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# *Coxiella burnetii* Infection of Marine Mammals in the Pacific Northwest, 1997–2010

**Gilbert J. Kersh,[1,6] Dyanna M. Lambourn,[2] Stephen A. Raverty,[3] Kelly A. Fitzpatrick,[1] Joshua S. Self,[1] Adrianne M. Akmajian,[2] Steven J. Jeffries,[2] Jessica Huggins,[4] Clifton P. Drew,[5] Sherif R. Zaki,[5] and Robert F. Massung[1]** [1] Centers for Disease Control and Prevention, Rickettsial Zoonoses Branch, 1600 Clifton Rd NE, Atlanta, Georgia, 30333, USA; [2] Washington Department of Fish and Wildlife, 7801 Phillips Rd SW, Lakewood, Washington 98498, USA; [3] Ministry of Agriculture Food and Fisheries, 1767 Angus Campbell Rd, Abbotsford, British Columbia, Canada V3G 2M3; [4] Cascadia Research Collective, 218½ W 4th Ave, Olympia, Washington 98501, USA; [5] Centers for Disease Control and Prevention, Infectious Disease Pathology Branch, 1600 Clifton Rd Atlanta, Georgia 30333, USA; [6] Corresponding author (email: gkersh@cdc.gov)

ABSTRACT: Q fever is a zoonotic disease caused by the bacterium *Coxiella burnetii*. Humans are commonly exposed via inhalation of aerosolized bacteria derived from the waste products of domesticated sheep and goats, and particularly from products generated during parturition. However, many other species can be infected with *C. burnetii*, and the host range and full zoonotic potential of *C. burnetii* is unknown. Two cases of *C. burnetii* infection in marine mammal placenta have been reported, but it is not known if this infection is common in marine mammals. To address this issue, placenta samples were collected from Pacific harbor seals (*Phoca vitulina richardsi*), harbor porpoises (*Phocoena phocoena*), and Steller sea lions (*Eumetopias jubatus*). *Coxiella burnetii* was detected by polymerase chain reaction (PCR) in the placentas of Pacific harbor seals (17/27), harbor porpoises (2/6), and Steller sea lions (1/2) collected in the Pacific Northwest. A serosurvey of 215 Pacific harbor seals sampled in inland and outer coastal areas of the Pacific Northwest showed that 34.0% (73/215) had antibodies against either Phase 1 or Phase 2 *C. burnetii*. These results suggest that *C. burnetii* infection is common among marine mammals in this region.

*Key words:* *Coxiella burnetii*, marine mammals, placenta, Q fever.

Q fever is a widespread zoonosis caused by infection with the Gram-negative bacterium *Coxiella burnetii*. The most common route of infection for humans is inhalation of airborne particles derived from infected animals (Maurin and Raoult, 1999). Sheep (*Ovies aries*) and goats (*Capra aegagrus hircus*) are the most common animal hosts linked to human infections, and *C. burnetii* derived from densely infected placentas are often the source of contaminated aerosols. However, a variety of species, including wild mammals, ticks, birds, and reptiles, can be infected with *C. burnetii* (McQuiston and Childs, 2002).

For marine mammals, infection with *C. burnetii* has been described in two case reports: an infection of a Pacific harbor seal (*Phoca vitulina richardsi*; Lapointe et al., 1999) and a Steller sea lion (*Eumetopias jubatus*; Kersh et al., 2010). Both animals were found on the Pacific coast of the USA, and the infection was noted only in the placenta. It is not known if these case reports are isolated incidents or indicate widespread infection of marine mammals with *C. burnetii*. To address this question, we examined 27 harbor seal, 6 harbor porpoise (*Phocoena phocoena*), and 2 Steller sea lion placentas for evidence of *C. burnetii* infection and performed a serosurvey of 215 live-captured harbor seals.

The placentas of 27 Pacific harbor seals were collected from beaches of Washington, USA, and British Columbia, Canada, between 2006 and 2010 (Table 1). The samples were collected from stranded seals where both the fetus and placenta were recovered from the deceased mother ($n=5$), from aborted fetuses or stillborn pups ($n=6$), and from placentas collected at or near rookeries or birth sites during the pupping season ($n=16$). For the last samples, the status of the associated pup is unknown in the majority of cases. Genomic DNA was purified from the placental tissues using a Qiagen QIAamp tissue protocol (Qiagen, Inc., Valencia, California, USA) and tested for *C. burnetii*

TABLE 1. Analysis of marine mammal placentas for *Coxiella burnettii* infection.[a]

| Identifier | Species | Date | Status of fetus | IS1111[b] | Com1[b] | IHC[c] | Placentitis[d] | Serum[e] |
|---|---|---|---|---|---|---|---|---|
| GI06-04 | Harbor seal | 3 Jun 2006 | 3rd trimester premature pup | 33 | 35.8 | neg | No | n.t. |
| SMI P No. 1 | Harbor seal | 12 Jul 2006 | Pup status unknown | 35.3 | undet. | neg | No | n.t. |
| SMI P No. 2 | Harbor seal | 12 Jul 2006 | Pup status unknown | 37 | undet. | neg | No | n.t. |
| SMI P No. 3 | Harbor seal | 12 Jul 2006 | Pup status unknown | 39 | undet. | n.t. | No | n.t. |
| SMI P No. 4 | Harbor seal | 12 Jul 2006 | Pup status unknown | 38.5 | undet. | neg | No | n.t. |
| SMI P No. 5 | Harbor seal | 12 Jul 2006 | Pup status unknown | undet. | undet. | neg | No | n.t. |
| SMI P No. 7 | Harbor seal | 12 Jul 2006 | Pup status unknown | 37 | undet. | pos | Yes | n.t. |
| GI P 2006 No. 1 | Harbor seal | 24 Jul 2006 | Pup status unknown | undet. | undet. | neg | No | n.t. |
| GI P 2006 No. 2 | Harbor seal | 24 Jul 2006 | Pup status unknown | 38 | undet. | neg | No | n.t. |
| GI P 06/3286 | Harbor seal | 1 Aug 2006 | Live birth, full term pup | 33.6 | 35.8 | neg | Yes | n.t. |
| WDFW0906-01 | Harbor seal | 7 Sep 2006 | Full term, stillborn | 38 | 38.8 | neg | No | n.t. |
| WDFW1106-03 | Harbor seal | 11 Nov 2006 | 2nd trimester, in mother | undet. | undet. | neg | No | pos |
| WDFW0207-01 | Harbor seal | 9 Feb 2007 | 2nd trimester, aborted | 35 | undet. | neg | No | n.t. |
| WDFW0607-01 | Harbor seal | 1 Jun 2007 | 3rd trimester, in mother | undet. | undet. | neg | No | n.t. |
| WB 2007 No. 3 | Harbor seal | 18 Jul 2007 | Pup status unknown | 36.1 | 38.6 | n.t. | n.t. | n.t. |
| WDFW2008-048 | Harbor seal | 21 May 2008 | 3rd trimester, in mother | undet. | undet. | n.t. | n.t. | n.t. |
| WDFW2008-053 | Harbor seal | 28 May 2008 | Near term, in mother | 36.3 | 32.4 | pos | Yes | pos |
| GI P 2008 No. 1 | Harbor seal | 1 Jul 2008 | Pup status unknown | 34.1 | undet. | n.t. | n.t. | n.t. |
| GI P 2008 No. 2 | Harbor seal | 15 Jul 2008 | Pup status unknown | 33.4 | 36.8 | n.t. | n.t. | n.t. |
| 09Pv11MayWI-10 | Harbor seal | 11 May 2009 | 3rd trimester, in mother | undet. | undet. | n.t. | n.t. | n.t. |
| CRC-P A | Harbor seal | 9 Jul 2009 | Pup status unknown | undet. | undet. | neg | No | n.t. |
| CRC-P B | Harbor seal | 9 Jul 2009 | Pup status unknown | 37.4 | undet. | neg | No | n.t. |
| GI09-35 | Harbor seal | 31 Jul 2009 | Full term, stillborn | 34.3 | 38 | neg | Yes | n.t. |
| CRC-963 | Harbor seal | 15 Aug 2009 | Full term, stillborn | 35.1 | 39 | neg | No | n.t. |
| WDFW2010-079 | Harbor seal | 18 Jun 2010 | Live, premature pup | undet. | undet. | n.t. | n.t. | n.t. |
| CRC-1055 | Harbor seal | 29 Jun 2010 | Full term, stillborn | undet. | undet. | n.t. | n.t. | n.t. |
| Plac-WB-072310 | Harbor seal | 26 Jul 2010 | Pup status unknown | undet. | undet. | n.t. | n.t. | n.t. |
| CRC-798 | Harbor porpoise | 11 Jul 2007 | Near term, in mother | 35 | 39.9 | n.t. | No | n.t. |
| CRC-835 | Harbor porpoise | 14 Mar 2008 | 3rd trimester, in mother | undet. | undet. | n.t. | No | n.t. |
| CRC-922 | Harbor porpoise | 13 Apr 2009 | 3rd trimester, in mother | 35.7 | undet. | n.t. | No | n.t. |
| CRC-1063 | Harbor porpoise | 9 Jul 2010 | Near term, in mother | undet. | undet. | n.t. | No | neg |

TABLE 1. Continued.

| Identifier | Species | Date | Status of fetus | IS1111[b] | Com1[b] | IHC[c] | Placentitis[d] | Serum[e] |
|---|---|---|---|---|---|---|---|---|
| WDFW2010-170 | Harbor porpoise | 15 Oct 2010 | 1st trimester, in mother | undet. | undet. | n.t. | No | pos |
| 10Pp31DecW1-07 | Harbor porpoise | 31 Dec 2010 | 2nd trimester, in mother | undet. | undet. | n.t. | n.t. | n.t. |
| WDFW0307-03 | Steller sea lion | 27 Mar 2007 | 2nd trimester, in mother | 37 | 38.5 | n.t. | Yes | n.t. |
| WDFW2010-200 | Steller sea lion | 20 Nov 2010 | 2nd trimester, in mother | undet. | undet. | n.t. | No | n.t. |

[a] Harbor seal (*Phoca vitulina richardsi*), harbor porpoise (*Phocoena phocoena*), and Steller sea lion (*Eumetopias jubatus*) placentas were collected in the Pacific Northwest, USA.

[b] The threshold cycle for quantitative polymerase chain reaction (PCR) targeting IS1111a or com1; undet. = undetermined; samples for which fluorescence did not cross the threshold after 40 cycles of PCR.

[c] pos = sample stained with anti–*C. burnetii* murine hyperimmune ascites fluid; n.t.= not tested; neg = no staining detected.

[d] Yes = sample showed evidence of placentitis based on H and E staining; no = no evidence of placentitis; n.t.= not tested.

[e] pos = serum sample was positive in indirect fluorescent antibody test using anti-dog secondary reagent; n.t.= not tested; neg = negative indirect fluorescent antibody test.

using quantitative *com1* and *IS1111a* polymerase chain reaction (PCR; Kersh et al., 2010). Of 27 samples, eight (30%) were positive for both *IS1111a* and *com1*, and 17 (63%) were positive for *IS1111a* only (Table 1). The *IS1111a* gene is multicopy, and PCR targeting this gene is expected to be more sensitive than PCR targeting the single-copy *com1*. For seven of the eight double-positive placenta samples, *IS1111a* PCR had a lower C(t) than the *com1* PCR, suggesting the *IS1111a* single-positive samples did not have enough *C. burnetii* DNA to be detected by *com1* PCR. The one placenta that had a lower C(t) value for *com1* compared to *IS1111a* may have an altered form of *IS1111a* that could be similar to the *C. burnetii* strain described previously in a Steller sea lion (Kersh et al., 2010). Eighteen of the 27 harbor seal placentas were also analyzed by immunohistochemistry (IHC), with two staining positive with anti–*C. burnetii* antibodies. The fact that so few of the samples were positive by IHC is probably due to the focal nature of the placental infection and the relatively low bacterial burden in most of the samples. Histologic examination revealed evidence for placentitis in four of these 18 placentas: WDFW2008-053, GI09-35, GI P-06/3286, and SMI P #7. Necrosis was also observed in GI-09-35, and diverse bacterial infiltrates were observed in WDFW2008-053, GI09-35, and GI P-06/3286.

Placentas were also collected from six dead, stranded harbor porpoises. Both the fetus and placenta were recovered from the deceased mother in all six cases. PCR analysis conducted on the harbor porpoise samples revealed one positive for *IS1111a* and *com1*, one positive for *IS1111a* only, and four negative (Table 1). We also performed PCR on two Steller sea lion placentas that were recovered upon necropsy of deceased mother and fetus; one was positive for both *IS1111a* and *com1*, and the other negative (Table 1). These results add to the description of a *C. burnetii*–infected Steller sea lion placenta (Kersh et al., 2010).

To examine a larger sample size and better determine the extent of *C. burnetii* exposure in the general population of harbor seals, we collected 215 serum samples from live, healthy, free-ranging harbor seals captured in the Pacific Northwest between 1997 and 2009, using either the beach seine technique (Jeffries et al., 1993) or by hand capture of individual seals from haulouts following boat or beach rushes. Harbor seal sera were tested by an indirect fluorescent antibody (IFA) test against Nine Mile Phase 1 and Phase 2 *C. burnetii* using a goat, anti-dog fluorescein isothiocyanate–conjugated secondary antibody. The cutoff for a positive result was set at 1:64 to exclude cross-reactive antibodies, similar to previous studies (Rousset et al., 2007). Serologic results from harbor seal haulout sites (Fig. 1) were grouped based on known harbor seal genetics (Lamont et al., 1996; Huber et al., 2010). Specifically, samples were grouped into two harbor seal stocks: The Washington (WA)/British Columbia (BC) inland water stock and the WA/Oregon (OR) outer coast stock. The WA/BC inland stock was sampled at two locations: South Puget Sound, WA ($n=60$), and San Juan, WA/Gulf Islands, BC ($n=55$; Fig. 1). The WA/OR outer coast stock was also sampled at two locations: The southern WA coast (Grays Harbor, WA [$n=25$], Columbia River, WA/OR [$n=25$]) and the central OR coast (Alsea Bay, OR [$n=50$]).

The IFA test detected anti–*C. burnetii* Phase 2 antibodies with a titer ≥1:64 in 48/215 (22.3%) samples. Anti–*C. burnetii* Phase 1 antibodies with a titer ≥1:64 were detected in 57/215 (26.5%) samples. A total of 73/215 (34.0%) samples had a titer ≥1:64 against either Phase 1 or Phase 2 *C. burnetii*, and 32/215 (14.9%) had a titer ≥1:64 against both Phase 1 and Phase 2 *C. burnetii*. Thus, 41 samples were positive for only one of the phases: 25/215 had a titer ≥1:64 against only Phase 1, and 16/215 had a titer ≥1:64 against only Phase 2. Samples specifically positive against Phase 2 tended to be weak (1:64 or 1:128), whereas samples only positive against Phase 1 had a broad distribution of titers (1:64 to 1:8192). The overall distribution of titers (Fig. 2) indicates that many animals had titers far greater than 1:64.

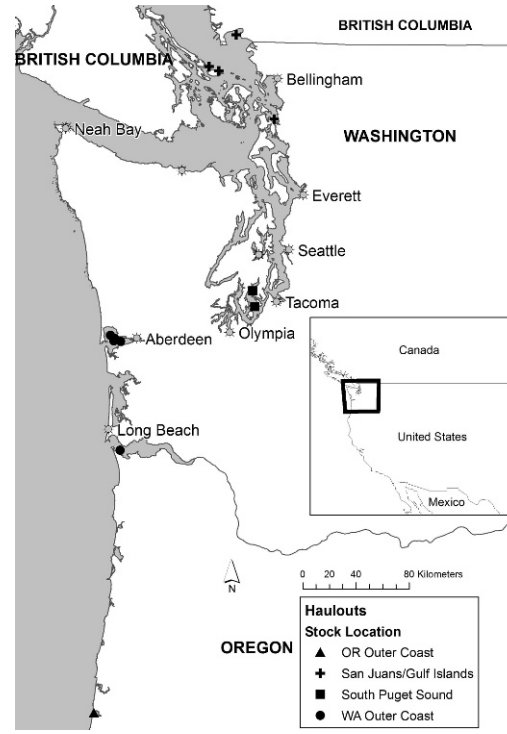


FIGURE 1. Marine mammal haulout sites where Pacific harbor seal serum samples were collected. Seals were grouped based on genetics into two stocks: the Washington/British Columbia (WA/BC) inland water stock and the Washington/Oregon (WA/OR) outer coast stock. The WA/BC inland stock was sampled at San Juan/Gulf Islands (✚), and South Puget Sound (■). The WA/OR outer coast stock was sampled at the Oregon outer coast (▲) and the Washington outer coast (●). The number of positive samples/number of samples tested for each location was San Juan/Gulf Islands (13/55), South Puget Sound (23/60), Oregon outer coast (12/50), and Washington outer coast (25/50).

In humans, anti-Phase 2 titers are usually detectable early in an acute infection, but anti-Phase 1 titers do not become elevated unless a chronic infection is present. The high percentage of harbor seals with elevated anti-Phase 1 titers presented here is unusual, particularly the 25 samples that were Phase 1 positive but Phase 2 negative. The reasons for this are not clear but could be related to repeated exposure to the

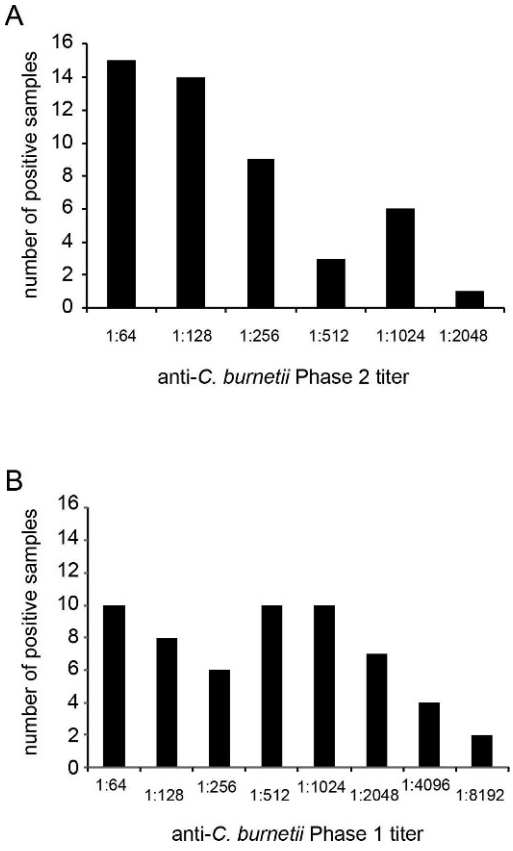


FIGURE 2. Distribution of antibody titers in Pacific harbor seals. The numbers of samples with specific titers are indicated. Samples were tested for reactivity against Phase 2 (A) and Phase 1 (B) *Coxiella burnetii*.

agent, or that strains that infect marine mammals have a greater propensity for inducing an anti–Phase 1 antibody response.

Results for the four locations were South Puget Sound, 38%; San Juan/Gulf Islands, 24%; southern WA coast, 50%; and central OR coast, 24%. Positive samples were found in each year tested (1997, 1999, 2000, 2007–2009). Statistically significant differences were found in prevalence of *Coxiella* antibody between the southern WA coast and the San Juan/Gulf Islands (Pearson's chi-square=7.88, $P=0.005$) and the central OR coast (Pearson's chi-square=7.25, $P=0.007$).

Previously, investigators have detected *C. burnetii* only in the placenta of marine mammals (Lapointe et al., 1999; Kersh et al., 2010), but nothing was known about the prevalence of infection in populations, particularly males. Our study included 115 female and 100 male harbor seals. The percentage of males with a titer ≥1:64 against Phase 1 *C. burnetii* was 29% (29/100) and 22% (22/100) against Phase 2. For females, 24.3% (28/115) had a titer ≥1:64 against Phase 1 *C. burnetii* and 22.6% (26/115) had a titer ≥1:64 against Phase 2 *C. burnetii*. This suggests that both male and female seals can be infected with *C. burnetii*, and that pregnancy is not a requirement for seroconversion. Positive titers were found in all age classes sampled. The percentage of pups <1 yr old (including premature, neonatal, nursing, and weaned pups) with a titer of ≥1:64 against either Phase 1 or Phase 2 was 37% (13/35). For yearlings/subadults, 18% (12/65) were positive against either Phase 1 or 2, and for adults (reproductively mature, over 4 yr) 41.7% (48/115) were positive on either Phase 1 or 2. Statistically significant differences were found between the percentage of positive adults and the percentage of positive yearlings/subadults (Pearson's chi-square=10.126, $P=0.0015$), and the difference between yearlings/subadults and pups <1 yr (Pearson's chi-square=4.234, $P=0.04$). The difference between adults and pups <1 yr was not statistically significant.

This study demonstrates that *C. burnetii* infection of marine mammals from coastal waters of OR and WA and inland waters of WA and BC is common and has been occurring since at least 1997. Evidence for infection of harbor seals, harbor porpoises, and Steller sea lions suggests that *C. burnetii* infection may occur in many marine mammal species.

The prevalence of antibody to *C. burnetii* in this population of harbor seals (34.0%) is lower than the average prevalence among domesticated goats in the USA (41.6%), but much higher than US sheep (16.5%; McQuiston and Childs, 2002). The antibody prevalence in harbor

seals was also higher than in most other free-ranging mammal species that have been reported, such as bears (*Ursus americanus*) (16.8%; Ruppanner et al., 1982), deer (*Odocoileus hemionus columbianus*) (22.2%), and mice (*Peromyscus boylei, Peromyscus maniculatus, Peromyscus truei*) (21.7%; Enright et al., 1971), although each of these studies was of limited scope and therefore may not be nationally representative. Our results identify Pacific harbor seals as a free-ranging species that is commonly infected with *C. burnetii*. The prevalence of *C. burnetii* exposure among marine mammals significantly expands the range of competent reservoirs of *C. burnetii* to species that are common in coastal and Arctic regions.

Whether infected marine mammals pose a health risk for humans is unknown. Given that some animals have a heavily infected placenta and harbor seal births take place on coastal beaches, docks, and other areas accessible to people, opportunities for human exposure exist. The possibility of widespread marine mammal infection suggests that seals may be a potential reservoir for human exposure. A recent case of chronic Q fever endocarditis was reported in a resident of Greenland (Koch et al., 2010). Although the source of infection was not identified, the primary animal exposure of the patient was to sled dogs and seals. Further studies are needed to define a human health risk based on exposure to *C. burnetii* from harbor seals and other marine mammals.

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