Alaska Department of Fish and Game Division of Wildlife Conservation

> Federal Aid in Wildlife Restoration Research Progress Report

> > 1 July 1994 - 30 June 1995

## Serologic Survey of Alaska Wildlife for Microbial Pathogens

Randall L. Zarnke



Grant W-24-3 Study 18.7 June 1995

Alaska Department of Fish and Game Division of Wildlife Conservation June 1995

## Serologic Survey of Alaska Wildlife for Microbial Pathogens

Randall L. Zarnke

Federal Aid in Wildlife Restoration Research Progress Report 1 July 1994–30 June 1995

> Grant W-24-3 Study 18.7

This is a progress report on continuing research. Information may be refined at a later date. If using information from this report, please credit author(s) and the Alaska Department of Fish and Game.

## STATE OF ALASKA Tony Knowles, Governor

## DEPARTMENT OF FISH AND GAME Frank Rue, Commissioner

## DIVISION OF WILDLIFE CONSERVATION Wayne L. Regelin, Acting Director

Persons intending to cite this material should receive permission from the author(s) and/or the Alaska Department of Fish and Game. Because most reports deal with preliminary results of continuing studies, conclusions are tentative and should be identified as such. Please give authors credit.

Additional copies of this report and other Division of Wildlife Conservation publications are available from:

> Publications Specialist ADF&G, Wildlife Conservation P.O. Box 25526 Juneau, AK 99802 (907) 465-4190

The Alaska Department of Fish and Game administers all programs and activities free from discrimination on the basis of race, religion, color, national origin, age, sex, marital status, pregnancy, parenthood, or disability. For information on alternative formats for this and other department publications, please contact the department ADA Coordinator at (voice) 907-465-4120, (TDD) 1-800-478-3648, or FAX 907-586-6595. Any person who believes she/he has been discriminated against should write to ADF&G, PO Box 25526, Juneau, AK 99802-5526 or O.E.O., U.S. Department of the Interior, Washington DC 20240.

## **PROGRESS REPORT (RESEARCH)**

State: <u>Alaska</u>

Cooperator: None

Project No.:W-24-3Project Title:Wildlife Research and ManagementStudy No.:18.7Study Title:Serologic Survey of Alaska Wildlife for<br/>Microbial Pathogens

Period Covered: <u>1 July 1994-30 June 1995</u>

## **SUMMARY**

Under the study title "Serologic Survey for Microbial Pathogens," 2 major projects have been completed. Formal manuscripts have been submitted to the *Journal of Wildlife Diseases*. These 2 manuscripts constitute the progress report for this reporting period (Appendix A and B).

- 1 Phocid herpesvirus-1 is a pathogenic agent for harbor seals (*Phoca vitulina*) and potentially other marine mammal species. A serologic survey of marine mammals from Alaska coastal areas revealed evidence of widespread exposure. There has been no evidence of mortality.
- 2 Trichinosis is a parasitic disease of carnivorous and omnivorous mammals. Lynx (*Felis lynx*) carcasses were examined for infection by the parasite *Trichinella* nativa.

#### CONTENTS

Pa	age
UMMARY	i
ACKGROUND	1
DBJECTIVES	1
1ETHODS	1
<b>PPENDIX A.</b> Serologic survey for phocid herpesvirus-1 in marine mammals from Alaska and Russia, 1978-1994	3
<b>PPENDIX B.</b> Prevalence of <i>Trichinella nativa</i> in lynx ( <i>Felis lynx</i> ) from Alaska, 1988-1993.	15

## BACKGROUND

Wildlife disease surveys of varying degrees of sophistication have been conducted by ADF&G since the early 1960s. In the early days these surveys were limited in scope, consisting of tests for evidence of 1 or 2 disease agents in 1 or 2 host species. Since the late 1970s, however, the surveys have been expanded to include up to 30 disease agents and 23 potential host species. Such a framework provides for a meaningful health profile of Alaska's wildlife.

### **OBJECTIVES**

The goal of this study is to monitor Alaska wildlife populations for evidence of previous exposure to infectious disease agents. Maximum benefit will be derived by keeping the survey as broad as possible, both in terms of disease agents and potential hosts. In an attempt to keep the cost of this study as low as possible, samples will be collected opportunistically in conjunction with other research and management operations.

#### **METHODS**

From 1970 through 1990 surveys have included approximately 9000 samples. Blood samples are usually collected in conjunction with other research and management projects. Preliminary preparation of samples is performed in Fairbanks before

shipment to laboratories in other parts of the world. When results are received in Fairbanks, the data are relayed to the contributor, in conjunction with an evaluation of potential management implications.

## Prepared by:

Randall L. Zarnke Wildlife Biologist II Approved by:

Wayne L. Regelin, Acting Director Division of Wildlife Conservation

Steven R. Peterson, Senior Staff Biologist Division of Wildlife Conservation

Submitted by:

Daniel J. Reed Research Coordinator APPENDIX A. Submitted to Journal of Wildlife Diseases (some minor format changes).

RH: Zarnke, et al. - Phocid herpesvirus-1 in Alaska marine mammals

## SEROLOGIC SURVEY FOR PHOCID HERPESVIRUS-1 IN MARINE MAMMALS FROM ALASKA AND RUSSIA, 1978-1994

- RANDALL L. ZARNKE<sup>1</sup>, Alaska Department of Fish and Game, 1300 College Road, Fairbanks, AK 99701 USA
- HELMA W. VOS, Seal Rehabilitation and Research Centre, Pieterburen, The Netherlands
- JAY M. VER HOEF, Alaska Department of Fish and Game, 1300 College Road, Fairbanks, AK 99701 USA
- ALBERT D. M. E. OSTERHAUS, Institute of Virology, Erasmus University, Rotterdam, The Netherlands

<sup>&</sup>lt;sup>1</sup> Send proofs to Randall L. Zarnke at address shown above

ABSTRACT: Blood samples were collected from 1125 mammals off the coast of Alaska and Russia during the period 1978-1994. Twelve species were represented, including sea otter (Enhydra lutris), river otter (Lutra canadensis), polar bear (Ursus maritimus), arctic fox (Alopex lagopus) and eight species of pinnipeds. Sera were tested for the presence of neutralizing antibodies to the 1984 Pieterburen isolate of phocid herpesvirus-1 (PhHV-1). Species-specific antibody prevalences ranged from 0%-77%. Prevalence was >70% for ringed seals (Pusa hispida), spotted seals (Phoca largha), and harbor seals (Phoca vitulina). Prevalence was <10% for sea otter, polar bear, and arctic fox. For each species, differences in antibody prevalence were not related to: 1) gender, 2) location of capture, or 3) year of collection. Antibody prevalence for walruses (Odobenus rosmarus) could be quantitatively predicted as a function of age. No evidence of PhHV-1 induced mortality has been detected in areas included in this survey. Based on results of this survey, PhHV-1 is not considered to be a significant mortality factor in mammals which inhabit the marine environment off the coast of Alaska or Russia.

Key words: Alaska, marine mammals, Russia, seal herpesvirus, serology

### INTRODUCTION

Phocid herpesvirus-1 (PhHV-1) was first identified in 1984 when it caused the deaths of 11 harbor seal (<u>Phoca vitulina</u>) pups in a nursery in the Netherlands (Osterhaus et al., 1985). Twelve additional pups which were ill during that epizootic subsequently recovered over the course of a 2-month period (Borst et al., 1986). Mortality due to PhHV-1 infection has only been observed in: 1) neonates or 2) seals acutely infected with phocine distemper virus (Osterhaus, pers. observ.).

Another distinct but related herpesvirus (PhHV-2) was recently isolated from harbor seals in the German Wadden Sea (Lebich et al., 1994). PhHV-1 is an alpha herpesvirus. Presumably, PhHV-2 is, as well.

Clinical signs of PhHV-1 disease included: 1) elevated body temperature, 2) inflammation of the oral mucosa, 3) nasal discharge, 4) coughing, 5) vomiting, 6) diarrhea, 7) anorexia, and 8) lethargy (Visser et al., 1991). Duration of the illness ranged from 1-6 days (Borst et al., 1986).

Interstitial pneumonia and necrosis of hepatic parenchyma were the primary histologic lesions. Less significant changes were also observed in kidneys, spleen, and lymph nodes (Borst et al., 1986).

Experimental evidence indicates that: 1) harbor seals can be infected by means of intranasal instillation of PhHV-1 or direct contact with infected animals, and 2) virus is shed in nasal and ocular discharge from naturally- and experimentally-infected animals (Horvat et al., 1989; Osterhaus, pers. observ.). Presumably, natural transmission occurs by means of aerosols or direct contact, as in other alpha herpesvirus infections.

Blood was collected from 49 free-ranging harbor seals which were involved in the 1988 phocine distemper virus (PDV) outbreak in the North Atlantic. Serologic tests revealed that approximately half of these seals had been exposed to PhHV-1 (Frey et al., 1989). Although PhHV-1 serum antibody prevalence was high following the 1988 PDV epizootic, PhHV-1 was isolated from only 4 of 112 harbor seals which died during the outbreak (Frey et al., 1989).

Herpesviruses have also been implicated in recent fatal and nonfatal infections of harbor seals in the North Pacific. Twenty-six harbor seals were collected during the investigation of the 1989 <u>Exxon Valdez</u> oil spill in Prince William Sound. One male had lesions on the penis and prepuce. Histologic examination of these lesions revealed intranuclear inclusion bodies typical of a herpesvirus (Spraker et al., 1994). The death of a single harbor seal off the coast of Washington state in 1990 was attributed to a herpesvirus. This diagnosis was based upon gross lesions, light microscopy, and electron microscopy (Zaucha, pers. comm.). Hepatic and adrenal necrosis were found in 12 harbor seal pups which died at a rehabilitation center in Sausalito, California during 1990. Herpesvirus virions were detected in these necrotic areas by use of electron microscopy (Lowenstine et al., 1992).

Herpesviruses have also been detected in other northern hemisphere marine mammal species including: harbor porpoise (<u>Phocoena phocoena</u>) (Kennedy et al., 1992), California sea lion (<u>Zalophus californianus</u>) (Kennedy-Stoskopf et al., 1986), and sea otter (<u>Enhydra lutris</u>) (Harris et al., 1990).

In the southern hemisphere, PhHV-1 antibody was also detected in sera from all of 25 apparently healthy Weddell seals (Leptonychotes weddelli) and all of three apparently healthy crabeater seals (Lobodon carcinophagus) from the eastern Weddell Sea (Harder et al., 1991).

The purpose of the current project was to determine the serum antibody prevalence of PhHV-1 in marine mammal populations off the coast of Alaska and Russia.

#### MATERIALS AND METHODS

Blood samples were collected from animals in portions of southeastern Alaska, the Gulf of Alaska, eastern and western Bering Sea, Chukchi Sea, and Beaufort Sea (Fig. 1). Standard descriptive data (date, sex, location) were recorded for each animal. For the purpose of comparison, samples were grouped by year of collection. Year of collection does not necessarily reflect year of exposure. Age determination for walruses (Odobenus rosmarus) was based on: 1) body conformation, 2) tusk size, and 3) facial characteristics (Fay, 1982). Age data were not available for other species.

Blood samples were collected by various investigators during studies of marine mammal population biology and ecology. Blood was routinely drawn from the extradural vein of pinnipeds and walruses. Samples were allowed to clot and then centrifuged. Serum was transferred to sterile vials. Sera were initially stored at -12C for several months and then transferred to -40 to -46C for periods lasting from several months to several years until the time of testing.

Sera were tested by means of a virus neutralization assay (Osterhaus et al., 1985). The test utilized: 1) seal kidney cells, 2) 50-100 TCID50 of the 1984 Pieterburen strain of PhHV1, and 3) 4 days of incubation before results were recorded on the basis of cytopathic changes.

Under normal test protocol, samples which neutralize PhHV-1 at a serum dilution of 1:5 or greater are considered indicative of previous natural exposure to the virus (Osterhaus et al., 1985). For the current study, a higher threshold dilution of 1:20 was selected. Samples that met or exceeded a titer of 20 will be referred to as "positive." All others will be referred to as "negative." This change in threshold was implemented in order to reduce the impacts of potential nonspecific neutralizing substances on test interpretation. If they are present, such substances can cause sera from "negative" animals to be incorrectly classified as being "positive." The higher threshold may have lead to an underestimation of actual antibody prevalences.

For walrus, a generalized linear model with a logit link (Agresti, 1990) was used to determine if there was a significant dependence of serum antibody prevalence on the following variables: 1) age, 2) sex, and 3) year of collection. All main and interaction effects of these variables were evaluated. During the modeling process, all higher order terms were removed from the model if they did not substantially (e.g., at P = 0.05) increase the "fit" of the model based on the likelihood ratio statistic. The SAS statistical software package was used to fit the model with maximum likelihood parameter estimates.

Confidence intervals for prevalence estimates were developed based on a binomial distribution (Bain and Englehardt, 1987).

#### RESULTS

Serum antibody prevalences are presented in Table 1. Prevalences were moderate to high in all pinniped species. Evidence of exposure was also found in small collections of sera from river otter and polar bear. These species also inhabit the marine environment. There was no evidence of exposure in sera from three arctic fox (Alopex lagopus).

Geographic location was not included in any logit models for predicting antibody status of walruses. With one exception, samples were collected from unique locations each year. Therefore, year of collection is confounded with location in the modeling process.

For walruses, neither gender nor year of collection had a significant effect on prevalence (P > 0.05). The only factor retained in the model was age (P = 0.04). Age is a continuous variable. Therefore, the final model is equivalent to logistic regression. The model predicts the probability of a "positive" test result as a function of age (Fig. 2):

$$\pi (AGE) = \frac{\exp (-0.5249 + 0.0428 \text{ x AGE})}{1 + \exp (-0.5249 + 0.0428 \text{ x AGE})}$$

## DISCUSSION

Many herpesviruses infect only a small number of closely-related host species. Therefore, it is possible that some of the results presented here may be due to cross reaction with herpesviruses other than PhHV-1. However, antibody titers are invariably much lower when sera are tested against heterologous alpha herpesviruses (Osterhaus et al., 1985). For purposes of this discussion, all "positive" test results were considered to be indicative of PhHV-1 exposure status.

Three general taxonomic groups were represented in the survey.

1) phocids -- ringed seal, spotted seal, harbor seal, bearded seal and ribbon seal;

2) odobenids and otariids - walrus, Steller sea lions (Eumetopias jubatus), and northern fur seal (Callorhinus ursinus); and

3) carnivores-river otter, sea otter, polar bear, and arctic fox.

Prevalences ranged from 22%-77% for the phocids, 34%-54% for the odobenids and otariids and 0%-43% for the carnivores. The three highest prevalences were seen in phocid seals. However, there was no clear-cut pattern among or between the three groups.

Harbor seals, Steller sea lions, and sea otters live primarily in the subarctic and temperate regions. In contrast, walrus, polar bear, and the remainder of the seal species live primarily in the arctic. There was no discernible pattern of prevalence between these two groups. Apparently, exposure of marine mammals to PhHV-1 or a related herpesvirus has been: 1) common, 2) geographically widespread, and 3) long term.

The logit model quantitatively predicts antibody prevalence as a function of age for walruses (Fig. 2). This model apparently reflects: 1) moderate exposure rates at an early age, and 2) continual opportunity for exposure throughout life.

The 73% antibody prevalence for harbor seals (Table 1) was substantially higher than the approximately 50% prevalence reported for this same species from the Wadden Sea (Frey et al., 1989). We know of no factors that could explain this apparent difference in exposure.

<u>Herpesvirus canis</u> infection in neonatal domestic dogs (<u>Canis familiaris</u>) is often fatal (Carmichael, 1970). Passive transfer of maternal antibody can mitigate the impact of infection. Dogs more than 2-3 weeks of age are usually able to survive infection (Carmichael, 1970). This change is attributed to development of an effective system of thermoregulation. Fatal PhHV-1 infections occur only in seals with immature or compromised immune systems. Other cohorts develop transient respiratory disease. Presumably, similar mechanisms determine the outcome of alpha herpesvirus infection in all species.

During the sample collection period, populations of the following species have declined: 1) Steller sea lion (Merrick et al., 1987), 2) northern fur seal (Fowler, 1990), and 3) harbor seal (Pitcher, 1990). No cases with clinical signs of PhHV-1 infection have been reported for either Steller sea lions or northern fur seals. A single case of fatal PhHV-1 infection in a harbor seal was recently reported from the coast of Washington state (Zaucha, pers. comm.). Thus, there is no apparent relationship between declines of these species and exposure to PhHV-1 or a related herpesvirus.

Census data suggest that populations of walrus (Gilbert, 1989) and polar bear (Amstrup et al., 1986) are stable. The geographic range of sea otters in Alaskan waters has expanded in recent decades (Rotterman and Simon-Jackson, 1988). This expansion is apparently a reflection of increased population size (Rotterman and Simon-Jackson, 1988). Population status for other species is unknown.

## **CONCLUSION**

Based upon serum antibody prevalences reported here, marine mammals in the waters of Alaska and Russia are commonly exposed to PhHV-1 or related herpesviruses. If this virus was highly pathogenic, epizootics would be common. There have been no documented PhHV-1 epizootics in these waters. Thus, we conclude that PhHV-1 has not been highly pathogenic in marine mammals from this region.

#### **ACKNOWLEDGMENTS**

The authors wish to thank the following individuals for collecting samples: Steve Amstrup, Vladimir Burkanov, John Burns, Don Calkins, Bob Elsner, Brian Fadely, Jim Faro, Francis Fay, Kathy Frost, Sue Hills, Jon Lewis, Richard Merrick, Bob Nelson, Lloyd Lowry, Ken Pitcher, Dan Reed, John Sease, Al Smith, Terry Spraker, Robert Suydam, Ken Taylor, and Pam Tuome.

## LITERATURE CITED

- Agresti, A. 1990. Categorical data analysis. John Wiley and Sons. New York, New York, USA. 558 pp. Amstrup, S. C., I. Stirling and J. W. Lentfer. 1986. Past and present status of polar bears in
- Alaska. Wildlife Society Bulletin 14: 241-254.
- Bain, L. J. and M. Englehardt. 1987. Introduction to probability and mathematical statistics. Duxbury Press. Boston, Massachusetts. USA. 565 pp. Borst G. H. A., H. C. Walvoort, P. J. H. Reijnders, J. S. van der Kamp and A. D. M. E.
- Osterhaus. 1986. An outbreak of herpesvirus infection in harbor seals (Phoca vitulina). Journal of Wildlife Diseases 22: 1-6.
- Carmichael, L. E. 1970. Herpesvirus canis: Aspects of pathogenesis and immune response. Journal of the American Veterinary Association 156: 1713-1724.
- Fay, F. H. 1982. Ecology and biology of the Pacific walrus, Odobenus rosmarus divergens. North American Fauna 74. U.S. Fish and Wildlife Service. Washington, D.C. USA. 279 pp.
- Fowler, C. W. 1990. Density dependence in northern fur seals (Callorhinus ursinus). Marine Mammal Science 6: 171-195.

- Frey, H. R., B. Liess, L. Haas, H. Lehmann and H. J. Marschall. 1989. Herpesvirus in harbour seals (<u>Phoca vitulina</u>): Isolation, partial characterization and distribution. Journal of Veterinary Medicine Series B 36: 699-708.
- Gilbert, J. R. 1989. Aerial census of Pacific walrus in the Chukchi Sea, 1985. Marine Mammal Science 5: 17-28.
- Harder, T. C., J. Plotz and B. Liess. 1991. Antibodies against european phocine herpesvirus isolates detected in sera of Antarctic seals. Polar Biology 11: 509-512.
- Harris, R. K., R. B. Moeller, T. P. Lipscomb, J. M. Pletcher, R. J. Haebler, P. A. Tuomi, C. R. McCormick, A. R. DeGange, D. Mulcahy and T. D. Williams. 1990. Identification of a herpes-like virus in sea otters during rehabilitation after the T/V Exxon Valdez oil spill. In: Sea otter symposium: Proceedings of a symposium to evaluate the response effort on behalf of sea otters after the T/V Exxon Valdez oil spill into Prince William Sound, Anchorage, Alaska, 17-19 April 1990. Biological Report 90(12). K. Bayha and J. Kennedy, eds. U.S. Fish and Wildlife Service. Anchorage, Alaska. 366-368 pp.
- Horvat, B., T. Wilhaus, H. R. Frey and B. Liess. 1989. Herpesvirus in harbour seals (<u>Phoca vitulina</u>): Transmission in homologous host. Journal of Veterinary Medicine Series B 36: 715-718.
- Kennedy, S., I. J. Lindstedt, M. M. McAliskey, S. A. McConnell and S. J. McCullough. 1992. Herpesviral encephalitis in a harbor porpoise (<u>Phocoena phocoena</u>). Journal of Zoo and Wildlife Medicine 23: 374-379.
- Kennedy-Stoskopf, S. M. K. Stoskopf, M. A. Eckhaus and J. D. Strandberg. 1986. Isolation of a retrovirus and a herpesvirus from a captive sea lion. Journal of Wildlife Diseases 22: 156-164.
- Lebich, M., T. C. Harder, H. R. Frey, I. K. G. Visser, A. D. M. E. Osterhaus and B. Liess. 1994. Comparative immunological characterization of type-specific and conserved Bcell epitopes of pinniped, felid and canid herpesviruses. Archives of Virology 136: 335-347.
- Lowenstine, L. J., L. J. Gage, D. M. Smith and C. FitzGerald. 1992. Mortality in neonatal Pacific harbor seals (Phoca vitulina richardsi) from the California coast: Possible role of a herpes-like virus infection. In: World Association of Veterinary Microbiologists, Immunologists and Specialists in Infectious Diseases 12th International Symposium Proceedings: New and Emerging Infectious Diseases. B. Osburn, G. Castrucci and C. Shore, eds. University of California-Davis. Davis, California. 243-247 pp.
- Merrick, R. L., T. R. Loughlin and D. G. Calkins. 1987. Decline in abundance of the northern sea lion, <u>Eumetopias jubatus</u>, in Alaska, 1956-86. Fishery Bulletin 85: 351-365.
- Osterhaus, A. D. M. E., H. Yang, H. E. M. Spijkers, J. Groen, J. S. Teppema and G. van Steenis. 1985. The isolation and partial characterization of a highly pathogenic herpesvirus from the harbour seal (<u>Phoca vitulina</u>). Archives of Virology 86: 239-251.
- Pitcher, K. W. 1990. Major decline in number of harbor seals, <u>Phoca vitulina richardsi</u>, on Tugidak Island, Gulf of Alaska. Marine Mammal Science 6: 121-134.
- Rotterman, L. M. and T. Simon-Jackson. 1988. Sea otter. Pages 237-271 in Selected marine mammals of Alaska: Species accounts with research and management recommendations. J. W. Lentfer, ed. U.S. Marine Mammal Commission. Washington, D.C.
- Spraker, T. R., L. F. Lowry and K. J. Frost. 1994. Gross necropsy and histopathological lesions found in harbor seals. <u>In</u>: Marine Mammals and the <u>Exxon Valdez</u>. T. R. Loughlin (ed.). Academic Press. New York, New York. In press.

Visser, I. K. G., J. S. Teppema and A. D. M. E. Osterhaus. 1991. Virus infections of seals and other pinnipeds. Reviews in Medical Microbiology 2: 105-114.

Species	Year(s) of collection	Prevalence	95% Confidence intervals	Locations collected
Ringed seal ( <u>Pusa</u> hispida)	1978-1992	10/13 <sup>a</sup> (77%)	46-95	<u>н,к</u> <sup>b</sup>
Spotted seal ( <u>Phoca</u> <u>largha</u> )	1978-1993	73/97 (75%)	65-83	H,J,K,N,O,P
Harbor seal ( <u>Phoca</u> <u>vitulina</u> )	1978-1994	161/219 (73%)	67-79	A,B,C,D,E,G
Walrus ( <u>Odobenus</u> <u>rosmarus</u> )	1981-1987	208/384 (54%)	49-59	F,I,M
Steller Sea lion ( <u>Eumetopias</u> jubatus)	1978-1993	41/88 (46%)	36-58	A,B,C,D,E,G
Bearded seal ( <u>Erignathus</u> <u>barbatus</u> )	1978-1990	11/24 (45%)	26-67	H,K
River otter ( <u>Lutra</u> <u>canadensis)</u>	1989	3/7 (43%)	10-82	В
Northern fur seal ( <u>Callorhinus</u> ursinus)	1980	63/185 (34%)	27-41	G
Ribbon seal (Histriophoca fasciata)	1978-1979	7/32 (22%)	10-40	Н
Sea otter ( <u>Enhydra</u> <u>lutris)</u>	1989	6/61 (10%)	4-20	В

Table 1. Serum antibody prevalence of phocid herpesvirus-1 in selected marine mammal species sampled off the coast of Alaska and Russia, 1978-1994.

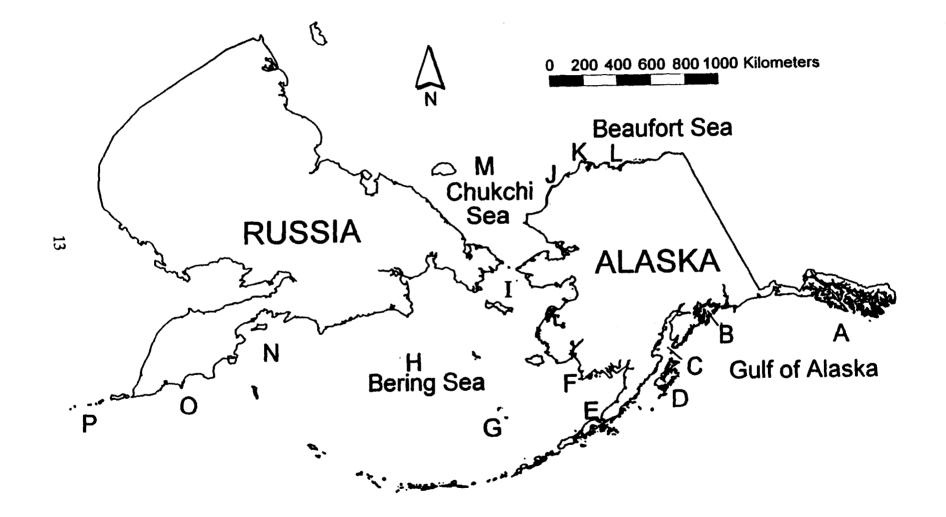
Table	1.	Continu	ed.

		<u> </u>	95%	95%	
Species	Year(s) of collection	Prevalence	Confidence intervals	Locations collected	
Polar bear ( <u>Ursus</u> maritimus)	1984	1/12 (8%)	0-38	K	
Arctic fox ( <u>Alopex</u> lagopus)	1 <b>99</b> 0	0/3 (0%)	0-71	L	

<sup>a</sup> Number of samples with significant levels of antibody/total number of samples tested. <sup>b</sup> A = Southeast Alaska; B = Prince William Sound; C = Cook Inlet; D = Kodiak Island; E = Alaska Peninsula; F = Kuskokwim Bay; G = Pribilof Islands; H = Bering Sea; I = Bering Strait; J = Northwest Alaska; K = Barrow; L = Prudhoe Bay; M = Chukchi Sea; N = Karaginsky Gulf; O = Southeast Kamchatka Peninsula; P = Kuril Islands.

Figure 1. Location of collection sites for marine mammals included in phocid herpesvirus-1 serologic survey.

- A -- Southeast Alaska
- B -- Prince William Sound
- C -- Cook Inlet
- D -- Kodiak Island E -- Alaska Peninsula
- F -- Kuskokwim Bay
- G -- Pribilof Islands
- H -- Bering Sea
- I -- Bering Strait J -- Northwest Alaska K -- Barrow
- L -- Prudhoe Bay
- M -- Chukchi Sea
- N -- Karaginsky Gulf O -- Southeast Kamchatka Peninsula P -- Kuril Islands



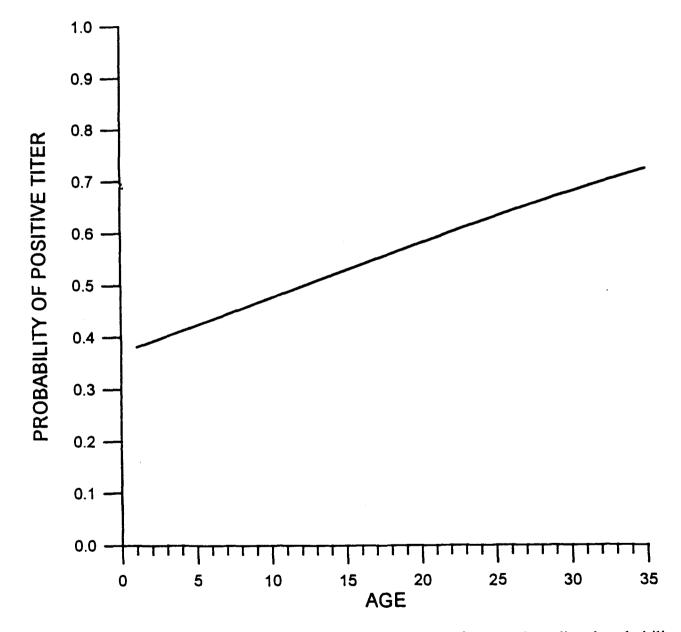


Figure 2. Relationship between walrus (<u>Odobenus rosmarus</u>) age and predicted probability of a serum sample exceeding threshold titer (>20) for phocid herpesvirus-1.

APPENDIX B. Submitted to Journal of Wildlife Diseases (some minor format changes).

## RH: ZARNKE ET AL. - LYNX TRICHINELLOSIS

## PREVALENCE OF <u>TRICHINELLA NATIVA</u> IN LYNX (<u>FELIS LYNX</u>) FROM ALASKA, 1988-1993

# RANDALL L. ZARNKE<sup>1</sup>, ALVIN A. GAJADHAR<sup>2</sup>, GREGORY B. TIFFIN<sup>3</sup>, AND JAY M. VER HOEF<sup>1</sup>

<sup>1</sup>Alaska Department of Fish and Game, 1300 College Road, Fairbanks, Alaska 99701 USA

<sup>2</sup>Agriculture and Agri-Food Canada, Health of Animals Laboratory, 116 Veterinary Road, Saskatoon, Saskatchewan S7N 2R3 CANADA

<sup>3</sup>Agriculture and Agri-Food Canada, Animal Diseases Research Institute, PO Box 640, Lethbridge, Alberta T1J 3Z4 CANADA

Corresponding author:

Dr Randall L. Zarnke, Alaska Department of Fish and Game, 1300 College Road, Fairbanks, Alaska 99701 USA

ABSTRACT: Lynx (<u>Felis lynx</u>) carcasses were collected during the 1989-1990 through 1992-1993 trapping seasons in Alaska. Seven areas were represented. Tongue samples were removed from 1065 carcasses. Specimens were examined for the presence of <u>Trichinella</u> nativa larvae by means of enzymatic digestion. Overall prevalence was 21%. Both prevalence and number of larvae per gram of host tissue were directly related to age of the host. Age-specific prevalence ranged from 4% for kittens up to 59% for lynx 5 yr of age and older. For infected lynx, intensity ranged from 0.27 larvae per gram of host tissue for kittens up to 2.35 larvae per gram for lynx 3 yr of age and older. Location-specific prevalence ranged from 19% to 27%. Year-specific prevalence ranged from 13% to 26%. Prevalence in both males and females was 21%.

Key words: Alaska, lynx, Felis lynx, trichinellosis, Trichinella nativa

#### INTRODUCTION

<u>Trichinella nativa</u> is a common parasite of free-ranging carnivores and omnivores, including lynx (Felis lynx) (Zimmerman, 1971). Transmission occurs by ingestion of infected meat.

Prevalence of trichinellosis in lynx ranged from 29% to 50% in Europe and the former Soviet Union (Churina et al., 1979; Horning, 1983; Brglez, 1989; A. Oksanen, pers. comm.). Prevalence in three widely separated areas of Canada was 3% (Smith and Snowdon, 1988). Prevalence in lynx from Alaska was 23% (Rausch et al., 1956). Most previous studies have been based on small sample sizes. The survey in Finland (A. Oksanen, pers. comm.) was an exception.

Humans in many areas of Alaska commonly eat lynx meat (Charnley, 1984). A case of human trichinellosis in Europe has been attributed to consumption of infected lynx meat (Horning, 1983). Therefore, a survey of <u>T. nativa</u> in lynx from Alaska was initiated in order to provide current data regarding the potential for human exposure from eating lynx meat.

The objective of this project was to determine the relationship of the following host parameters to prevalence: 1) geographic location, 2) age, 3) gender, and 4) year of collection. In addition, intensity of infection (number of <u>T. nativa</u> larvae per gram of host tissue) was determined.

#### MATERIALS AND METHODS

Seven areas of Alaska were represented (Fig. 1). Approximate boundaries of each area are as follows:

1) Central Arctic - 150 to 156 W; 67 to 68 30 N

2) Eastern Arctic - 144 to 146 W; 66 30 to 67 30 N

3) Central Interior - 145 to 149 W; 64 to 66 N

4) Eastern Interior - 141 to 143 W; 63 to 65 N

5) Eastern Border - 141 to 143 W; 62 to 63 N

6) Southeast Mainland - 143 to 145 W; 61 to 62 30 N

7) Southcentral - 145 to 146 W; 62 to 63 N

Carcasses were obtained from trappers. Lynx trapping seasons in Alaska normally begin in November or December and end in January or February of the following year. Therefore, collection periods will be indicated as a 2 yr period. For example, the current study began with the 1989-1990 season and ended with the 1992-1993 season. Gender was determined by examination of internal sex organs. Age was determined by counting cementum annuli of a canine tooth (Crowe, 1972). Most animals were represented by entire carcasses. However, only heads were available for 107 animals collected during the 1989-1990 and 1990-1991 seasons from the Eastern Arctic study area.

T. nativa larvae encyst in muscle tissue. Common diagnostic specimens include tongue, masseter, and diaphragm. Preliminary study demonstrated that tongue and masseter provided nearly identical results and were equally suitable. For this study, tongue was selected due to amount of tissue available and ease of collection.

Frozen tongues were shipped to the Animal Diseases Research Institute in Lethbridge, Alberta, Canada for analysis. Ten gram portions of each tongue were chopped into small pieces and subjected to enzymatic digestion (Schad et al., 1984). Resulting digested material was cleaned and examined microscopically for the presence of <u>T. nativa</u> larvae. Specimens and animals which harbored <u>T. nativa</u> larvae will be referred to as "positive." Specimens and animals with no <u>T. nativa</u> larvae will be referred to as "negative." For ease of evaluation, results were grouped by year of collection.

Selected isolates were identified to the species level based on species-specific regions within the excised expansion segment of the large subunit ribosomal DNA. The DNA was amplified by specific polymerase chain reaction primers. Products were identified by agarose gel electrophoresis (Zarlenga and Dame, 1992).

Data were analyzed by a logit generalized linear model (Agresti, 1990) to test for relationship of both prevalence and intensity to the following independent variables: 1) age, 2) gender, 3) geographic location, and 4) year of collection. All four independent variables were categorical. All main and pairwise interaction effects were considered in the model. Effects which were not significant ( $\alpha > 0.05$ ) were removed until the most parsimonious model was obtained. The final model contains only those effects, and possible interactions, which are significant with a log-likelihood ratio statistic at  $\alpha$  0.05.

#### RESULTS

Samples were collected from 1065 lynx. Prevalence was not significantly affected by interactions of independent variables. Prevalence was 21% in both males (110 positive of 512 tested) and females (89 positive of 428 tested). Age was the only independent variable which was significantly ( $\mathbf{P} < 0.0001$ ) related to prevalence (Table 1). The number of <u>T. nativa</u> larvae per gram of lynx tissue (LPG) was weakly related ( $\underline{P} = 0.072$ ) to age of lynx (Table 1). Only positive animals were included in this analysis. There were no statistically significant differences in prevalence between study areas (Table 2).

Year-specific prevalence of trichinellosis in lynx was as follows:

1) 17 positive of 129 tested (13%) for the 1989-1990 season 2) 41 positive of 210 tested (20%) for the 1990-1991 season

3) 84 positive of 419 tested (20%) for the 1991-1992 season

4) 75 positive of 285 tested (26%) for the 1992-1993 season

There were no significant differences in year-specific prevalences.

### DISCUSSION

Prevalence was essentially identical in male and female segments of the populations. Transmission of T. nativa occurs by means of ingesting infected meat. Therefore, it can be inferred that these two cohorts have similar feeding habits.

The logit model revealed that age was significantly correlated to prevalence (Table 1). Presumably, opportunities for exposure to T. nativa are available throughout the life of a lynx. Apparently, exposure is cumulative with each additional year of life.

The LPG increased in each age cohort (Table 1). T. nativa larvae do not reproduce in situ. Thus, LPG can increase only if additional larvae are ingested. Based on these data, it appears that lynx are subjected to repeated exposure throughout their lives.

Transmission of trichinellosis from lynx to other potential hosts may be at least partially dependent on these repeated exposures. Viability of T. nativa larvae in situ declines as time passes (Marquardt and Demaree, 1985). In the absence of repeated exposure, infectivity of lynx meat might decline to a negligible level.

Although there were no statistically significant differences in prevalences between study areas, prevalence was highest in lynx from the Central Arctic study area (Table 2).

The mean age of lynx collected in the Central Arctic region was noticeably higher compared with other areas. For example, the mean age of lynx collected during the 1990-1991 season in the Central Arctic study area was  $5.8 \text{ yr} \pm 1.2 \text{ yr}$  (n = 10). By contrast, the mean age of animals collected from the Eastern Border study area during the 1990 to 1991 season was  $1.6 \text{ yr} \pm 0.2 \text{ yr}$  (n = 38). As stated above, age is the factor most highly-correlated to prevalence (Table 1). Therefore, it is not surprising that prevalence was highest in the region where mean age was also highest.

Chronology of initial exposure to  $\underline{T}$ , <u>nativa</u> cannot be determined by means of enzymatic digestion. Therefore, there is no way of knowing the precise year when the adult lynx represented in this study were initially exposed. Year-of-exposure is obvious for kittens. Therefore, year-specific prevalence for the kitten cohort could be readily calculated. Unfortunately, the number of kittens included in each yearly collection was inadequate to allow a meaningful statistical evaluation.

Populations of both lynx and snowshoe hares (<u>Lepus americanus</u>) experience a predictable 10-yr cycle of abundance (Brand et al., 1976). This similarity in population dynamics is due to the strong dependence of lynx upon hares as a food source (Keith, 1963). In years of hare abundance, lynx subsist primarily on a diet of hares (Brand and Keith, 1979). Hares are primarily herbivorous. Therefore, trichinellosis is rare in hares (Rausch et al., 1956). Thus, exposure of lynx to <u>T. nativa</u> would theoretically be low in years of hare abundance.

Conversely, in years when hares are scarce lynx presumably use other animals as food sources. Alternate prey species might include red fox (<u>Vulpes vulpes</u>), marten (<u>Martes americana</u>) or even other lynx. Prevalence of trichinellosis is higher in these omnivorous and carnivorous species (Rausch et al., 1956). Thus, there is a greater opportunity for transmission of trichinellosis to lynx.

Hare and lynx populations peaked in 1990-1991 in many areas of Alaska (Abbott, 1993). Both were declining in the last 2 yr of this study. Data presented above suggest a minor increase in prevalence of trichinellosis in lynx during this time frame. However, there were no statistically significant differences in year-specific prevalences. The current survey did not cover an adequate time period to clearly elucidate a chronologic pattern, if any exists. Year-specific patterns of prevalence may have been evident if the survey covered 15 to 20 yr.

In addition, there was no apparent year-specific pattern of prevalence for any of the individual study areas. Lynx and hare population cycles are grossly synchronous throughout Alaska (Stephenson and Karczmarczyk, 1989). However, there may be minor differences where lynx population density in one area peaks 1 to 2 yr prior to the population in another region (Stephenson and Karczmarczyk, 1989). Therefore, combining test results from all study areas may effectively mask differences in year-specific prevalence which might occur at individual areas. The logit model is capable of detecting complex interactions of multiple causative factors referred to above such as: 1) location, 2) age, and/or 3) year of collection. No such relationships were evident.

## **Management Implications**

Trichinellosis poses no apparent threat to the long-term viability of lynx or other wildlife populations. However, there is potential for transmission of <u>T</u>. <u>nativa</u> from lynx to humans (Horning, 1983). Therefore, results of this study and cooking recommendations for lynx meat will be shared with trappers and other consumers.

#### ACKNOWLEDGMENTS

The following individuals generously contributed their time by collecting and submitting specimens: Toby Boudreau, Terry Doyle, Steve DuBois, Robin Eagan, Craig Gardner, Howard Golden, Danny Grangaard, Thomas Lowy, Karen Ogden, Craig Perham, Chris Scranton, and Shelli Swanson. Dr David Worley of Montana State University provided invaluable advice and assistance during the early stages of this project. The authors thank the Alaska Trappers Association for publicizing and facilitating the carcass collection program. This project was supported by US Federal Aid in Wildlife Restoration Projects.

## LITERATURE CITED

- ABBOTT, S. M. (editor). 1993. Furbearers. Federal aid in wildlife restoration survey and inventory management report. Project W-23-3 and W-23-4. Alaska Department of Fish and Game, Juneau, Alaska, 303 pp.
- AGRESTI, A. 1990. Categorical data analysis. John Wiley and Sons. New York, New York, 558 pp.
- BRAND, C. J. AND L. B KEITH. 1979. Lynx demography during a snowshoe hare decline in Alberta. The Journal of Wildlife Management 43: 827-849.

AND C. A. FISCHER. 1976. Lynx responses to changing snowshoe hare densities in central Alberta. The Journal of Wildlife Management 40: 416-428.

- BRGLEZ, J. 1989. The incidence of trichinellosis in some wild animals in Yugoslavia. In Proceedings of the Seventh International Conference on Trichinellosis. C.  $\overline{E}$ . Tanner, A. R. Martinez-Fernandez and F. Bolas-Fernandez (editors). Consejo Superior de Investigaciones Científicas Press. Madrid, Spain. pp. 412-415.
- CHARNLEY, S. 1984. Human ecology of two central Kuskokwim communities: Chuathbaluk and Sleetmute. Alaska Department of Fish and Game. Subsistence Division Technical Paper Number 81, Juneau, Alaska, 391 pp.
- CHURINA, N. V., Y. M. MALAFEEV, AND A. I. RUSSKOVA. 1979. Trichinelliasis in lynx in the Central Urals and Trans-Ural region. Ekologicheskie issledovaniya v lesnykh i lugovykh biogeotsenosakh ravnin Zaural'ya. Informatsionnye materialy Talitskogo Statsionara, 1979. Sverdlovsk, Russia, pp. 51-53. CROWE, D. M. 1972. The presence of annuli in bobcat tooth cementum layers. The
- Journal of Wildlife Management 36: 1330-1332.
- HORNING, B. 1983. Short report concerning Trichinella research in Switzerland (1979-1982). Wiadomosci Parazytologiczne 29: 638-640.
- KEITH, L. B. 1963. Wildlife's ten year cycle. University of Wisconsin Press, Madison, Wisconsin, 201 pp.

MARQUARDT, W. C. AND R. S. DEMAREE, Jr. 1985. Parasitology. MacMillan Publishing Company, New York, New York, 446 pp. RAUSCH, R., B. B. BABERO, R. V. RAUSCH, AND E. L. SCHILLER. 1956. Studies on

- the helminth fauna of Alaska. XXVII. The occurrence of larvae of Trichinella spiralis in Alaskan mammals. The Journal of Parasitology 42: 259-271.
- SCHAD, G. A., D. A. LEIBY, AND K. D. MURRELL. 1984. Distribution, prevalence and intensity of <u>Trichinella spiralis</u> in furbearing animals of Pennsylvania. The Journal of Parasitology 70: 372-377.
- SMITH, H. J. AND K. E. SNOWDON. 1988. Sylvatic trichinosis in Canada. Canadian Journal of Veterinary Research 52: 488-489.
- STEPHENSON, R. O. AND P. KARCZMARCZYK. 1989. Development of techniques for evaluating lynx population status in Alaska. Federal aid in wildlife restoration

research final report. Project W-23-1, Study 7.13. Alaska Department of Fish and Game, Juneau, Alaska, 95 pp.

- ZARLENGÁ, D. T. AND J. B. DÁME. 1992. The identification and characterization of a break within the large subunit ribosomal DNA of <u>Trichinella spiralis</u>: comparison of gap sequences within the genus. Molecular and Biochemical Parasitology 51: 281-290.
- ZIMMERMAN, W. J. 1971. Trichinosis. In Parasitic diseases of wild animals. J. W. Davis and R. C. Anderson (editors). Iowa State University Press, Ames, Iowa, pp. 127-139.

Received for publication 3 October 1994.

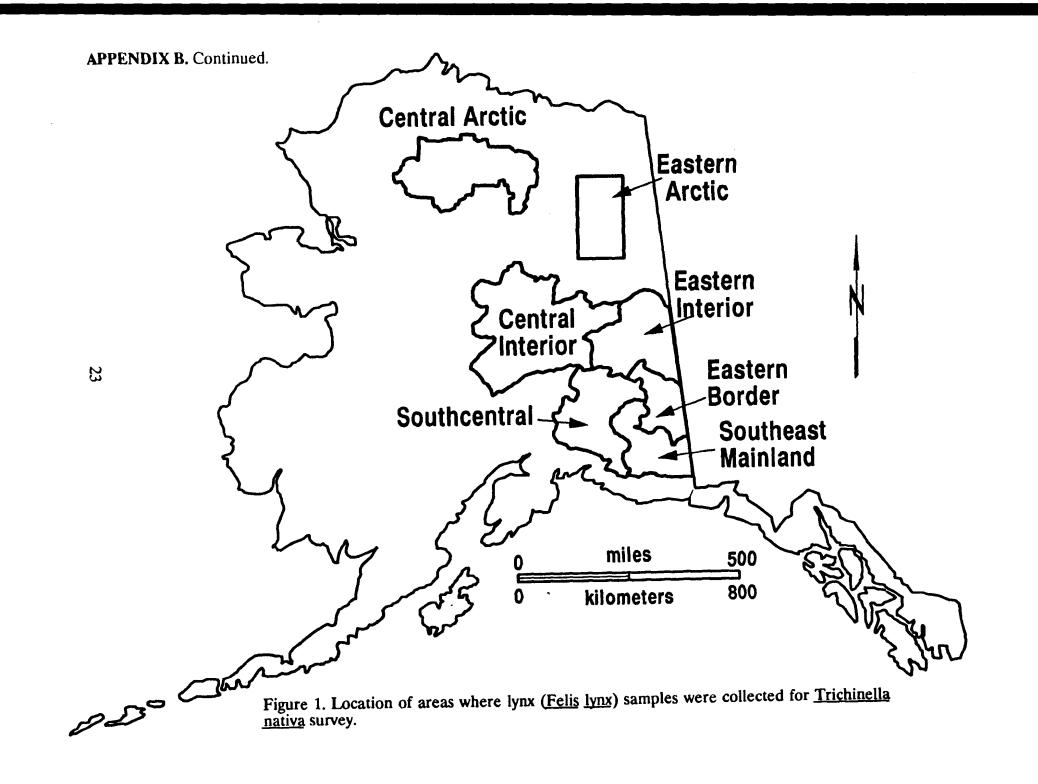
Age	Number with <u>Trichinella nativa</u> larvae	Number sampled	Percent	Mean intensity of samples with <u>Trichinella nativa</u> larvae
Kitten <sup>a</sup> 1 2 3 4	4 58 91 19 7	90 341 338 74 17 29	4 17 27 26 41 59	0.27 <sup>b</sup> 1.84 2.11 2.35 <sup>c</sup>

Table 1. Age-specific prevalence and intensity of <u>Trichinella nativa</u> infection in lynx (<u>Felis</u> <u>lynx</u>) from Alaska, 1988 to 1993.

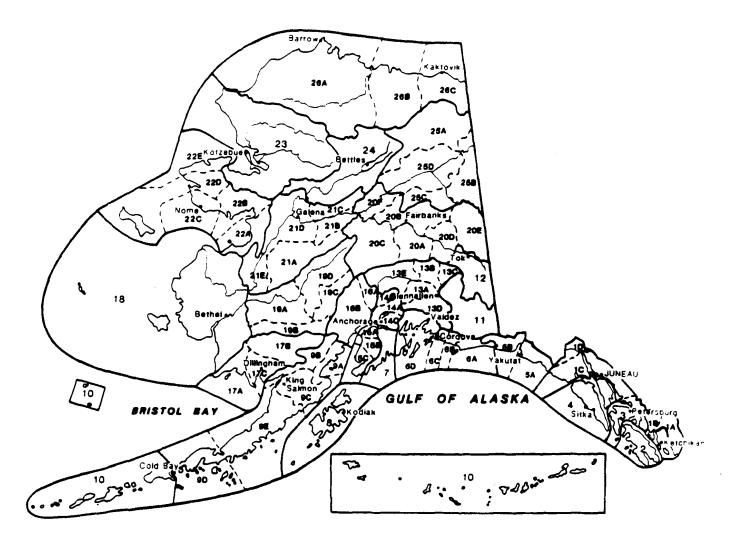
<sup>a</sup> Age determined by counting cementum annuli of tooth. <sup>b</sup> Number of <u>Trichinella nativa</u> larvae per gram of lynx tissue. <sup>c</sup> Mean value for all lynx 3 yr.

Location	Number of samples with <u>Trichinella nativa</u> larvae	Number sampled	Percent
Central Arctic	28	103	27
Southcentral	38	157	24
Eastern Interior	23	105	22
Eastern Border	37	171	22
Southeast Mainland	14	68	21
Central Interior	63	328	19
Eastern Arctic	25	133	19

Table 2. Location-specific prevalence of <u>Trichinella nativa</u> infection of lynx (<u>Felis lynx</u>) from Alaska, 1988 to 1993.

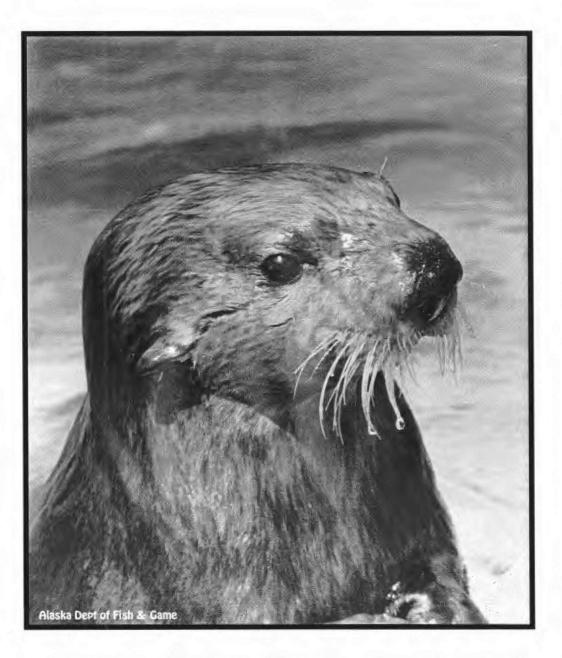


## Alaska's Game Management Units



The Federal Aid in Wildlife Restoration Program consists of funds from a 10% to 11% manufacturer's excise tax collected from the sales of handguns, sporting rifles, shotguns, ammunition, and archery equipment. The FederalAid program allots funds back to states through a formula based on each state's geographic area and number of paid hunting license holders. Alaska receives a maximum 5% of revenues collected each year. TheAlaska Department of Fish and Game uses federal aid funds to help restore, conserve, and manage wild birds and mammals to benefit the

public. These funds are also used to educate hunters to develop the skills, knowledge, and attitudes for responsible hunting. Seventy-five percent of the funds for this report are from Federal Aid.



The Alaska Department of Fish and Game administers all programs and activities free from discrimination based on race, color, national origin, age, sex, religion, marital status, pregnancy, parenthood, or disability. The department administers all programs and activities in compliance with Title VI of the Civil Rights Act of 1964, Section 504 of the Rehabilitation Act of 1973, Title II of the Americans with Disabilities Act of 1990, the Age Discrimination Act of 1975, and Title IX of the Education Amendments of 1972.

If you believe you have been discriminated against in any program, activity, or facility, or if you desire further information please write to ADF&G, P.O. Box 25526, Juneau, AK 99802-5526; U.S. Fish and Wildlife Service, 4040 N. Fairfax Drive, Suite 300 Webb, Arlington, VA 22203 or O.E.O., U.S. Department of the Interior, Washington DC 20240.

For information on alternative formats for this and other department publications, please contact the department ADA Coordinator at (voice) 907-465-6077, (TDD) 907-465-3646, or (FAX) 907-465-6078.