

AN ABSTRACT OF THE THESIS OF

Jack A. Mortenson, D.V.M. for the degree of Master of Science in Wildlife Science  
presented on November 18, 1998. Title: Serologic Survey of Infectious Disease Agents in  
Black Bears (*Ursus americanus*) of California, Oregon, and Washington.

Abstract approved: \_\_\_\_\_

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The causes of natural mortality and disease in free ranging black bears, *Ursus americanus*, in California, Oregon, and Washington are poorly known. Life history components, such as scavenging and overlapping habitat with many species of carnivores, potentially expose bears to a wide range of infectious disease agents. To date, no disease has been identified that appears to greatly influence black bear population dynamics. The objectives of this study were to determine the prevalence rates of exposure to selected infectious disease agents in black bears at six study sites of California, Oregon, and Washington, and to assess if age, sex, study area, or year of sampling are related to the prevalence of specific diseases.

One hundred and ninety nine black bear serum samples were collected between 1993 and 1997 and tested for selected viral and bacterial disease agents. Antibody prevalence was 0% for bluetongue virus, 12.6% (24/190) for *Borrelia burgdorferi* (Lyme disease), 0% for *Brucella* spp., 0% for *Dirofilaria immitis* (heartworm), 4.8% (8/165) for canine distemper virus, 4.5% (9/198) for *Ehrlichia equi*, 0% for epizootic hemorrhagic disease virus, 9% (8/88) for *Francisella tularensis* (tularemia), 1.8% (3/165) for canine infectious hepatitis virus, 2.5% (5/198) for *Trichinella spiralis*, 45% (89/198) for *Toxoplasma gondii* and 5.5% (11/198) for *Yersinia pestis* (plague). Prevalence differences were observed among study sites. Lyme disease and plague antibodies were detected only in black bears from California and Oregon. *E. equi* antibody detection was highest from California bears. This is the first report of *E. equi* in the Ursidae family, and the first report of morbillivirus in black bears.

These data do not support the relationship reported in other studies of rising prevalence rates with increased age of bears. The potential implications of diseases transmitted by translocated bears or re-introduced sympatric carnivores should be considered before management decisions are made.

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**Serologic Survey of Infectious Disease Agents in Black Bears  
(*Ursus americanus*) of California, Oregon, and Washington**

by

**Jack A. Mortenson, D.V.M.**

**A THESIS**

**submitted to**

**Oregon State University**

**in partial fulfillment of  
the requirements for the  
degree of**

**Master of Science**

**Presented November 18, 1998  
Commencement June 1999**

Master of Science thesis of Jack A. Mortenson, D.V.M. presented on November 18, 1998

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## ACKNOWLEDGMENTS

Financial support making this research possible was provided by Oregon Department of Fish and Wildlife Project 11900 and Federal Aid Project W-90-R3 through Dr. DeWaine Jackson's efforts. I would like to thank Wildlife Safari and Jim Phelps for financial assistance for my graduate studies during the 1996-1997 academic year.

Providing guidance, criticism, and pointing out early in my graduate work "to build on what you already know", I thank my major professor, Erik Fritzell, for his help and commitment to me.

This project would not have been possible without the work of the field biologists that collected the majority of blood samples. Dave Immel providing the majority of the Oregon samples, Tim Burton in California, and Gary Koehler in Washington. A special thanks to Dave Immel for his interest in bear diseases and his patience with my many questions.

Many thanks are owed to both Dr. Bruno Chomel for the serological analysis and Dr. Emilio DeBess for assistance in the statistical analysis. They both shared freely with me their knowledge and time.

A special thanks to Richard Green for his help in retrieving and sending samples from the Oregon Department of Fish and Wildlife Laboratory- Corvallis, Rocky Baker from Oregon State University, College of Veterinary Medicine Diagnostic Laboratory for advice on serology testing, and Bob Gilman for providing trapped bears for necropsies.

And to my family, Angel and Andrea, thank you for allowing me to put my love for learning ahead of family time during my graduate studies.

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# **Serologic Survey of Infectious Disease Agents in Black Bears (*Ursus americanus*) of California, Oregon, and Washington**

## **Chapter 1 Introduction**

Diseases impair normal body functions of animals and have direct and indirect influences on the distribution, longevity, and reproductive success of individuals and populations of animals. Diseases originate from both infectious agents such as viruses, bacteria, and parasites and non-infectious sources such as toxins, trauma, and nutrition. Disease-causing agents are normal components of every ecosystem in which wild mammals live, move and reproduce. In only the rarest of instances are individuals found not to harbor some kind of parasitic or infectious organisms. But, in most cases these agents have little obvious effect on their hosts. The impact of disease agents may be variable depending on the mammal involved, the nature of the agent, and the environment in which the interaction takes place (Yuill, 1987). That is, diseases are a part of a spectrum of factors such as food habits, population dynamics, and habitat requirements that affect the management puzzle of wildlife populations (Bolen and Robinson, 1995).

Infection resulting in acute mortality is difficult to detect in wild animal populations. Despite the large number of diseases that are known to affect animals, many ecologists have not recognized disease as a significance factor affecting natural populations of wild animals (Scott, 1988). When infection influences the reproduction of the host or reduces the average life span, but does not cause acute mortality, it is difficult to detect in a host population without a detailed demographic and epidemiology study, which compares features among a variety of populations. However, the limits of disease detection should not be interpreted as a lack of disease in wild animal populations (Scott, 1988).

Diseases and pathogens are receiving increasing recognition as sources of mortality in animal populations (Loehle, 1995) and as regulators of population dynamics in wildlife. For example, diseases of wildlife have significant management implications in some National Parks due to increasing interactions between wildlife and domestic animals (Aguirre and Starkey, 1994). Both types of animals may serve as reservoirs, or as vectors for pathogens that ultimately affect wildlife and domestic populations, plus humans (Bolen and Robinson, 1995).

Exposure to infectious diseases and the role of diseases in the population ecology of the American black bear (*Ursus americanus*) are poorly known. There are few published studies on infectious diseases in black bears, and any epidemiological role bears may serve to other wildlife, domestic animals, and humans is unknown. Causes of natural mortality and disease in free-ranging black bears remain relatively unknown (Binninger et al., 1980).

The black bear's life history characteristics such as food selection, distribution, and habitat use, potentially expose them to a wide range of infectious disease agents. Black bears eat a variety of plant and animal matter (Pelton, 1982), because they are both scavengers and predators. Berries, grasses, tubers, small mammals, fish and insects typically make up the bulk of the diet, but for some individual bears, animal carcasses and human refuse often may be consumed.

The distribution of black bears in California, Oregon and Washington is relatively contiguous from the east slopes of the Cascade Range and the Sierra Nevada to the coast in all three states with the exceptions of the major central valleys (Verts and Carraway, 1998). The eastern portions of all three states have bear populations in forested habitats at higher elevations. These populations are mainly in the northeast corners of northern California and Oregon, and the northeast and southeast corners of Washington. Black bear habitat in northern California, Oregon, and Washington overlaps with that of many other carnivores including: coyotes (*Canis latrans*), cougars (*Felis concolor*), bobcats (*Lynx rufus*), gray fox (*Urocyon cinereoargenteus*), red fox (*Vulpes vulpes*), raccoon (*Procyon lotor*), *Mustelidae* species, and domestic dogs and cats. In addition, bears inhabit areas where domestic livestock such as cattle and sheep are common.

Examples of diseases to which bears are exposed include, but are not limited to: toxoplasmosis, tuberculosis, Q-fever, plague, leptospirosis, trichinosis, infectious canine hepatitis, rabies, tularemia, Lyme disease, brucellosis, and heartworm disease, plus a wide variety of parasitic infections. Although many diseases have been reported for black bears, none appear to contribute greatly to the natural regulation of black bear populations (Pelton, 1982). Bear populations may be increasing in California, Oregon, and Washington, due to declining hunter-harvest and abundant habitat. Expanding populations can increase the likelihood of infectious diseases, thus becoming significant sources of morbidity and mortality. For expanding populations in other species, disease can be an effective regulatory mechanism (Loehle, 1995).

Seroepidemiologic surveys of wildlife populations have been used widely to identify the occurrence of pathogens in wildlife, and to anticipate the risk of exposure to humans and domestic animals to specific pathogens (Stauber et al., 1980). Although few serological surveys of black bears in the Pacific Northwest have been done, several researchers outside this region have described the prevalence rates of infectious disease agents within sampled populations. By surveying black bear populations in the above three states, we may gain a better understanding of the diseases to which they are exposed. This understanding may have increased value through time as black bear populations change, or are exposed to new environmental conditions. The selection of infectious agents assessed was based on known exposure in other black bear populations, requests from Oregon Department of Fish and Wildlife biologists, and discussions with wildlife veterinarians regarding emerging and newly recognized diseases.

The objectives of this study were (1) to determine the prevalence rates of exposure to selected infectious disease agents in black bears at six study areas of California, Oregon, and Washington; (2) to determine if age, study area, year of sampling, or sex of the bears were related to the prevalence of specific diseases; and (3) to determine if pathological changes associated with specific diseases could be detected upon necropsy.

This thesis is written in a format to facilitate submission for publication. The remainder of the inductive chapter contains a summary of findings of previous black bear disease studies and a general description of each disease and infectious agent surveyed for in this study. The second chapter is a manuscript written to conform to the Journal of Wildlife Diseases requirements. Separate chapters follow for management implications, bibliography, and appendices.

### **Literature Review**

Seventeen studies of diseases in North American black bear populations have been published. Diseases which are known to infect black bears and have been reported at a low prevalence rates (<15.0%) include: brucellosis, Rocky Mountain spotted fever, St. Louis encephalitis, western equine encephalitis, trichinosis, infectious canine hepatitis, and botulism. Higher prevalence (15-20%) has been reported for tularemia, Lyme disease, Q-fever, and leptospirosis with up to an 80% prevalence for toxoplasmosis. Although there was no relationship between age and sex with disease prevalence rates in most studies, it was noted in two studies that adult male black bears have higher titers for toxoplasmosis. Studies summarized below have demonstrated exposure to infectious disease agents in black bear populations in the following states and two Canadian provinces, see also Appendix 1.

#### Alaska

Chomel et al., (1995) reported a prevalence rate of 15% for antibodies against *Toxoplasma gondii* in 40 black bears. There was no significant association noted for infection with age or sex.

### California

Approximately 150 bears were screened for six zoonotic diseases in three counties by Ruppanner et al., (1982). Twenty-seven percent were positive for *Toxoplasma gondii*; 17% had antibodies against *Coxiella burnetii* (Q-fever); 13% were seropositive for *Trichinella spiralis*; 15% had antibodies against *Yersinia pestis* (plague); only 2% had antibodies against *Clostridium botulinum*, with 16% testing positive for *Leptospira interrogans* serovars. Thirty-six percent of 69 black bears were seropositive for *Yersinia pestis* (plague) reported by Clover et al., (1989). Smith et al., (1984) reported 23% of 203 black bears taken from seven counties were seropositive for plague. Drew et al., (1992) tested 180 black bears from 6 counties for *Brucella* spp. with one positive result.

### Idaho

Binninger et al., (1980) reported positive serologic results for toxoplasmosis at a prevalence of 8% out of 256 bears. It was speculated that higher prevalence in older bears was due to longer exposure times and highest in males because of extensive home ranges. Other prevalence rates reported were as follows: 19% tularemia, 5% brucellosis, 1% leptospirosis, 13% trichinosis, 6% Q-fever, 1% St. Louis encephalitis, 1% western equine encephalitis, and 2% Rocky Mountain Spotted Fever.

### Montana/Wyoming

Worley et al., (1974) reported a 11.9% prevalence of *Trichinella spiralis* in black bears collected in Glacier and Yellowstone National Parks.

### Pennsylvania

Of 2056 hunter-harvested black bears, 1.8% were infected with *Trichinella spiralis* (Schad et al., 1986). Infected bears ranged from < 1 year of age to fourteen years of age; no sex, age, or weight related differences were observed. Briscoe et al., (1993) reported a 80% prevalence rate for *Toxoplasma gondii* out of 665 hunter-harvested black bears.

No significant difference was found between sexes, but a higher infection rate was noted in adult bears. Dubey et al., (1995) reported a 78.6% prevalence rate of *T. gondii* in 28 black bears with a higher prevalence rate in adults.

#### Wisconsin

Kazmierczak et al., (1988) cultured *Borrelia burgdorferi* (Lyme disease) from 3 out of 18 hunter-harvested black bears, the first report within the Ursidae family.

#### Washington

One of 33 black bears in northeastern Washington had a positive antibody titer to canine adenovirus type 1(CAV-1)(Foreyt et al., 1986). Clinical infections of canine infectious hepatitis (CAV-1) have not been reported in wild bears, but reports (Pursell et al., 1983; Collins et al., 1984) indicate that captive black bears are susceptible to lethal infections.

#### Newfoundland, Canada

One of 158 black bears tested for *Trichinella spiralis* larvae was positive (Butler and Khan, 1992).

#### Ontario, Canada

Thirteen black bears were found to be free of *Trichinella spiralis* larvae in a study surveying many wildlife species (Dick et al., 1986). The authors suggested the epizootiology of trichinosis in wildlife is complex and may depend on strain differences in the parasite. Addison et al., (1979) reported a prevalence of 1.7% of trichinosis in 59 black bears in Algonquin Park, Ontario.



## **Disease Descriptions**

The following is a brief review of each disease tested for in this study. Unless specifically cited, the general information contained here is from the following: (Appel, 1987; Davis et al., 1981; Fraser et al., 1986.)

### Bluetongue/Epizootic Hemorrhagic Disease

Bluetongue (BLU) and epizootic hemorrhagic disease (EHD) are closely related infectious, often fatal, viral diseases of wild and domestic ruminants (Hoff and Trainer, 1981). The viruses belong to Reoviridae family (subgroup Orbiviruses) and are insect transmitted. The disease is common in tropical, subtropical, and some temperate regions of the world. The consequences of the virus infection differ among ruminant hosts. The exact distribution of BLU and EHD in the United States is difficult to determine. Generally, EHD is found east of the Mississippi River. BLU is seen more in the western states, but scattered outbreaks have occurred in the eastern states. The virus is probably endemic in the southeastern states. Clinical signs include fever, edema of the head, hyperemia of the oral region, ulcerations of the tongue and dental pad, lameness, and bloody diarrhea. The role of non-artiodactyl wildlife in the epizootiology of EHD and BLU has not been determined (Hoff and Trainer, 1978). Serological surveys have been conducted among North American ungulates, but none have been reported for carnivores.

Domestic dogs are susceptible to BLU with mortality and abortion as suggested following administration of a vaccine that was BLU contaminated (Evermann et al., 1994; Wilbur et al., 1994; Akita et al., 1994) and later documented experimentally (Brown et al., 1996). Serological titers have been detected in African carnivores (Alexander et al., 1994), but no naturally occurring clinical cases have been documented.

### Brucellosis

Cattle, swine, goats, and dogs are affected by bacteria of the genus *Brucella* and characterized by abortion in the female, orchitis in the male, and infertility in both sexes (Fraser et al., 1986). The disease has been described in a wide range of wildlife including ungulates, carnivores and smaller mammal species, and is present throughout the world. Transmission is congenital, venereal or by ingestion of infective materials. All ages and both sexes appear to be equally susceptible.

### Canine Distemper

Canine distemper in dogs and other carnivores has been known worldwide for centuries, with the virus first isolated by Carre in 1905 (Appel and Summers, 1995). The virus is a member of the Paramyxoviridae family and is part of the morbillivirus genus. Many different genera of Carnivora are susceptible to canine distemper with varying rates of mortality: *Ailuridae*, *Canidae*, *Hyaenidae*, *Mustelidae*, *Procyonidae*, *Ursidae*, *Viverridae*, and *Felidae* (Montali et al., 1987).

Canine distemper is mainly transmitted via aerosolized respiratory secretions, directly from animal to animal, and is a highly contagious disease with the virus rapidly inactivated outside the host. Clinical signs involve the respiratory, gastro-intestinal, and central nervous systems. The virus can affect animals of all ages; however, young carnivores are most susceptible. Immunity appears to be long lasting and potentially lifelong with no persistent shedding of the virus.

### Ehrlichiosis

Several species of the genus *Ehrlichia* cause clinical and subclinical infections. These bacteria are obligate intracellular rickettsial organisms that parasitize circulating leukocytes or thrombocytes of the host animal. Host species currently recognized include the dog, horse, and humans. Although ehrlichial diseases were once considered rare in the United States, this is no longer true. Diagnoses of ehrlichial diseases are being made with increasing frequency in part because of improving diagnostic tests, expanding geographic occurrence, and increasing clinical awareness (Woody and Hoskins, 1991).

Disease caused by *Ehrlichia canis* was first recognized in the United States in 1962. By 1980, the disease had been reported in 10 states, and by 1982, there was serologic evidence of the disease in 34 states (Woody and Hoskins, 1991).

*Ehrlichia equi* is thought to be transmitted to dogs primarily by ticks. Transmission by direct blood inoculation is possible. The vector, reservoir, incubation period, and pathogenesis of *Ehrlichia equi* infection in the dog are unknown but are assumed to be similar to *Ehrlichia canis*. Clinical signs include thrombocytopenia, anemia, lameness, and generalized lymphadenomegaly.

### Heartworm

A clinical or subclinical disease caused by the filarial worm, *Dirofilaria immitis*, with adults primarily occurring in the right ventricle and pulmonary artery. Micro-filariae usually are in the blood. The disease has a cosmopolitan distribution at sea level in the tropics and subtropics. In North America, it once appeared to be endemic mainly in the Southeastern United States. It is now common in wide areas of the Atlantic states, the Midwest, and Canada where high mosquito densities occur. It is common on the West Coast of the United States, and has been reported in northern California and southern Oregon. Clinical signs, including gradual weight loss, coughing, and decreased exercise tolerance, are related to endarteritis and thromboembolization from live and dead adult heartworms in the pulmonary arteries. Periodic acute inflammation and fibrosis obstruct and mechanically interfere with blood flow, resulting in pulmonary hypertension and secondary right-heart enlargement. Naturally occurring heartworm infections have been described in foxes (Hubert et al., 1980; Simmons et al., 1980; Wixsom et al., 1991), raccoons (Fox, 1941; Hubert et al., 1980; Snyder et al., 1989), wolverines (*Gulo gulo*) (Williams, 1976), coyotes (Agostine and Jones, 1982; Wixsom et al., 1991), and black bears (Johnson, 1975; Crum et al., 1978).

### Infectious Canine Hepatitis

This disease is caused by canine adenovirus Type 1 (CAV-1), belonging to the Adenoviridae family. The disease was formerly known as “epizootic fox encephalitis” (Green et al., 1930), but Rubarth (1947) suggested that the two diseases seen in foxes and dogs had the same causative agent that was later cultured by Cabasso et al., (1954). Infection with CAV-1 occurs worldwide in the Canidae family. Other carnivores are reported to be susceptible to infection, including raccoons, skunks, and bears. Endemic disease outbreaks have been reported among bears (Green and Stulberg, 1947; Kapp and Lehoczki, 1966).

Transmission of CAV-1 is by direct contact, ingestion of urine, feces, or saliva from infected animals. Because CAV-1 can survive for several weeks at either 20°C or frozen, infected urine probably is the most important source of virus for transmission (Poppensiek and Baker, 1951). Clinical signs include fever, conjunctivitis, jaundice, ocular lesions and acute death and affect animals of all ages with mortality being the highest in neonates. In dogs that have recovered from clinical disease, virus can be shed in the urine for at least 6 months, possibly a year, after infection.

### Lyme

A spirochete, *Borrelia burgdorferi* (Burgdorfer et al., 1982), causes Lyme disease, which is transmitted by ticks of the *Ixodes* family. The species responsible for the most Lyme disease transmission in North America is *Ixodes scapularis*, which serves as a vector primarily in the northeastern and northcentral United States. The main vector in the West is the western black-legged tick, *I. pacificus* (Lane et al., 1991). The white-footed mouse, *Peromyscus leucopus*, appears to be the most important reservoir host in the northeastern United States (Donohue et al., 1987). Norway rats (*Rattus norvegicus*) and meadow voles (*Microtus pennsylvanicus*) can apparently serve as reservoirs in the absence of *P. leucopus* (Smith et al., 1993). *Borreliae* have been isolated from several additional mammal species, but reservoir competence has been studied in only a few (Mather, 1993).

Clinical signs include fever, lameness, cutaneous lesions, and lymphadenopathy. Animals infected with Lyme spirochetes are frequently asymptomatic, even in species that often show symptoms (Wright and Nielson, 1990).

### Plague

This zoonotic, highly infectious disease is caused by the bacteria *Yersinia pestis*. Wild rodents are primarily affected and vary considerably in their susceptibility. Fleas are the main transmission vector, feeding and regurgitating the bacteria on hosts. This organism occurs in Asia, South America, Africa, Europe and in North America from the Pacific Ocean east to Kansas and Texas, and also in parts of Mexico and Canada.

In the western states, sylvatic (wild) plague is generally maintained in desert and grassland communities; however, there is a great deal of interaction between the host and the environment in determining the distribution. Clinical signs include swollen lymph glands, fever, internal hemorrhaging, and pneumonia. Past research has suggested that wild carnivores and omnivores may serve as sentinels to aid in identifying the geographical and the temporal distribution of sylvatic plague by ingesting plague-infected prey or carrion (Barnes, 1982).

### Toxoplasmosis

*Toxoplasma gondii* is an intracellular parasitic protozoan which belongs to the subphylum Sporozoa. It has worldwide distribution and infects a wide variety of mammals, birds and reptiles. However, it frequently is found in the absence of any recognizable disease, and often exposure to it can be detected only by positive serologic reactions (Sanger, 1971).

Severe clinical infection has been seen in a variety of non-domestic animals (Lappin et al., 1991). Domestic and free-ranging felids are the only known definitive hosts (Zarnke et al., 1997), and shed oocysts in feces following the completion of an enteroepithelial cycle.

Oral-fecal infection of other hosts such as bears can occur following exposure to plants contaminated by *Toxoplasma* oocysts derived from cat feces, or infected carcasses from a wide range of intermediate hosts. Clinical signs involve the respiratory, reproductive and central nervous systems. Encysted *Toxoplasma* can survive for years in the skeletal muscle of these animals and cause an acute *T. gondii* infection when the infected meat is consumed by humans or wildlife (Dubey and Beattie, 1988).

### Trichinosis

The nematode parasite *Trichinella spiralis* is infectious to all mammals and is distributed worldwide. Transmission occurs by ingestion of carcass meat that contains encysted larvae. *Trichinella* spp. can only be transmitted by ingestion of infected muscle tissue from another host (Bailey and Schantz, 1990). Therefore, strict carnivorous species generally have a higher prevalence than omnivorous species (Franchimont et al., 1993). The larvae undergo a reproductive stage within the intestinal mucosa and release larvae, which migrate to skeletal muscle to encyst. Larvae remain viable in the cysts for years and will continue their development when ingested by another suitable host. Clinical signs are associated with the gastrointestinal and skeletal muscle systems. This disease has public health significance because of bear meat consumption by humans.

### Tularemia

*Francisella tularensis* is the causative bacteria of this plague-like disease. It is seen in North America, Middle East, Asia and Europe, with the main hosts being wild lagomorphs and rodents. The organism can be transmitted by direct contact, contact by environmental contamination, or by a range of ectoparasites. Clinical signs are not commonly seen, but can include stupor, muscle spasms and anorexia. Dead and moribund animals are more typically found. Pathological changes are related to a generalized septicemia affecting the lungs, liver and other organs with caseous necrosis present. This disease is of zoonotic concern to biologists, trappers and hunters who have direct exposure to the organism or bear ectoparasites.

## Chapter 2

### Serologic Survey of Infectious Disease Agents in Black Bears (*Ursus americanus*) of California, Oregon, and Washington

#### Introduction

Exposure to infectious diseases and the role of diseases in the population ecology of the black bear (*Ursus americanus*) are poorly known. There are few published studies on infectious diseases in black bears, and any epidemiological role bears may serve to other wildlife, domestic animals, and humans is unknown. Causes of natural mortality and disease in free-ranging American black bears remain relatively unknown (Binninger et al., 1980).

The black bear's life history characteristics, such as food selection, distribution, and habitat use, potentially expose them to a wide range of infectious disease agents. Black bears eat a variety of plant and animal matter (Pelton, 1982), because they are both scavengers and predators. Berries, grasses, tubers, small mammals, fish and insects typically make up the bulk of the diet, but for some individual bears, animal carcasses and human refuse often may be consumed.

The distribution of black bears in California, Oregon and Washington is relatively contiguous from the east slopes of the Cascade Range and the Sierra Nevada to the coast in all three states with the exceptions of the major central valleys (Verts and Carraway, 1998). The eastern portions of all three states have bear populations in forested habitats at higher elevations. These populations are mainly in the northeast corners of northern California and Oregon, and the northeast and southeast corners of Washington. Black bear habitat in northern California, Oregon, and Washington overlaps with that of many other carnivores including: coyotes (*Canis latrans*), cougars (*Felis concolor*), bobcats (*Lynx rufus*), gray fox (*Urocyon cinereoargenteus*), red fox (*Vulpes vulpes*), raccoon (*Procyon lotor*), *Mustelidae* species, and domestic dogs and cats. In addition, bears inhabit areas where domestic livestock such as cattle and sheep are common.

Examples of diseases to which bears are exposed include, but are not limited to, toxoplasmosis, tuberculosis, Q-fever, plague, leptospirosis, trichinosis, infectious canine hepatitis, rabies, tularemia, Lyme disease, brucellosis, and heartworm disease, plus a wide variety of parasitic infections.

Although many diseases have been reported for black bears, none appear to contribute greatly to the natural regulation of black bear populations (Pelton, 1982). Bear populations may be increasing in California, Oregon, and Washington, due to declining hunter harvest and abundant habitat. Expanding populations can increase the likelihood of infectious diseases becoming significant sources of morbidity and mortality. For expanding populations, disease can be an effective regulatory mechanism in some species (Loehle, 1995).

Seroepidemiologic surveys of wildlife populations have been used widely to identify the occurrence of known pathogens in wildlife, and to anticipate the risk of exposure to humans and domestic animals to specific pathogens (Stauber et al., 1980). Although few serological surveys of black bears in the Pacific Northwest have been done, several previous studies in other states have described the prevalence rates of infectious disease agents within sampled populations. By surveying black bear populations in the above three states, we may gain a better understanding of the diseases to which they are exposed. This understanding may have increased value through time as black bear populations change, or are exposed to new environmental conditions. The selection of infectious agents assessed was based on known exposure in other black bear populations, and requests and discussions with wildlife veterinarians and biologists regarding emerging and newly recognized diseases.

The objectives of this study were to determine (1) the prevalence rates of exposure to selected infectious disease agents in black bears at six study areas of California, Oregon, and Washington; and (2) if age, study area, year of sampling, or sex of the bears were associated to the prevalence of specific diseases.



## Methods

Whole blood samples were collected from black bears from six different regions in California, Oregon and Washington (Figure 1). All bears were leg snared and anesthetized to place radio collars on them, except at one site in which bears were legally harvested. Samples from California were collected by California Department of Fish and Game personnel in 1993-1997 from the Shasta National Forest (SHA) (n = 10) and Klamath National Forest (KLA) (n = 37) sites during bear population ecology studies. Oregon samples were collected by two different methods. The Willamette National Forest (WIL) site (n = 93) samples were obtained by Oregon Department of Fish and Wildlife biologists in 1993-1997 during bear population ecology studies. Samples from the Coast Range (COA) site (n = 9) came from damage-causing bears killed by private trappers in 1997, and were obtained at the time of necropsy. Blood samples from the two sites in Washington, Olympic National Park (OLY) (n = 23) and Snoqualmie National Forest (SNO) (n = 27), were obtained in 1996-1997 by Washington Department of Fish and Wildlife biologists during bear population ecology studies.

Blood samples were collected by venipuncture and serum was separated by centrifugation within 24 hours (Appendix 2). Serum was stored for up to 2 years at -20°C, and shipped frozen overnight to laboratories for analysis. A premolar tooth was extracted from 131 (85 males, 46 females) immobilized bears to estimate age by examining cementum annuli (Stoneberg and Jonkel, 1960) by Gary Matson's Laboratory, Milltown, MT.

Diseases, organisms causing them, and test type are listed in Table 1. All serologic tests were performed at the School of Veterinary Medicine (SVM), University of California, Davis, California except the following: heartworm was done at the School of Medicine, University of California, Davis, California; canine distemper (serum neutralization) and infectious canine hepatitis tests on samples from 1993-1995 were done at the Rhone-Merieux Laboratory (RML), Lyon, France; canine distemper (ELISA) and infectious canine hepatitis tests on samples from 1996-1997 were done at the Washington Animal Disease Diagnostic Laboratory (WADDL), Pullman, Washington.

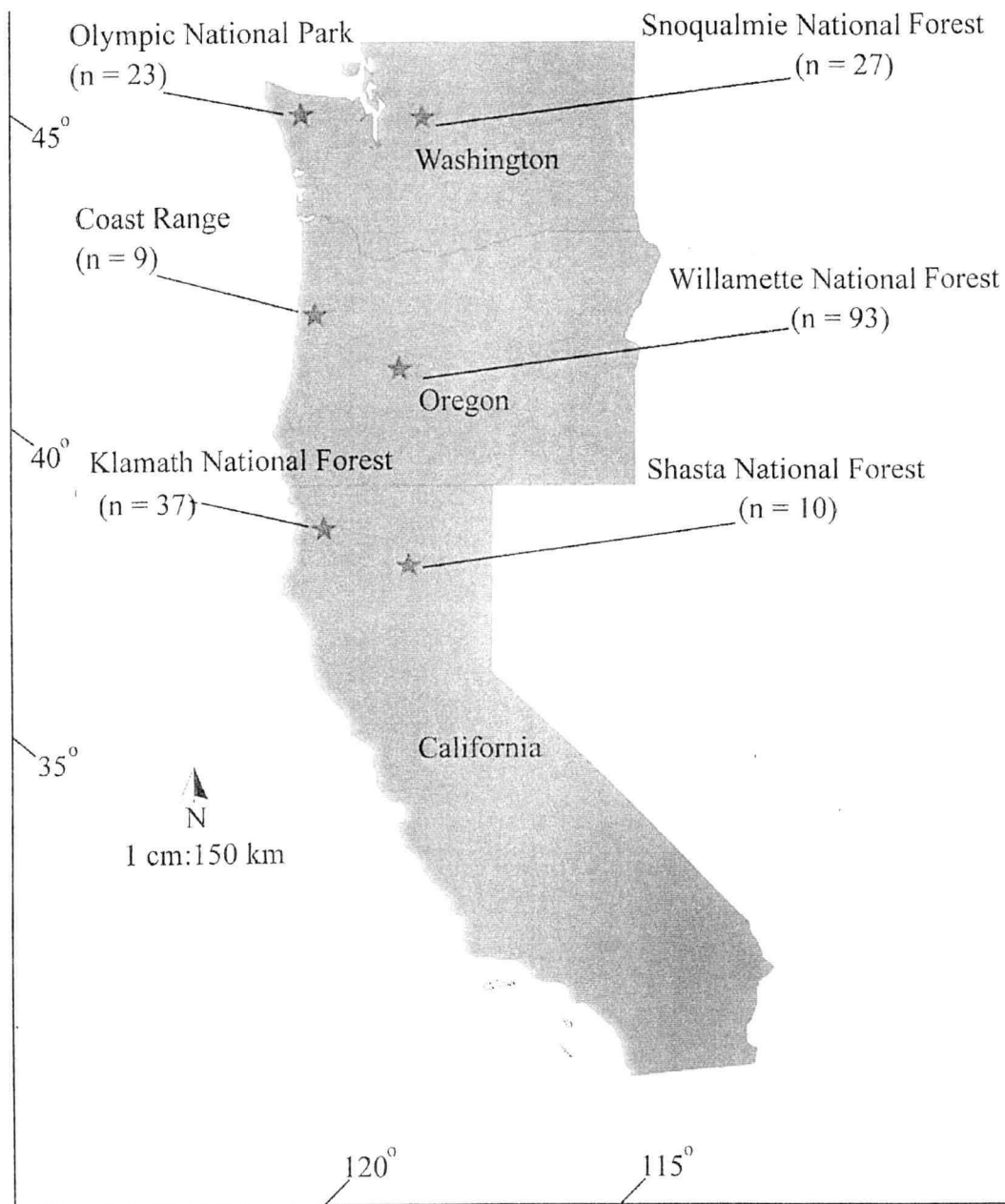


Figure 1. Locations of study sites in California, Oregon and Washington, 1993-1997.

Table 1. Disease name, organism tested for, and type of test used on black bear serum samples from California, Oregon and Washington, 1993-1997.

<u>Disease</u>	<u>Organism</u>	<u>Test</u>
Bluetongue	<i>Reoviridal/Orbiviruses</i>	Competitive ELISA
Brucellosis	<i>Brucella abortus</i>	Brucellosis card test
Distemper	<i>Morbillivirus</i>	ELISA/serum neutralization
Ehrlichiosis	<i>Ehrlichia equi</i>	Immunofluorescent assay
Epizootic Hemorrhagic Disease	<i>Reoviridal/Orbiviruses</i>	Agar immunodiffusion test
Heartworm	<i>Dirofilaria immitis</i>	ELISA
Infectious Canine Hepatitis	<i>Adenovirus (CAV-1)</i>	Serum neutralization
Lyme	<i>Borrelia burgdorferi</i>	Western blots
Plague	<i>Yersinia pestis</i>	Passive hemagglutinin
Toxoplasmosis	<i>Toxoplasma gondii</i>	Latex agglutination
Trichinosis	<i>Trichinella spiralis</i>	ELISA
Tularemia	<i>Francisella tularensis</i>	Slide agglutination

Each serum sample was tested for all of the 12 disease agents except in the following cases: Bluetongue/EHD were tested for in 37 and 40 sera respectively as a random survey of the six study sites to determine if any positive samples were present; tularemia was tested for in just the 88 samples from 1993-1995; 35 samples were not tested for canine distemper and infectious canine hepatitis due to limited sera volume. Laboratory accuracy was subjectively tested by assessing *Toxoplasma gondii* titers by several different methods. Four serum samples were split and submitted to both SVM and WADDL in 1997 and 1998 for comparison. Two serum samples were diluted with sterile phosphate buffer saline, 0.1 M solution, to 50% and 25% concentrations and assessed for serial dilution of the titer results. Three serum samples were each split and submitted as six separate samples.

Statistical analyses to determine associations between overall antibody prevalence and regions, age, sex and years of sample collection were done using Epi Info software, version 6.02 (Dean et al., 1994). Frequency distributions were obtained and chi-square statistics using the Yate's correction factor (Ott, 1993) for 2 X 2 contingency tables and stratified analyses were calculated to obtain measures of association, and statistical significance of such associations. Sampled bears were placed in age groups of two year intervals to assess relationships of age to prevalence of disease.

Necropsies were performed on 10 bears obtained from private animal damage control trappers in the spring and summer of 1997. These bears were killed in the northern coastal range of Oregon, and were transported to the Oregon State University Veterinary College, Corvallis, Oregon within eight hours of death. Gross necropsies, histopathology on general tissues (Appendix 3), and gastrointestinal parasitology were done on each bear, with immunofluorescent assay (IFA) testing for rabies on 6 bears. Three bears were not tested for rabies due to extensive brain trauma secondary to euthanasia.

### Bluetongue - *Reovirus/Orbivirus*

A commercially available competitive ELISA test was used (Blueplate special, DiaXotics, Inc., Wilton, Connecticut). A positive titer was considered 1:64 or greater.

### Brucellosis - *Brucella abortus*

The buffered acidified card antigen test was used (Veterinary Services, Animal and Plant Health Inspection Services, U.S.D.A.) (Alton et al., 1975; Angus and Barton, 1984). Any positive serum was run a second time, to prevent any non-specific reactions and to verify test specificity.

### Distemper - *Morbillivirus*

Competitive ELISA: One-hundred microliters (ml) per well of capture monoclonal antibodies, at a 1:1500 dilution in carbonate bicarbonate buffer, pH 9.6, were bound to 96-well flat bottom microtitration plates by overnight incubation at room temperature. On cell culture plates, 50 ml of distemper virus and 50 ml of each serum dilution (0.9, 1.8 and 2.7) and respective controls were incubated and shaken for one hour (hr) at 37 °C. After three washes of the ELISA plates, 50 ml of the virus-serum mix were transferred on the monoclonal antibody sensitized plates then incubated and shaken for one hr at 37° C.

Then 50 ml of the monoclonal antibody marked with peroxydase were added in each well and the plates shaken and incubated for one hr at 37°C. The plates were washed three times before 100 ml of substrate, orthphenylene diamine, were added. The reaction was stopped after 25 min. with 50 ml of 2.5 Molar H<sub>2</sub>SO<sub>4</sub> solution. Microtitration plates are read at 490 nm wavelength. Results were expressed as optical density (O.D.) percent compare to the control without serum (100%). Serum titers were given as log of the inverse of the dilution with a 50% O.D.

To validate the ELISA test and define the cut-off point for seropositivity, 23 serum samples of bears with an ELISA titer of 0.8 or greater were also tested by the serum neutralization test (Appel and Robson, 1973). Titers by serum neutralization were usually lower than by ELISA, which led to defining the cut off point at 1.0 or greater.

Serum neutralization: Serum was heat inactivated then incubated with the Rockborn strain of canine distemper virus for 60 min. at 25<sup>0</sup>C. At the end of the incubation period, kidney cells were added to microtiter plates and incubated for 5 days. Titers of 1:5 or greater were considered positive (Follmann et al., 1996).

#### Ehrlichiosis- *Ehrlichia equi*

Immunoflorescent assay techniques were used as described by Barlough et al. (1996), except that the was goat anti-canine IgG (whole molecule) conjugate was used at a 1:100 dilution. A positive titer was considered 1:10 or higher.

#### Epizootic Hemorrhagic Disease - *Reovirus/Orbivirus*

An agar immunodiffusion test was performed in 96-well tissue culture microtitration plates using Vero-M or BHK cell line cultures using 400 to 600 median tissue culture infective doses (TCID<sub>50</sub>) of each virus per 0.1 ml. The test serum was heat inactivated and screened at a final dilution of 1:10 against each of the EHD types. The virus-serum mixtures were incubated for 1 hr. at 37°C, cell suspension was added, and the plates were incubated in a CO<sub>2</sub> incubator. A serum was classified as positive if at least 75% of the cell sheet remained intact.

#### Heartworm - *Dirofilaria immitis*

An ELISA test was used and run against the gamma heartworm antigen. This is a commercial test made by Synbiotix, San Diego, California (Dirocheck ELISA).

#### Infectious Canine Hepatitis - *Adenovirus* - (CAV-1)

A microtiter serum neutralization test was used to evaluate bear sera for CAV-1 neutralizing antibodies. Standard laboratory procedures as previously described by Carmichael et al. (1963) were followed using canine adenovirus type-2 and the Mandin-Darby canine kidney cell line (MDCK) (100,000 cells per ml.). Cytopathic effect on culture plates was read seven days after infection, and titers were expressed in log<sub>10</sub> protective dose 50% on MDCK.

If 1 or more virus strains were neutralized by a 1:10 dilution of the test serum, a serum titration was performed against each strain that reacted to confirm the serotyping and establish the end-point titer of the serum (Person, et al. 1984).

#### Lyme disease - *Borrelia burgdorferi*

Initial western blotting was based on the *Borrelia burgdorferi* SON 188 whole cell lysate. A culture of *Borrelia burgdorferi* SON 188 was washed in phosphate buffered saline supplemented with 5 mM MgCl<sub>2</sub>. The amount of protein in the culture was quantitated with the bicinchoninic acid assay (Pierce, Rockford, IL). The spirochetes were lysed in SDS-PAGE sample buffer (62.5 mM Tris, 2% SDS, 10% glycerol, 5% 2-mercaptoethanol [pH 6.8]), and 70 mg of protein were loaded into a curtain-comb 10% acrylamide gel. The proteins were separated by electrophoresis and transferred to a nitrocellulose membrane. The membrane was blocked with Blotto (50 mM Tris, 150 mM NaCl, 0.05% Tween 20, and 5% non-fat dry milk) and cut into strips for blotting. Then, 300 ml of a 1:100 dilution (in Blotto) of each serum sample were added to each strip of membrane. The incubation period was approximately 12 hr. The remainder of the protocol mirrors that of Maniatis et al., (1989). The secondary antibody, raccoon anti-bear conjugated to horseradish peroxidase, was diluted 1:250 in Blotto.

Secondary western blotting was used for samples showing multiple banding with whole cell lysate. The antigen, P39, used in these blots is indicative of *B. burgdorferi* infection. The blot was performed with a recombinant P39 fusion protein (derived from *B. burgdorferi* SON 188) in a manner similar to that described above and by Maniatis et al. (1989). For the controls (anti-P39 fusion protein), the secondary antibody was conjugated to alkaline phosphatase, and the development of these control bolts proceeded in a manner consistent with the Maniatis et al., (1989) protocol for blots involving alkaline phosphatase. No *B. burgdorferi* positive bear serum was provided as a control.

### Plague - *Yersinia pestis*

All serum samples were tested by the passive hemagglutination (PHA) and inhibition (PHI) test as described by the World Health Organization Expert Committee on Plague (1970). Titers of 1:16 or greater were reported as positive (Suzuki and Hotta, 1979).

### Toxoplasmosis - *Toxoplasma gondii*

A commercially available latex agglutination test was used (Toxotest-MT , Eiken Chemical Co, Ltd., Tokyo, Japan). A titer of 64 or greater was considered positive (Chomel et al., 1995).

### Trichinosis - *Trichinella spiralis*

An enzyme linked immunosorbent assay (ELISA) based on the official United States Department of Agriculture) method for pseudorabies (Snyder et al., 1977; Behymer et al., 1985) was used with some modifications. Fifty ml per well of a *Trichinella spiralis* ES antigen, 5 mg/ml, produced by the Veterinary Diagnostics Laboratory, Ames, Iowa, USA at 1:1000 dilution in carbonate bicarbonate buffer, pH 9.6, was bound to 96-well flat bottom microtitration Linbro/Titertek plates (Flow Laboratories, Inc., McLean, Virginia) by overnight incubation at 4°C. Bear sera were diluted 1:40 in tris buffer (pH 7.4) containing 5% skim milk and 0.05% Tween 20 and 0.01% bovine serum albumine fraction V (Sigma Chemical Co., St. Louis, Missouri, USA). The peroxydase conjugate was a raccoon anti-bear antibody at 1:500 raccoon anti-bear IgG (Antibodies Inc., Davis, California). The substrate was 2, 2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid, (Sigma Chemical Co., St. Louis, Missouri). The reaction was stopped after 30 min. with 100 ml of a 0.1 M solution of hydrofluoric acid (pH 3.3).



Each plate contained known positive and negative control sera. The positive control serum was selected from a bear which was both positive by bentonite flocculation and ELISA (optical density (O.P.) = 1.0), and the negative controls were selected from 3 bears both negative by ELISA (O.D. < 0.1) and bentonite flocculation. Each serum was tested in duplicate and the mean of the two absorbance values calculated. Microtitration plates were read at 410 and 450 nm wavelengths respectively for test and reference on a microELISA autoreader (MR 50000, Dynatech Laboratories, Inc., Chantilly, Virginia). The cut-off point for a positive test was determined at an optical density of 0.2, which is the mean O.D. of the negative control population (all brown bears {*Ursus arctos*} from Kodiak Island, Alaska) plus three standard deviations (Richardson et al., 1983; Magnarelli et al., 1991).

#### Tularemia- *Francisella tularensis*

A commercially available slide agglutination test (Difco Laboratories, Detroit, Michigan) was used with the laboratory protocol as described by Owen (1970). Any agglutination at a titer greater or equal to 1:20 was considered as positive. Any serum with a titer of 1:20 or greater was re-tested to eliminate any non-specific reactions.

## Results

I obtained 199 samples from 123 males and 76 females. Ages ranged from 1 to 22 years with 22.1% juveniles (less than or equal to 2 years), 72.4% adults and 5.5% unknown.

Prevalence rates varied by infectious disease agent, sex and study site (Table 2 and 3). Variation ranged from 0 to 56.8% , and was highest in general for *Toxoplasma gondii*. Bears from the Klamath and Willamette National Forests had the greatest measurable exposure to the disease agents tested for, 7 of 12 and 8 of 12, respectively. Bears from the other 4 study sites had exposure to 1 or 2 disease agents. There was no evidence of mean positive titers or prevalence rates increasing with age for any of the infectious disease agents. A difference between male and female prevalence rates was determined with only one disease agent.

Bluetongue- All 37 sera samples tested for antibodies were negative.

Brucella- No antibodies were detected in the 196 samples tested.

Canine Distemper- Of the 165 bears tested, 8 (4.8%) had antibodies against morbillivirus. Positive samples were found in four of the study sites, with none detected in the Snoqualmie or Shasta National Forest sites. There was no significant difference in the prevalence rate between male (3.9%, 4/104) and female (6.6%, 4/61) bears. Only those bears 7 and 8 years old at the Willamette National Forest site had positive titers more frequently than expected ( $\chi^2 = 4.34$ , d.f.= 1, P= 0.029). The titer values ranged from 1:80 to 1:320.

Epizootic Hemorrhagic Disease- Specific antibodies were not detected in the 40 samples tested.

Ehrlichiosis- Nine (4.5%) of the 198 samples tested for *Ehrlichia equi* antibodies were positive; these came from the Klamath (18.9%, 7/37) and Willamette National Forest (2.2%, 2/93) sites. Overall prevalence rates of males (3.3%, 4/122) and females (6.6%, 5/76), but neither sex or age were associated with positive sera samples. The titer values ranged from 1:10 to 1:80.

Heartworm- None of the 190 bears tested had heartworm (*D. immitis*) antibodies.

Disease	SHA	KLA	WIL	COA	OLY	SNO	Overall % Positive	Total Sample #
Bluetongue	No Test	0 (0/8)	0 (0/8)	0 (0/3)	0 (0/7)	0 (0/11)	0	37
Brucellosis	0 (0/10)	0 (0/37)	0 (0/93)	0 (0/7)	0 (0/23)	0 (0/26)	0	196
Canine Distemper	0 (0/10)	9.1 (1/11)	9 (5/85)	11 (1/9)	4.4 (1/23)	0 (0/27)	4.8 (8)	165
EHD	No Test	0 (0/11)	0 (0/8)	0 (0/3)	0 (0/7)	0 (0/11)	0	40
Ehrlichiosis	0 (0/10)	18.9 (7/37)	2.2 (2/93)	0 (0/8)	0 (0/23)	0 (0/27)	4.5 (9)	198
Heartworm	0 (0/10)	0 (0/31)	0 (0/91)	0 (0/8)	0 (0/23)	0 (0/27)	0	190
ICH	0 (0/10)	0 (0/11)	3.5 (3/85)	0 (0/9)	0 (0/23)	0 (0/27)	1.8 (3)	165
Lyme	0 (0/10)	54.8 (17/31)	7.7 (7/91)	0 (0/8)	0 (0/23)	0 (0/27)	12.6 (24)	190
Plague	30 (3/10)	8.1 (3/37)	5.4 (5/93)	0 (0/8)	0 (0/23)	0 (0/27)	5.5 (11)	198
Toxoplasmosis	10 (1/10)	56.8 (21/37)	45.2 (42/93)	50 (4/8)	43.5 (10/23)	40.7 (11/27)	45 (89)	198
Trichinosis	0 (0/10)	8.1 (3/37)	2.2 (2/93)	0 (0/8)	0 (0/23)	0 (0/27)	2.5 (5)	198
Tularemia	0 (0/10)	41.2 (7/17)	1.6 (1/61)	No Test	No Test	No Test	9 (8)	88

EHD = epizootic hemorrhagic disease

ICH = infectious canine hepatitis

COA= Coast Range

KLA= Klamath National Forest

OLY= Olympic National Park

SHA= Shasta National Forest

SNO= Snoqualmie National Forest

WIL= Willamette National Forest

Table 2. Prevalence of diseases and their infectious agents tested for in black bear serum samples from six study sites in California, Oregon and Washington, 1993-1997.

Table 3. Prevalence of diseases and their infectious agents tested in male and female black bear serum samples from six study sites in California, Oregon and Washington, 1993-1997.

Agent	Male	Female
Bluetongue	0	0
Brucellosis	0	0
Canine Distemper	3.9 (4/104)	6.6 (4/61)
EHD	0	0
Ehrlichiosis	3.3 (4/122)	6.6 (5/76)
Heartworm	0	0
ICH	2.9 (3/104)	0
Lyme	11.1 (13/117)	15.1 (11/73)
Plague	7.4 (9/122)	2.6 (2/76)
Toxoplasmosis	39.3 (48/122)	54.0 (41/76)
Trichinosis	2.5 (3/122)	2.6 (2/76)
Tularemia	8.8 (5/57)	9.7 (3/31)

EHD = epizootic hemorrhagic disease

ICH = infectious canine hepatitis

Infectious Canine Hepatitis- Overall prevalence rate of antibodies to canine adenovirus type-1 was 1.8% (3 of 165 samples). All positive samples came from male bears from the Willamette National Forest site. There was no association between positive titers and age or sex. The titer values were 1.7 or > 3.1.

Lyme- Twenty-four (12.6%) of the 198 samples tested were positive for *Borrelia burgdorferi* antibodies. Positive samples were found at the Klamath (54.8%, 17/31) and Willamette National Forest (7.7%, 7/91) sites. Overall prevalence rates for males (11.1%, 13/117) and females (15.1%, 11/73), were not statistically different. Sampled bears 3 and 4 years of age had serum antibodies more frequently than expected ( $\chi^2 = 13.06$ , d.f. = 1,  $P = 0.0003$ ). The annual prevalence rate in the Klamath National Forest site varied from 0% in 1994 to 85% in 1997. The prevalence rate in the Willamette National Forest site varied from 0% for 1993-1995 to 23.1% in 1996 and 22.2% in 1997 (Figure 2).

Plague- One hundred and ninety eight samples were tested for *Yersinia pestis* antibodies with 11 (5.5%) positive sera from the Klamath (8.1%, 3/37), Shasta (30%, 3/10), and Willamette National Forest (5.4%, 5/93) sites. There was no significant difference between male (7.4%, 9/122) and female (2.6%, 2/76) bears in the prevalence rate and there was no detectable association with age. Positive titers ranged from 1:16 to 1:256.

Toxoplasmosis- A total of 89 (45.0%) of 198 samples had antibodies against *Toxoplasma gondii* with positives found at all six study sites (Table 2). The prevalence rate for males (39.3%, 48/122) was significantly lower in comparison to females (54.0%, 41/76) both overall, and specifically at the Snoqualmie National Forest site, ( $\chi^2 = 3.47$ , d.f. = 1,  $P = 0.063$ ) and ( $\chi^2 = 4.8$ , d.f. = 1,  $P = 0.028$ ) respectively. Overall, young bears 1 to 2 years of age were more likely to have positive titers ( $\chi^2 = 4.58$ , d.f. = 1,  $P = 0.032$ ). At the Klamath site, 3 and 4 year old bears ( $\chi^2 = 5.75$ , d.f. = 1,  $P = 0.017$ ) and 1 and 2 year old bears at the Willamette National Forest site ( $\chi^2 = 4.88$ , d.f. = 1,  $P = 0.027$ ) had positive titers more frequently than expected. The prevalence rate increased at the Willamette National Forest site over the five years of sampling from 29.6% to 55% (Figure 3). The titers ranged from 1:64 to 1:1024.

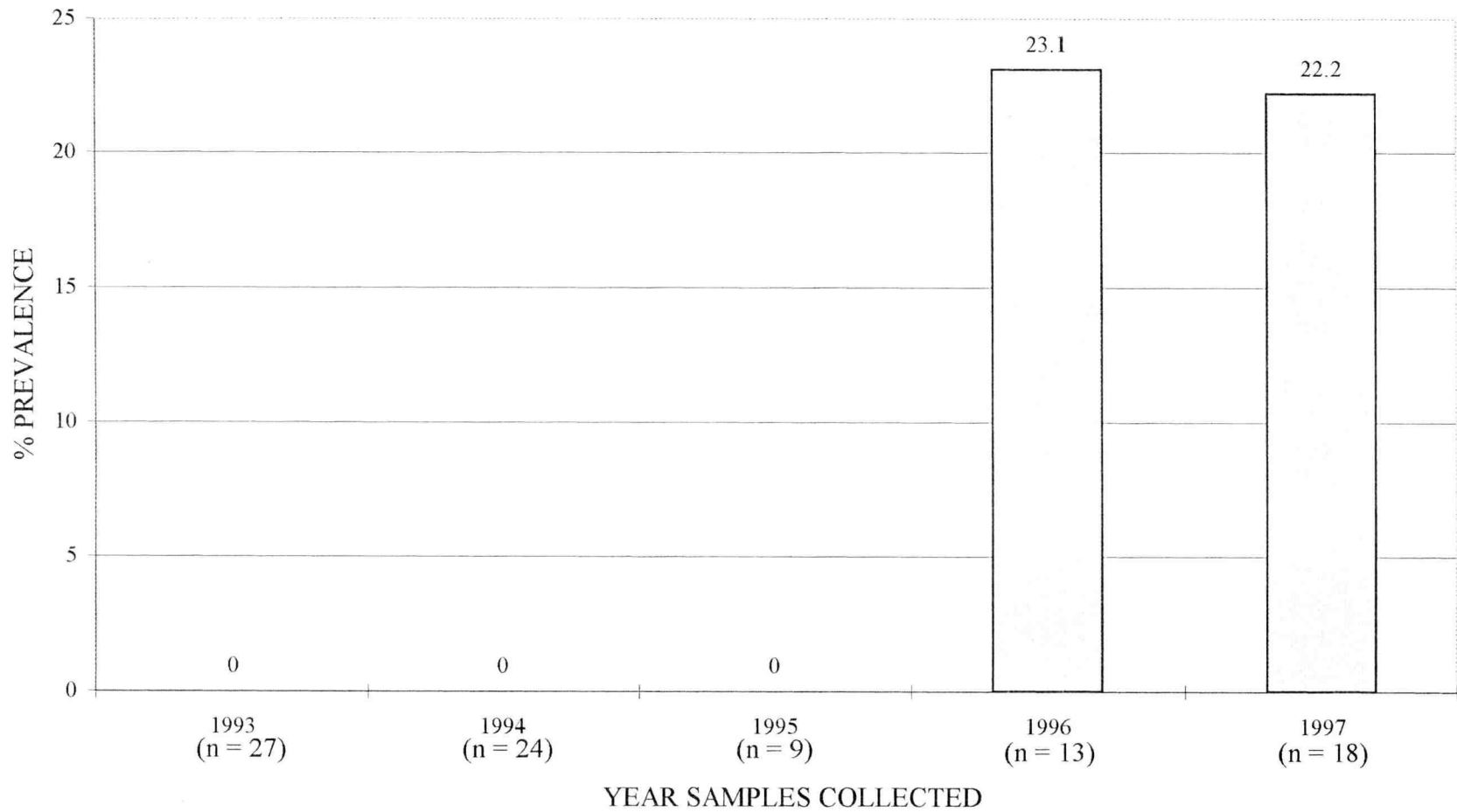


Figure 2. *Borrelia burgdorferi* (Lyme disease) antibody prevalence in black bears for the Willamette National Forest site, 1993-1997.

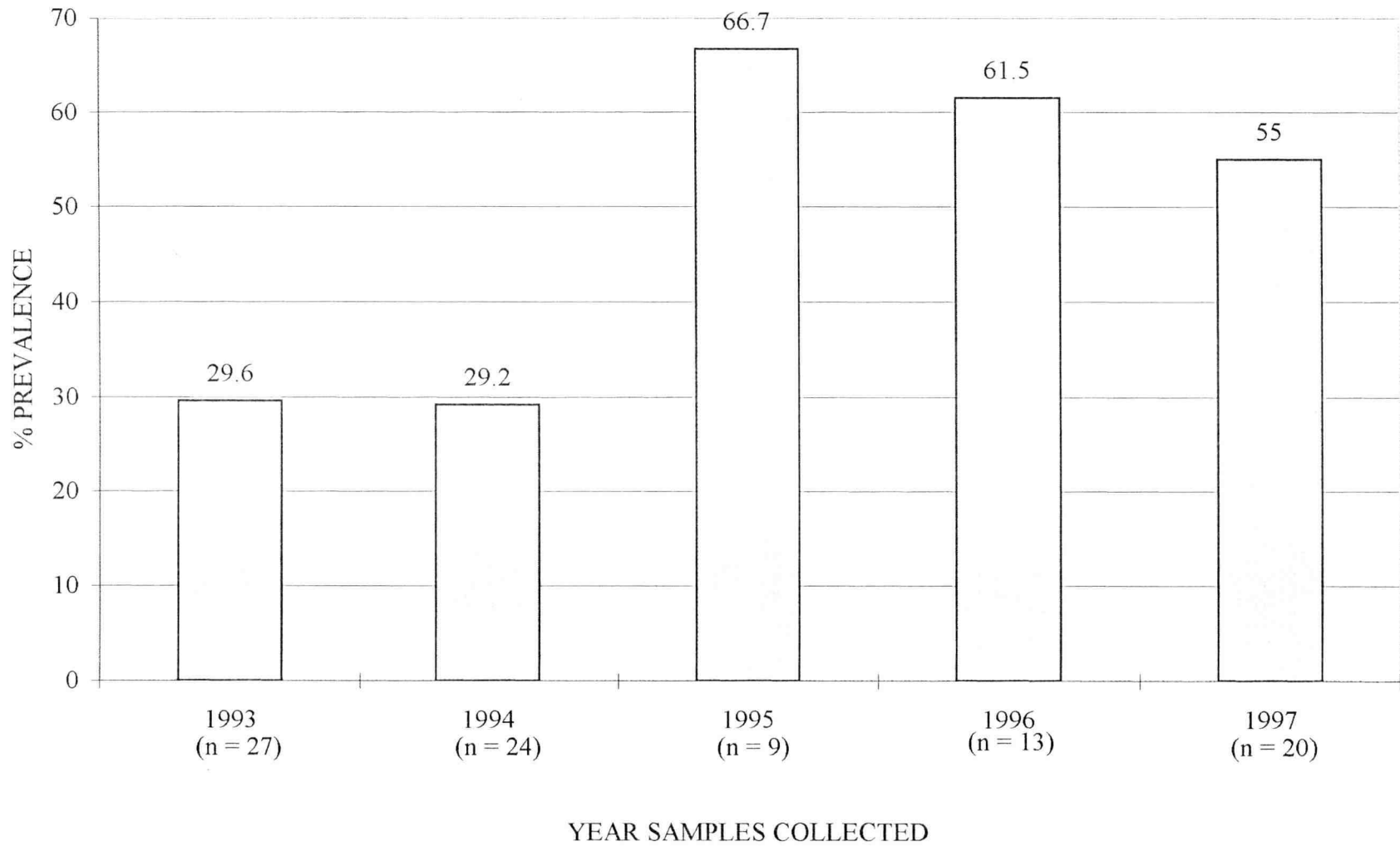


Figure 3. *Toxoplasma gondii* (Toxoplasmosis) antibody prevalence in black bears for the Willamette National Forest site, 1993-1997.

Subjective assessment of lab accuracy for *Toxoplasma gondii* titers was considered to be adequate. The four samples re-submitted one year later had different titer levels between laboratories, but it appeared to be due to different test kits and a dilution factor. The three split samples submitted as duplicates yielded identical titers, and two 50% and 25% diluted samples produced appropriately diluted titer levels.

Trichinosis- Five (2.5%) of 198 samples were positive for *Trichinella spiralis* antibodies. Only two of the study sites, Klamath and Willamette National Forests, had positive sera. Male bears tested had a 2.5% (3/122) rate and females had a 2.6% (2/76) prevalence rate, but there was no significant difference by sex or age of bears with the prevalence of this disease agent overall or by study site. Positive O.D. titers ranged from 0.2 to 1.16.

Tularemia- Eighty eight samples were tested for *Francisella tularensis* antibodies with 8 (9.0%) being positive. Sera from three sites were tested, which included the Klamath, Shasta and the Willamette National Forest (Table 2). Male prevalence rates (8.8%, 5/57) were not significantly different from female rates (9.7%, 3/31). Bears 3 and 4 years old were more often positive across all sampled bears, and specifically for the Klamath National Forest study site, ( $\chi^2 = 3.69$ , d.f.= 1, P= 0.028) and ( $\chi^2 = 9.94$ , d.f.= 1, P= 0.002), respectively. The titers ranged from 1:40 to 1:320.

Necropsy findings for each bear is listed in Appendix 4. Significant findings include generalized emaciation; gastro-intestinal parasites (*Cryptosporidium*, *Baylisascaris procyonis*, nematode and fungus); large amounts of cambium in several stomachs; focal areas of hemorrhage in the heart, adrenal glands, and brain; focal myofibrillar degeneration of the masseter muscle; multifocal mineralization of the kidney; lipidosis of the adrenal gland; focal histiocytosis of the lung; and multifocal granulomas in the liver. All six bears IFA tested for rabies were negative.



## Discussion

Exposure to assessed disease agents was limited at four of the study sites, but more extensive at the remaining two. There was no apparent geographic, sample size bias, or other related variable to explain this pattern. Age and sex were associated with a higher than expected prevalence rate for exposure to several disease agents.

This study did not detect exposure of black bears to orbiviruses, but further serological surveys should be conducted on carnivores from regions in which bluetongue (BLU) and epizootic hemorrhagic disease (EHD) are better documented in wild and domestic ruminant populations. BLU/EHD serogroups of orbiviruses principally infect domestic and wild ruminants worldwide; however evidence of infections may occur naturally in African carnivores (Alexander et al., 1994). Morbidity and mortality of wild ungulates in North America from these viruses have typically followed introduction of domestic species, specifically sheep (Hoff and Trainer, 1981). The route of transmission for bears could be from the vector, *Culicoides* midges, or ingestion of meat. If bears scavenge sheep or wildlife carcasses, they may come in contact with the virus. However, Alexander et al., (1994) postulated that carnivores that are scavengers would probably not be sero-positive because of their scavenging of meat and skin rather than ingestion of organs such as spleen and liver where the virus is more concentrated, which are rapidly consumed by the predators that killed the prey.

Concern about transmission of infectious diseases to wildlife by elk and deer in game farms in the western states was the motivation to look for *Brucella abortus* in bear populations. Both Binninger et al., (1980) and Drew et al., (1992) reported low prevalence rates of *Brucella* spp. in black bears, and the role of black bears as a reservoir of brucellosis is unlikely. I found no positive samples from 196 bears from three states; thus it does not appear bears would be a good amplifying or sentinel species for this disease.

I found a 4.8% prevalence rate for morbillivirus infections. This is the first record for antibody presence in wild black bears. Little is known regarding the epizootiology of morbillivirus infections in wild carnivores, although it has been identified in wild Italian brown bears (Marsillio et al., 1997). The relationship between antibody prevalence for this virus and bears 7 to 8 years of age is most likely related to sampling artifact. Five of the 8 positive bears were from the Willamette National Forest study site in Oregon. These five bears shared overlapping home ranges as determined with radio telemetry, and one was a translocated nuisance bear from a garbage disposal site on the Oregon coast (Dave Immel, pers. comm.). The translocation occurred two years before the blood samples were taken. Because the most likely mode of virus transmission is directly from animal to animal, exposure could have occurred from a wild or domestic canid, raccoon, or mustelid either before or after the translocation. No blood sample was taken at the time of the translocation.

Serum antibodies to *Ehrlichia equi* in black bears are reported here for the first time. Since this bacteria is endemic to northern California it is interesting to note it was not found at the Shasta National Forest study site. Because *E. equi* is a member of the *E. phagocytophilia* geno-group, which is responsible for human granulocytic ehrlichiosis and is transmitted by *Ixodes* spp. ticks, it should be considered a zoonotic potential when handling bears.

I found no evidence of exposure to *Dirofilaria immitis* in all sampled bears. This is surprising given bears at the California study sites are in endemic areas for heartworm infections in domestic dogs (Steinhauer, 1995). There are also confirmed cases in domestic dogs in the northern Oregon Coast Range, but this is not considered an endemic area (Wood, 1992). No confirmed cases of heartworm in resident dogs were determined in a serological survey by Foreyt and Lagerquist, (1991) in Washington, even though 8 species of mosquitoes known to occur in state have been identified as *D. immitis* vectors in other areas of the U.S. The true prevalence rate in all three states may be higher due to the serological test not detecting the small antigen amount from a low number of worms or immature worms.

Bear populations sampled in this study are relatively unexposed to infectious canine hepatitis (ICH) adenovirus, with just three bears from the Willamette National Forest site in Oregon having titers. Foreyt et al., (1986) reported a similar low prevalence in northeastern Washington bears. Wild canids are presumed to be the most likely source of infection for grizzly bears in Alaska, (Zarnke and Evans, 1989) with transmission by urine, feces or saliva. The prevalence rate for ICH in wild canids in the Pacific states is unknown, but would be of interest due the wildlife management practices of re-introduction and translocations. Any future re-introduction efforts for wild canids should consider the exposure rates of known infectious diseases for sympatric carnivores. This may have relevance to the efforts of re-introducing wolves into Olympic National Park and grizzly bears into the North Cascades of Washington. Currently, orphaned bear cubs are re-introduced from wildlife rehabilitation centers in all three states. This represents a potential epizootiology of ICH in wild black bear populations and in Washington grizzly bear populations as well.

*Borrelia burgdorferi* prevalence rate of 12.6% for bears in this study is comparable to the 16.6% rate reported by Kazmierczak et al., (1988) in Wisconsin. Because Western Blot techniques were used for the serology, the potential of cross-reactivity between other *Borrelia* spp. is not of concern. There may be a relationship between prevalence and bears 3 to 4 years of age, but I have no explanation for it. The prevalence rate increased at both study sites where positive samples were found. This may reflect the changing ecology of the organism or transmission vector. It is not known if black bears or other wild carnivores serve as reservoirs for *B. burgdorferi* or develop blood spirochete levels sufficient to infect *Ixodes* spp. tick vectors (Kazmierczak and Burgess, 1989). The zoonotic threat to humans is not known and would depend on the type of ticks parasitizing black bears. *Ixodes neotomae* appears to maintain the enzootic cycle of *B. burgdorferi* and does not bite humans in northern California. *Ixodes pacificus* is more likely to feed on large mammals and is the primary vector of *B. burgdorferi* to humans (Brown and Lane, 1992).

Previous surveys for *Yersinia pestis* in California black bears reported 15%, (Ruppanner et al., 1982), 23%, (Smith et al., 1984), and 36%, (Clover et al., 1989), comparable to those found at the Klamath and Shasta National Forest study sites. Few fleas were not found on bears in this study, thus the most likely source of exposure to the organism was from preying on infected rodents (Clover et al., 1989).

The overall prevalence rate of 45% for *Toxoplasma gondii* is much higher than found elsewhere (Binninger et al., 1980; Chomel et al., 1995; and Ruppanner et al., 1982) However, it is much less than the rates Biscoe et al., (1993), or Dubey et al., (1995) reported. Nearly all of the above authors reported older male bears having the highest prevalence rates of *T. gondii*, and speculate this is due to longer exposure and larger home ranges. This generally does not hold true for other infectious diseases surveyed in bear populations in North America such as plague, (Clover et al., 1989), lyme disease, (Kazmierczak et al., 1988), tularemia, (Binninger et al., 1980), or trichinella, (Schad et al., 1986).

There is a wide range of prevalence rates of exposure to *Trichinella spiralis* depending on the geographic location. Binninger et al., (1980) in Idaho and Ruppanner et al., (1982) in California reported 13% rates, compared 2.5 % in this study. Because trichinellosis is transmitted by consumption of infected meat, Zarnke et al., (1997) speculated that food availability and habits of bears are related to exposure to *T. spiralis*. The source of the infections are unknown, but are likely due to scavenging or ingestion of infected rodents rather than domestic swine. This is supported by Worley et al., (1974) and Schad et al., (1984) with their findings of a higher prevalence of infection in carnivores from more remote regions as compared to more urban or human-accessible areas.

Only bears from 3 sites were tested for *Francisella tularensis* in 1993-1995. Antibodies were found in 9.0% of the samples as compared to 19% in Idaho (Binninger et al., 1980). Prevalence rates were highest in bears 3 to 4 years of age in this study, without apparent cause. Because the organism can be transmitted by a wide range of ectoparasites, the disease has zoonotic potential.

### Laboratory testing

The accuracy of serological surveys are dependent on ability of laboratory tests to detect antigens or antibodies in blood or serum samples, plus the methodologies and quality control individual laboratories employ. Using serological tests developed for domestic animal use for wildlife studies requires an assumption that the specificity and sensitivity of these tests remain relatively unchanged. Interpretation of serological data must be done with caution. Providing evidence of exposure to an organism does not demonstrate the pathogenicity involved in individual animals or wildlife populations.

For each organism tested for there are usually several options for serological tests. Choosing tests in which sensitivity and specificity are high aid in the accuracy of the determined prevalence rates of exposure. Agglutination tests are the least accurate by allowing cross- reactivity of organisms, and Western Blot is the most accurate of the tests used due to specific band width analysis.

In an effort to subjectively assess laboratory accuracy, I sent several paired and diluted samples to two different laboratories to test for serum *Toxoplasma gondii* antibodies. Results were similar, except with the samples re-submitted one year later for one of the laboratories. Results for the four samples were negative in 1997 and then positive in 1998. The laboratory director thought it was due to two different types of diagnostic agglutination kits.

## Sampling Issues

Determining sample size in wildlife surveys is often determined by the statement, "How many samples can we get!". This is usually borne out of a finite amount of funding and a realization that large sample sizes on live animals are frequently difficult to obtain. Sample size remains an important question however, because the collection of more than are needed is a waste of resources and the collection of too few may lead to results with little or no value (Wobeser, 1994). A general formula to estimate sample size for a proportion (the prevalence of a disease) is  $n = z^2 p(1-p)/d^2$ .

$d$  = the maximum difference allowed between the estimated and true proportion

$n$  = size of sample randomly selected from the population

$p$  = true proportion of disease in the population

$z$  = the area under a normal curve for the desired confidence level

DiGiacomo and Koepsell (1986) provide a useful table showing sample sizes required to estimate prevalence with various  $d$  values (Table 4). The calculations based on this table have the following assumptions; random sampling of the population, the disease agent is distributed randomly throughout the population, and true proportion (prevalence) of the disease in the population can be accurately estimated before the study begins.

Because the prevalence rates of disease agents from my study sites are from 0 to 56.8%, I needed both very small and very large sample sizes to determine prevalence with a low tolerable error. For example, based on past serological surveys (Clover et al., 1989; Ruppanner et al., 1982; Smith et al., 1984) *Yersinia pestis* antibodies were present at 36%, 15%, and 23% respectively. Using a 5% maximal tolerable error, I would have needed a minimum of 246 serum sample to have 95% confidence that the true prevalence of the population was estimated.

None of the sampling assumptions were met in my study. The sampled population is from bears caught in snares. This is believed to produce a male bias in the sampled bears due to sex difference in foraging behavior. It is unlikely that exposure to all the disease agents is equally likely to occur in both sexes and all age groups in the population. For example, exposure to *Toxoplasma gondii* in this study is higher in young female bears.

The last condition of estimating the true population prevalence rate before the study begins relies on the results of previous similar studies. This was not possible for all the infectious disease agents I assessed.

After reviewing the methods of estimating sample size, my goal was to obtain at least 20 serum sample from each study site. This was based on a potential prevalence rate of 5% and using a 10% maximum tolerable error in the estimated prevalence at a 95% confidence level. At four of the six study sites, I was able to obtain > 20 samples. The fifth site was the Shasta National Forest in California. Less sampling effort on the part of the biologists was the cause of a small size as compared to the other California site. The sixth was the Coast Range site in Oregon at which bears were trapped because of tree damage in commercial plantations. The year I sampled this site had limited tree damage, which reduced the number of bears to sample.

Table 4: Samples needed to estimate the prevalence of disease in a population.

Estimated prevalence (%) <sup>a</sup>	<u>Maximal tolerable error in estimated prevalence<sup>b</sup></u>			
	0.05	0.10	0.15	0.20
1	16			
2	31			
5	73	19		
10	139	35	16	
15	196	49	22	13
20	246	62	28	16
25	288	72	32	18
30	323	81	36	21
35	350	88	39	22
40	369	93	41	24
45	381	96	43	24
50	384	96	43	24

<sup>a</sup> For prevalences greater than 0.50, use 1- estimated prevalence. <sup>b</sup> At 95% confidence level.

### Chapter 3

#### Management Implications

Seroprevalence to infectious disease agents reflects an exposure rate, but does not reflect the overall effect on a population. Controlled studies of an wildlife species accounting for confounding variables would be needed to demonstrate this. Few studies have documented the effects of disease on population dynamics, and these are mainly on ungulates. Given the difficulty in carrying out such research, and recognizing that much of the effect of diseases is subclinical and would require comparing an exposed versus a non-exposed population, the likelihood of obtaining data on population affects is unlikely. Seroprevalence also can assess the risk of zoonotic potential or transmission of diseases to domestic animals.

Black bears in all three states are being exposed, and are mounting immune responses to a wide variety of infectious disease agents. There is evidence of increased exposure to both *Borrelia burgdorferi* and *Toxoplasma gondii* over time at specific study sites. This study reports for the first time in wild black bears antibodies to morbillivirus (canine distemper) and *Ehrlichia equi*. These diseases reflect the potential for changes in prevalence and importance to a population over time. It would be beneficial to monitor key species and diseases for incidence changes to predict future impact on populations.

Nuisance black bears in all three states are currently considered for translocation as a part of management goals, and orphan cubs are re-introduced into the wild from rehabilitation centers. Translocated bears can potentially transmit infectious diseases to naive populations if conditions for the disease agent are favorable. These conditions include factors such as a hospitable environment, suitable number of hosts and any required vectors. Any black bear being translocated or re-introduced could have blood drawn at the time of handling for testing for appropriate disease agents and serum storage for potential future analysis. Bears from high risk areas, such as regions where repeated distemper outbreaks in other wildlife species, or open dump sites where high rodent populations exist, would not be good candidates for re-introduction or translocation.



With the recognition that infectious diseases are density-dependent, management practices that discourage density increases could help in reducing infectious disease transmission. Also, if the re-introduction of other carnivores is being considered, their immune status and any native species known to share similar infectious diseases should be considered.

The zoonotic potential for several diseases exists in black bear populations in all six study sites. It is reasonable to infer similar prevalence rates in the general populations of the regions of the study sites. Humans handling bears such as biologists, hunters, trappers and butchers should be made aware of the potential of disease transmission. Specifically the organisms *Francisella tularensis*, *Trichinella spiralis*, *Toxoplasma gondii*, *Yersinia pestis*, *Borrelia burgdorferi*, and *Ehrlichia equi* are of zoonotic concern. Exposure prevalence from black bears in this study to these disease agents ranged from low to high, however the potential for zoonotic transmission based on reported cases of human illnesses currently appears to be quite low.

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## **APPENDICES**

Author	Brucella	Borrelia	CAV-1	Clostrid	Coxiella	Lepto	RMSF	SLE	Toxo	Trich	Tula	WEE	Yersinia
Addison, 1979										1.7			
Binninger et al., 1980	5				6	1	2	1	8	13	19	1	
Biscoe et al., 1993									80				
Bulter/Khan, 1992										0.6			
Chomel et al., 1995									15				
Clover et al., 1989													36
Dick et al., 1980										0			
Drew et al., 1992	0.6												
Dubey et al., 1995									78.6				
Foreyt et al., 1986			3										
Kazmierczak et al., 1988		16.6											
Ruppanner et al., 1982				2	17	16			27	13			15
Schad et al., 1986										1.8			
Smith et al., 1984													23
Worley et al., 1974										11.9			

Brucella = *Brucella* species

Borrelia = *Borrelia burgdorferi* (Lyme)

CAV-1 = infectious canine hepatitis adenovirus

Clostrid. = *Clostridium botulinum*

Coxiella = *Coxiella burnetii* (Q-fever)

Lept. = *Leptospira interrogans*

RMSF = Rocky Mountain Spotted Fever

St Louis Enceph. = St Louis Encephalitis

Toxo. = *Toxoplasma gondii*

Trich. = *Trichinella spiralis*

Tula. = *Francisella tularensis* (tularemia)

WEE = Western Equine Encephalitis

Yersinia = *Yersinia pestis*

Appendix 1. Prevalence of selected infectious disease agents in black bears from published literature.

Appendix 2. Collection and storage protocol for blood samples collected from black bears at six study sites in California, Oregon and Washington, 1993-1997.

1) Equipment -

Red top vacutainer tubes

Vacutainer needles 20ga x1 1/4"

Vacutainer needle holder

Alcohol

Cotton swabs

2) Instructions -

A) Obstruct venous flow proximal to sampling site on the cephalic or medial saphenous vein with a tourniquet or hand pressure.

B) Prep site with alcohol soaked swab.

C) Collect blood in 2 red top tubes (10 mls total) with vacutainer equipment.

D) Spin blood down in centrifuge within 24 hrs of sampling and pipette off serum into a plastic storage vial labeled with bear's ID #.

E) Place in freezer at -20°C for storage.

Appendix 3. Necropsy sampling protocol for bears necropsied from the Coast Range site, 1997.

Gross necropsy: body systems examined

External:

- dermal
- auditory
- reproductive
- oral
- general condition

Internal:

- cardiovascular
- urinary
- reproductive
- respiratory
- gastrointestinal
- central nervous
- lymphatic
- skeletal muscle

Samples collected from each bear

- whole coagulated blood - attempt serum collection
- diaphragm, tongue, masseter - stored in freezer
- fecal samples- large/small intestine
- frozen liver and kidney- possible toxicology analysis
- general organs and diaphragm, tongue, masseter muscles - formalin
  - heart
  - lung
  - liver/gall bladder
  - kidney
  - pancreas
  - skin
  - spleen
  - eye
  - lymph nodes (3)
  - stomach/intestines
  - adrenal gland
  - urinary bladder
  - ovaries/uterus/testicles
  - brain

Appendix 4. Necropsy reports from black bears harvested from the Coast Range site, 1997.

ID Number: 20433

1534

Date of Capture: 05/12/97

Location of Capture: 12S/07W/06

Sex: Male

Estimated Weight: 70 kg

Gross Necropsy Findings:

emaciated body condition

large volume of cambium in stomach

no other abnormalities

Rabies Exam: FA- negative

Parasite Exam: Low numbers of *Cryptosporidium* species

Histological Findings:

heart-one focal area of endocardial hemorrhage

masseter muscle-a few foci of myofibrillar degeneration with infiltration by macrophages

intestines-one section with *Cryptosporidium* species

testicles-immature or aspermia

ID Number: 20251

1528

Date of Capture: 05/12/97

Location of Capture: 12S/09W/34

Sex: female

Estimated Weight: 27kg

Gross Necropsy Findings:

emaciated body condition

small volume of cambium in stomach

no other abnormalities

Rabies Exam: not done

Parasite Exam: *Baylisascaris procyonis*

Histological Findings:

lung-three focal areas of alveolar histiocytosis

liver-a few multifocal small granulomas- past infections?

kidney- multifocal areas of tubular mineralization

ID Number: 21018

1531

Date of Capture: 05/19/97

Location of Capture: 08S/07W/09

Sex: male

Estimated Weight: 125kg

Gross Necropsy Findings:

moderately obese body condition

beaver carcass and plastic bag in stomach

very rigid airways in lungs and generalized purple discoloration of lung lobes

Rabies Exam: not done

Parasite Exam: negative

Histological Findings:

adrenal gland- multifocal acute hemorrhage in cortex

ID Number: 20432

1533

Date of Capture: 05/24/97

Location of Capture: 12S/07W/07

Sex: male

Estimated Weight: 90kg

Gross Necropsy Findings:

emaciated body condition

large amount of grass in stomach

no other abnormalities

Rabies Exam: not done

Parasite Exam: ascaridia species

Histological Findings:

skin- local epidermal crust and nematode larvae



ID Number: 20448

1529

Date of Capture: 05/26/97

Location of Capture: 04S/06W/32

Sex: Male

Estimated Weight: 125kg

Gross Necropsy Findings:

emaciated body condition

large amount of cambium in stomach

all 10 hind claws broken off at base

front right foot missing- snare related

Rabies Exam: not done

Parasite Exam: negative

Histological Findings:

skin- nematodes in hair follicles

ID Number: 20260

1532

Date of Capture: 05/29/97

Location of Capture: 08S/09W/22

Sex: male

Estimated Weight: 36kg

Gross Necropsy Findings:

small amount of grass in stomach

emaciated body condition

no other abnormalities

Rabies Exam: FA- negative

Parasite Exam: negative

Histological Findings:

no abnormal findings

ID Number: 20434  
1547  
Date of Capture: 05/30/97  
Location of Capture: 13S/09W/16  
Sex: male  
Estimated Weight: 90kg  
Gross Necropsy Findings:  
    emaciated body condition  
    beaver in stomach  
    no other abnormalities

Rabies Exam: FA- negative  
Parasite Exam: negative

Histological Findings:  
    adrenal gland- multifocal acute hemorrhage  
    skin- dermatophytosis (superficial fungal infection)

ID Number: 20198  
1530  
Date of Capture: 05/30/97  
Location of Capture: 04S/06W/32  
Sex: male  
Estimated Weight: 136kg  
Gross Necropsy Findings:  
    grass in stomach  
    moderately obese  
    no other abnormalities

Rabies Exam: FA- negative  
Parasite Exam: negative

Histological Findings:  
    skin- moderate numbers of fungus organisms on superficial epithelium and hair shafts  
    brain- multifocal acute hemorrhage  
    generalized autolysis of tissues

ID Number: 5  
1546  
Date of Capture: 05/30/97  
Location of Capture: 10S/07W/35  
Sex: female  
Estimated Weight: 36kg  
Gross Necropsy Findings:  
    emaciated body condition  
    stomach empty  
    no other abnormalities

Rabies Exam: FA- negative  
Parasite Exam: negative

Histological Findings:  
    kidney- multifocal mineralization within medulla  
    adrenal gland-cortical and medullary lipidosis

ID Number: 20435  
1548  
Date of Capture: 06/05/97  
Location of Capture: 13S/08w/30  
Sex: male  
Estimated Weight: 36kg  
Gross Necropsy Findings:  
    emaciated body condition  
    beaver and a small amount of cambium in stomach

Rabies Exam: FA- negative  
Parasite Exam: negative

Histological Findings:  
    tissues lost