitate identification of the largest number of potentially infected animals.

The prevalence of brucellosis in free-ranging ruminants and black bear in California in this survey is very low which is consistent with results of previous reports and reviews for these species (Binninger et al., 1980; Moore and Schnurrenberger, 1981; McCorquodale and DiGiacomo,

TABLE 3. Results of serologic testing for antibodies to *Brucella* spp. in California black bear and ruminants, 1977 to 1989.

	Serologic test used							
		STP						
Species (n)	BAPA	1:25	1:50	1:100	1:200	Rivanol	Card	Status
Black Bear (1)	+	+	+	+	+	+1:25	ND*	Reactor
Mule Deer (1)	+	ND				+1:200	ND	Reactor
Blacktail Deer (1)	+	ND				+1:100	+	Reactor
Bighorn Sheep (3)	ND	+	+			ND	−	Suspect
Mule Deer (1)	+	ND				ND	ND	Suspect

* ND indicates test not performed.

TABLE 4. Results of serologic testing for antibodies to *Brucella* spp. in California feral swine, 1977 to 1989.

Number of samples	Number of tests run	Number of positive test results	Test						Number of reactors
			BAPA	STT	SPT	Card	Rivanol	CF	
326	1	0	−	ND*	ND	ND	ND	ND	0
25	1	1	+	ND	ND	ND	ND	ND	0
59	2	0	−	−	ND	ND	ND	ND	0
10	2	1	+	−	ND	ND	ND	ND	0
1	2	2	+	+	ND	ND	ND	ND	1
8	3	0	−	−	ND	ND	ND	−	0
4	3	1	+	−	ND	ND	ND	−	0
3	3	2	+	+	ND	ND	ND	−	3
8	4	0	−	−	ND	−	−	ND	0
1	4	2	+	+	ND	−	−	ND	1
1	4	3	+	−	ND	+	+	ND	1
1	4	4	+	+	ND	+	+	ND	1
38	5	0	−	−	−	−	−	ND	0
9	5	1	+	−	−	−	−	ND	0
1	5	1	−	−	+	−	−	ND	0
7	5	2	+	−	−	+	−	ND	7
2	5	3	+	+	−	+	−	ND	2
2	5	5	+	+	+	+	+	ND	2
100	6	0	−	−	−	−	−	−	0
2	6	1	−	+	−	−	−	−	2
1	6	2	+	−	−	+	−	−	1
2	6	3	+	−	−	+	−	+	2

* ND = Test not performed.

1985; Tessaro, 1986). Isolated cases of exposure, and possibly infection, may occur, but the disease is not considered important in the demographics of free-ranging ruminant or black bear populations in California. It is unlikely that these species are a reservoir of brucellosis for domestic livestock.

The cause of the serologic reactions in bighorn sheep in this study is unknown. The reactions could be due to exposure to infected cattle or sheep on open range, non-specific cross reactions, or reactions with antibodies against other Gram negative organisms. The negative ELISA results for bighorn sheep in this study may indicate that *B. ovis* is not present in free-ranging bighorns or that the ELISA reagents used are not applicable to bighorn sheep. Scrotal palpation of bighorn sheep rams in future capture and handling operations is suggested. Experimental infections of *B. ovis* and ELISA testing in bighorn sheep is needed to clarify the disease status in free-ranging bighorns.

Prevalence of feral swine reactors in this study was low but similar to that reported by Zygmont et al. (1982) and Corn et al. (1986). Other studies have found higher prevalence (Wood et al., 1976; Becker et al., 1978; Clark et al., 1983). The criteria of Zygmont et al. (1982) for defining reactor swine could not be used for all samples in this survey because of the variety of test batteries used, and only three feral swine would be considered reactors. The more liberal test interpretation used here, especially with the variable test batteries, provided an easily applied classification to indicate the prevalence of brucellosis in feral swine.

The high number of reactor swine in two counties may reflect an area of enzootic feral swine brucellosis or the intensive sample collection efforts in the area. Continued testing of feral swine in this area and intensified testing of feral swine in other areas is needed to better define the distribution of the disease in California.

Brucellosis in feral swine in at least one location in California has several zoonotic and epidemiological implications. The population and distribution of feral swine is growing rapidly. Hunters in California harvest approximately 30,000 to 50,000 feral swine annually (E. T. Loft, pers. comm.) and field dressing of these animals may be a source of *B. suis* to humans. Relocation of infected feral swine could spread the disease to other swine populations in the state. Given the difficulty in interpretation of serologic tests in swine, the eradication of brucellosis may be difficult if the disease becomes widespread in feral swine populations.

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