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

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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.

Keywords

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

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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* 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.

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 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 chisquare=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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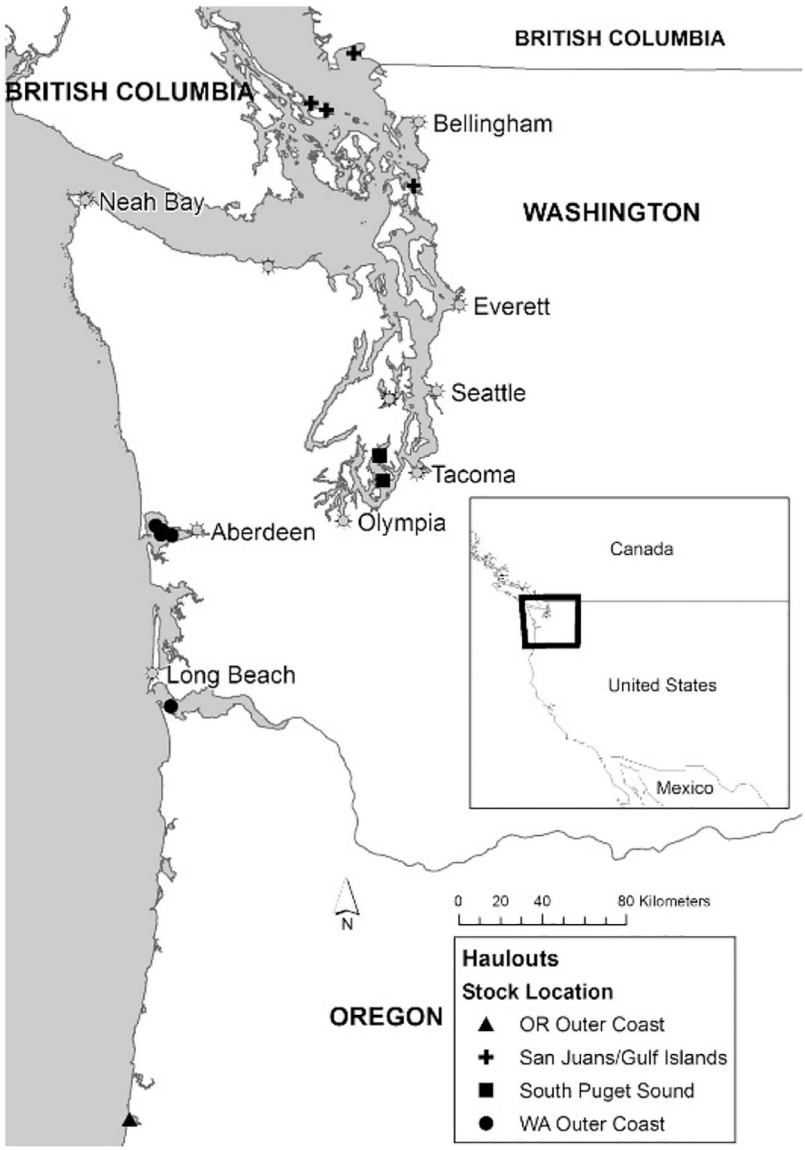

Figure 1.

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).

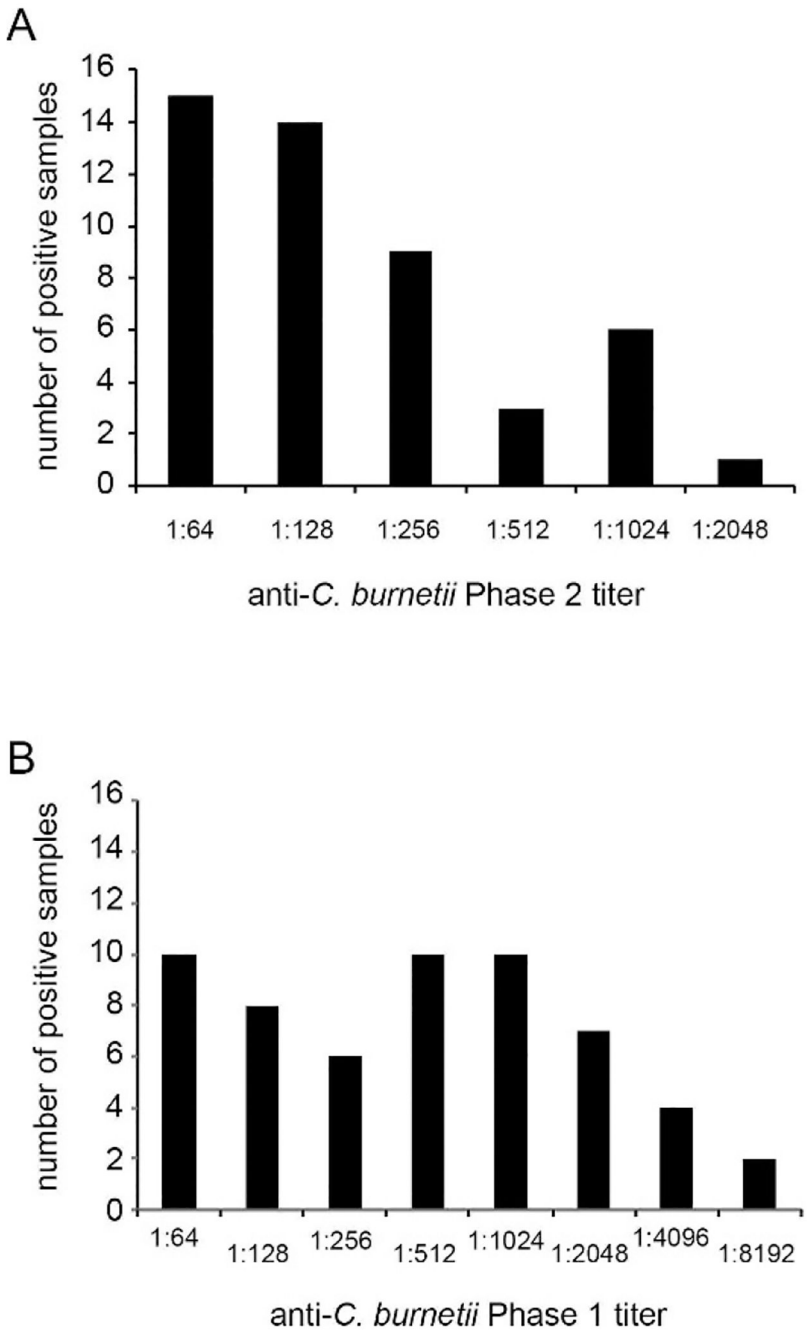

Figure 2.

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*.

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.

Identifier	Species	Date	Status of fetus	IS1111[b]	Com1[b]	IHC[c]	Placentitis[d]	Serum[e]
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
WDFW2010-170	Harbor porpoise	15 Oct 2010	1st trimester, in mother	undet.	undet.	n.t.	No	pos
10Pp31DecWI-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.