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VIRAL AND BACTERIAL SEROLOGY OF FREE-RANGING PACIFIC WALRUS

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ABSTRACT: Serum or heparinized plasma samples were obtained between 1994 and 1996 from 20 male and 20 female adult free-ranging Pacific walrus (*Odobenus rosmarus divergens*) from St. Lawrence Island and Round Island, Alaska. Samples were screened for antibodies to some potentially pathogenic bacteria and viruses. No sample had detectable antibody to *Brucella* spp. Three of 40 (8%) had low antibody titers to *Leptospira interrogans* serovars. Phocine distemper virus antibodies were not detected. Serologic responses to one or more caliciviruses (San Miguel sea lion virus 12 or vesicular exanthema of swine serotypes E54, F55, G55, 1934B) were detected in 18% (seven of 40) walrus. Antibodies to one or more subtypes of influenza A virus (H10, N2, N3, N5, N6, N7) were detected in 21% (eight of 38). Periodic screening of free-ranging populations for exposure to infectious diseases has become an important component of bio-monitoring programs to facilitate understanding and detecting trends in marine mammal populations.

Key words: *Brucella,* calicivirus, influenza A virus, *Leptospira interrogans, Odobenus rosmarus divergens,* phocine distemper virus, serologic survey, walrus.

INTRODUCTION

Leptospira spp. (Smith et al., 1977; Gulland, 1999), *Brucella* spp. (Foster et al., 1996; Nielson et al., 1996, 2001; Tryland et al., 1999), phocine distemper virus (PDV) (Osterhaus et al., 1988; Duignan et al., 1995, 1997; Kennedy, 1998), caliciviruses (Barlough et al., 1986a, c), influenza A virus (Geraci et al., 1982; Hinshaw et al., 1984; Callan et al., 1995; Danner et al, 1998), and phocid herpesvirus-1 (Zarnke et al., 1997) have been documented in free-ranging pinniped populations. Some have been responsible for significant pinniped morbidity or mortality. There have been limited studies of bacterial or viral pathogens in either Pacific walrus (*Odobenus rosmarus divergens*) (Fay, 1982; Smith et al., 1983; Barlough et al., 1986c, 1987b, 1988; Osterhaus et al., 1988; Zarnke et al., 1997; Danner et al., 1998) or Atlantic walrus (*O. rosmarus rosmarus*) (Duignan et al., 1994; Nielsen et al., 1996, 2000, 2001). Each study focused on one, or at most two, agents (calicivirus, PDV, *Brucella* spp., serovars of *Leptospira*, influenza A, or phocid herpesvirus-1), and most were conducted in the late 1970s or early 1980s. The objectives of this study were to determine antibody levels to potential pinniped pathogens in samples from free ranging Pacific walrus obtained between 1994–96 and to compare these findings to previous serologic studies of Pacific and Atlantic walrus. Testing was conducted for *Leptospira interrogans* serovars, *Brucella* spp., PDV, influenza A virus, and calicivirus strains. Determining the disease exposure of free-ranging animals was intended as a first step in the establishment of a broader population biomonitoring program. Such data and programs are increasingly useful in detecting and understanding changes in marine mammal populations and ultimately to a more thorough understanding of overall marine ecosystem health (Becker et al., 1994).

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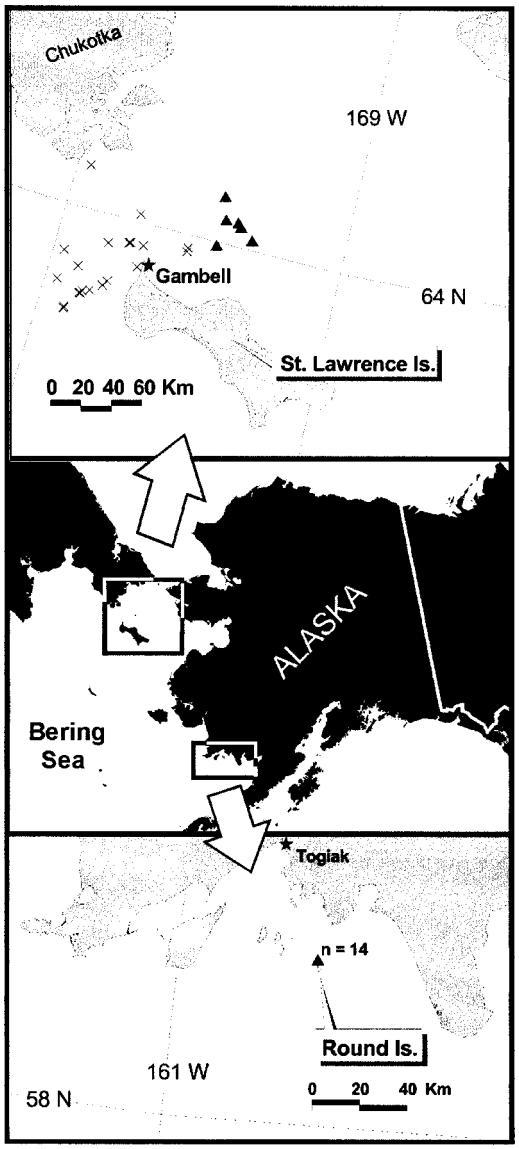

FIGURE 1. Locations where Pacific walrus serum samples were collected (X females, ▲males).

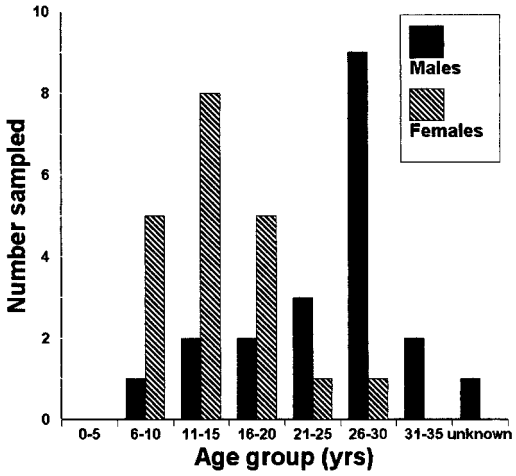

FIGURE 2. Age distribution of sampled Pacific walrus.

MATERIAL AND METHODS

Blood samples were collected from 20 male and 20 female adult Pacific walrus harvested by native Alaskans during May 1994, 1995, and 1996 near Gambell, St. Lawrence Island (63°46′47″N, 171°44′30″W) (six males and 20 females) and during October 1995 and 1996 on Round Island (58°37′05″N, 159°59′50″W) (14 males), Alaska, USA (Fig. 1). Collection of blood samples was authorized by Fish and Wildlife Service permits (PRT − 778099, 786616, 792101) or the Fish and Wildlife Service Walrus Program.

Samples were collected into plain or sodium heparin tubes from either freely flowing wounds or from the heart within 30 min postmortem and held at ambient temperature (−5 C to 5 C) until processed. Samples were centrifuged within 9 hr of collection. Time from centrifugation to freezing of serum or plasma varied from 0.25 to 12.5 hr (mean = 2.1 hr). In the field, plasma and sera were stored in chest freezers, or portable liquid nitrogen tanks, for 2 to 3 wk until transferred to a freezer (−20 C or −70 C) for intermediate storage prior to shipment by overnight delivery to laboratories. Walrus were 6- to 31-yr-old (Fig. 2), based on visual counts of lower canine cementum growth layer groups (Fay, 1982; Garlich-Miller et al., 1993).

Leptospira serology was performed for five serovars of *L. interrogans* (canicola, hardjo, grippotyphosa, icterohaemorrhagiae/copenhageni, pomona). Testing was conducted at the New York State Diagnostic Laboratory (College of Veterinary Medicine, Ithaca, New York, USA) by the microscopic agglutination test (MAT) (Cole et al., 1979; Rubin et al., 1981; Ellinghausen et al., 1984). Samples were considered positive if 50% or more of live *Leptospira* agglutinated at the screening dilution of 1:100. Titers of positive sera were determined by assay of serial dilutions of the sample. In order to better define the serologic responses, positive sera were retested with a panel of 18 *L. interrogans* serovars (the initial five and serovars australis, autumnalis, ballum, bataviae, bratislava, icterohaemorrhagiae/icterohaemor-

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rhagiae, javanica, pyrogenes, saxkoebing, sejroe, szwajizak, tarassovi, and wolffi).

Brucella spp., PDV, and calicivirus serology was performed at the Foreign Animal Disease Diagnostic Laboratory (FADDL; USDA, APHIS-VS-NVSL, Plum Island, New York). Undiluted samples were tested by the card test (Miller et al., 1999) for antibodies to *B. abortus*. If any granularity was observed, the sample was also tested by the tube agglutination test (Miller et al., 1999). Samples agglutinating completely at 1:25 or incompletely at 1:50 were considered to be positive for antibody to *Brucella* spp.

Samples were serially diluted from 1:20 to 1:160 and tested by virus neutralization test for antibody to PDV (Duignan et al., 1994). Control wells observed to be contaminated by bacteria or to be non-specifically toxic to Vero cells were recorded as toxic and the sample was regarded as negative at that dilution. Test wells exhibiting cytopathogenic effects typical of the virus were recorded as positive, and the endpoint titer of the sample was calculated by the Spearman-Karber method (Cottral, 1978). Samples were considered to be positive for antibody to PDV if the endpoint titer was ≥64.

Samples were serially diluted from 1:20 to 1:180 and tested by virus neutralization for antibody to a panel of caliciviruses (San Miguel sea lion virus [SMSV] strains 1, 2, 4–13 and vesicular exanthema of swine virus [VES] strains A48, B51, C52, D53, E54, F55, G55, H54, I55, J56, K54, 1934B, Tillamook, and Walrus) (O'Hara et al., 1998.). Determination of the toxicity and the endpoint titer of the sample were as described for PDV serologic testing. Samples were considered to be positive for antibody to a serotype of calicivirus if the endpoint titer was ≥32.

Thirty-eight undiluted samples were tested at the FADDL for antibodies to influenza A virus by the agar gel immunodiffusion (AGID) test. Samples precipitating the test antigen and forming a line of identity with the reference reagent serum were classified as positive. Agar gel immunodiffusion positive samples were further tested at the National Veterinary Services Laboratories (NVSL, Ames, Iowa, USA) by the hemagglutination-inhibition (hemagglutinin subtypes H1–H14) (Palmer et al., 1975) and neuraminidase-inhibition (neuraminidase subtypes N1–N9) (Van Deusen et al., 1983) tests to determine the subtype specificity of antibodies to the hemagglutinin and neuraminidase, respectively. Hemagglutination-inhibition titers of 8 or higher were considered positive and indicative of previous exposure to influenza A virus. For neuraminidase-inhibition testing a 1:2 dilution of sample was used and any inhibition was considered a positive test result.

Comparisons of the frequencies of positive antibody titers to *L. interrogans* serovars, calicivirus, and influenza A were analyzed for statistical significance ($P < 0.05$) by the Fisher exact test for sex and collection location and by the Chi squared test for year of collection (Sokal and Rohlf, 1969). Sample sizes for ages or age classes were too small to test for significant differences.

RESULTS

None of the walrus had detectable antibodies to four *L. interrogans* serovars (canicola, hardjo, icterohaemorrhagiae/copenhageni, pomona). Eight percent (three of 40 walrus; all three were males sampled on Round Island) had low titers (100 or 200) to *L. interrogans* serovar grippotyphosa. Sera were available from two of these three positive animals for further testing. When retested, neither had antibodies to these five serovars, but each was positive (titers of 100) for *L. interrogans* serovar icterohaemorrhagiae/icterohaemorrhagiae. In addition, one also had a titer of 100 to *L. interrogans* serovar ballum. There was a significant difference in the number of positive responses to *L. interrogans* serovars in walrus sampled at Round Island (3/14) compared to those sampled at Gambell (0/26) (Fisher exact test, $P = 0.04$). There was no significant difference in the frequency of titers to *L. interrogans* serovars by sex or collection year.

No walrus had antibody titers to *Brucella* spp. Twenty-seven walrus were negative for PDV antibodies and samples from the remaining 13 walrus were toxic.

Eight of 38 (21%) walrus were AGID-positive for influenza A antibodies. The positive animals included four female and two male walrus from St. Lawrence Island, and two males from Round Island. Each of these walrus had antibodies specific for hemagglutinin subtype H10, with titers ranging from 8 to ≥32. In addition, two walrus had antibodies to neuraminidase subtypes N7; two were positive for subtypes N2, N3, and N7; one was positive

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for subtypes N2 and N7; one was positive for subtypes N2, N3, N5, N6, and N7; and two were negative for specific neuraminidase antibodies. There were no statistically significant differences in the frequency of influenza A antibody titers in walrus on the basis of sex, collection location, or year of sampling.

Antibodies to one or more calicivirus strains were present in 18% (seven of 40) walrus. Five females sampled at St. Lawrence Island had antibodies: one had antibodies to SMSV 12 (titer of 57) and VES 1934B (titer of 57); one each to VES F55 (titer of 140) or VES E54 (titer of 140); and two to VES G55 (titers of 60; ≥ 230). Two males, one each from St. Lawrence and Round Islands, had antibodies to SMSV 12 (titers of 180; ≥ 230). There was a significant difference (X^2 = 7.48, df = 2; P = 0.02) in the frequency of calicivirus test results by year of collection. There was no significant difference in the frequency of calicivirus antibody titers between males or females or by collection location.

DISCUSSION

This serologic study updates and extends the assessment of bacterial and viral exposure of Pacific walrus in the Bering Straight and Bristol Bay regions. In our study walrus infrequently had titers (≥100) to *L. interrogans* serovars. None had titers to the known pinniped pathogen *L. interrogans* serovar pomona. Three (all from Round Island) had low titers to *L. interrogans* serovars grippotyphosa, icterohaemorrhagiae/icterohaemorrhagiae, or ballum. Inconsistent antibody titers detected in repeat analyses of the same samples were likely due to low initial antibody titer and antibody loss during sample freeze thaw cycles (P. McDonough, pers. comm.). These low antibody titers may represent exposure to *L. interrogans* serovars grippotyphosa, icterohaemorrhagiae/icterohaemorrhagiae, or ballum; cross-reaction between serovars of *Leptospira* which were not assayed for in this study; or nonspecific responses. Based upon histologic review of kidneys from 170 walrus from this population, few if any had evidence of renal disease (D. Seagars and T. Lipscomb, unpubl. data).

No antibodies against *L. interrogans* serovar pomona were found in a 1976 survey of 55 Pacific walrus (Fay, 1982). *Leptospira interrogans* serovar pomona infection is common in northern fur seals (*Callorhinus ursinus*) (Smith et al., 1977) and California sea lions (*Zalophus californianus*) (Gulland, 1999). California sea lions with antibodies to *L. interrogans* serovars ballum, grippotyphosa, icterohaemorrhagiae (Gulland et al., 1996; Godinez at al., 1999), and infection with *L. interrogans* serovar grippotyphosa have been reported (Stamper et al., 1998). Pacific harbor seals (*Phoca vitulina richardsi*) with renal disease have recently been shown to be infected with *L. interrogans* serovar grippotyphosa, as have asymptomatic elephant seals (*Mirounga angustirostris*) (Stamper et al., 1998). Steller sea lions (*Eumetopias jubatus*) also have serologic evidence of *Leptospira* exposure (Gulland, 1999). There was a significantly greater frequency of antibodies to *L. interrogans* serovars in walrus sampled on Round Island; therefore it is worth noting that there is a Steller sea lion haulout on the south side of Round Island. It is possible walrus at this location may come into contact with Steller sea lions and be exposed to *L. interrogans* serovars more frequently than the walrus sampled near St. Lawrence Island.

Both Atlantic and Pacific pinnipeds recently have been found with cultural or serologic evidence of *Brucella* spp. infection (Foster et al., 1996; Nielson et al., 1996; Garner et al., 1997; Tryland et al., 1999; Nielson et al., 2001). In contrast to Atlantic walrus (Nielson et al., 1996, 2001), none of the Pacific walrus in this study had *Brucella* spp. antibodies. This may indicate either lack of exposure to *Brucella* spp. or result from the less sensitive card and tube agglutination tests utilized in the present study compared to the more sensitive en-

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zyme linked immunosorbent assay used to test Atlantic walrus (Nielson et al., 1996, 2001).

Infection with PDV has been implicated in harbor (*P. vitulina*) and gray seal (*Halichoerus grypus*) epizootics in 1988 and 1992 (Osterhaus et al., 1988; Duignan et al., 1995; Kennedy, 1998). Antibodies to PDV have been documented in Atlantic walrus (Duignan et al., 1994; Nielson et al., 2000), but not in Pacific walrus or a number of other Arctic pinniped species tested (Osterhaus et al., 1988). Other eastern Arctic species have antibodies to PDV (Duignan et al., 1997). None of the Pacific walrus in this study had antibodies to PDV, thus, at this time it does not appears that PDV has been introduced into the North American Pacific coast pinniped populations (Duignan et al., 1995; Kennedy, 1998; Ham-Lamme et al., 1999). If PDV was introduced to the immunologically naive Pacific walrus population, there is a risk of devastating population consequences (Duignan et al., 1994, 1995; Ham-Lamme et al., 1999).

Eight of 38 (21%) walrus had influenza A virus antibody. Based upon virus isolation or serologic surveys, multiple serotypes of Influenza A virus have been documented in Atlantic harbor and gray seals (Webster et al., 1981; Geraci et al., 1982; Hinshaw et al., 1984; Callan et al., 1995). No serologic evidence of influenza was found in northern fur seals sampled from the Bering Sea, Pacific Ocean, and Sea of Okhotsk (Webster et al., 1981). In a survey of Alaskan pinnipeds, including Pacific walrus, only one ringed seal (*Phoca hispida*) had antibody to influenza A (Danner et al., 1998), in contrast to the frequency of influenza A antibody detection in our study. The influenza A virus strains of these pinniped species are closely related to avian influenza A virus strains. There may be interchange of influenza A viruses between marine mammals and aquatic birds (Webster et al., 1981; Geraci et al., 1982; Hinshaw et al., 1984; Callan et al., 1995; Danner et al., 1998), similar to what has been postulated for calicivirus transmission between birds and marine mammals (Poet et al., 1996). Pacific walrus occasionally consume sea birds (Fay et al., 1990), and many terrestrial walrus haulout sites are adjacent to sea bird colonies. Antibodies to influenza A virus detected in the Pacific walrus in this study were against serotypes different than those previously reported from pinnipeds. The walruses in our study were probably exposed to multiple serotypes of influenza A virus.

None of 54 walrus sampled in a 1976 study had SMSV antibodies (Fay, 1982). No walrus in our study had antibody to a calicivirus (Walrus) originally isolated from Pacific walrus feces (Smith et al., 1983) or to several other caliciviruses (SMSV 5, 6, 7, 8, Tillamook) that have been reported in Pacific walrus (Barlough et al., 1986c, 1987b, 1988). There was serologic evidence of exposure to several other caliciviruses (VES E54, F55, G55, 1934B, SMSV 12), comparable to other studies of pinnipeds from the eastern Pacific, Arctic, or Bering Seas (Smith et al., 1983; Barlough et al., 1986a, 1986b, 1986c, 1987a, 1987b, 1988). These calicivirus strains have been documented in domestic swine (VES E54, F55, G55, 1934B) or California sea lions and northern fur seals (SMSV 12) (Barlough et al., 1986b). In addition, bowhead whales (*Balaena mysticetus*) harvested in Alaska had antibodies to VES F55, 1934B, and SMSV 12 (O'Hara et al., 1998). Some of these calicivirus strains may be enzootic in multiple marine mammal species in the Arctic ecosystem. Walrus may play a role in the northern cycle of calicivirus transmission (Barlough et al., 1986a). Although there was a statistical difference in serologic responses based on collection year, the power was low and this result should be interpreted with caution. Our results are consistent with a population exposed to several marine caliciviruses.

Leptospira, Brucella, caliciviruses, and influenza A virus are potentially zoonotic pathogens. Pinnipeds infected with these

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pathogens may pose a threat to Inupiat and Yupik hunters who use them as food sources. Such zoonotic infections have occurred in biologists and veterinarians working with influenza A infected pinnipeds who developed viral conjunctivitis (Webster et al., 1981). People exposed to *Leptospira* infected pinnipeds have developed either clinical leptospirosis or antibodies (Gulland, 1999). In addition, investigators of calicivirus infected pinnipeds have developed calicivirus antibodies (Barlough et al., 1986a).

Our study is the first survey to examine the exposure of walrus to multiple infectious agents. These data contribute to knowledge of Pacific walrus exposure to potentially pathogenic bacteria and viruses, updates and expands previous surveys, and establishes baseline exposure to a range of infectious agents. There were too few samples to adequately assess differences in exposure based on age, sex, or collection location. The results suggest that the Pacific walrus population has exposure to multiple strains of influenza A viruses and caliciviruses, but limited exposure to *Leptospira* spp., *Brucella* spp., and PDV. Populations without previous exposure to infectious agents are more susceptible to significant health consequences when first exposed. It has been suggested that pinniped populations having high burdens of organochlorine contaminants are most susceptible to infectious disease (de Swart et al., 1996; Ross et al., 1996; Nakata et al., 1997), although this has not been demonstrated in actual disease outbreaks (Kennedy, 1998). As with other naive pinniped populations, the Pacific walrus population could be adversely affected if novel pathogens were introduced (Duignan et al., 1994, 1995; Danner et al, 1998; Kennedy, 1998; Ham-Lamme et al., 1999); this could have adverse consequences for both the walrus population and Native Alaskan subsistence. Fortunately, the Pacific walrus population has low levels of PCB, DDT, dieldrin, chlordanes, and related compounds (Taylor et al., 1989; Seagars and Garlich-Miller, 2001), and animals appear to be in relatively good body condition based on life history studies and hunter observations. Thus this population may be less susceptible to an epizootic than more highly contaminated pinniped populations.

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