Zoonotic diseases without pandemic potential, like brucellosis, are in need of innovative One Health approaches

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ANTHC's Center for Climate and Health
CDC Arctic Investigations Program in Anchorage Alaska
April 17, 2018
Tromsø
69° 40'58"N - 18° 56'34"E
« Brucellosis is the most common bacterial zoonosis, with over 500 000 new cases globally every year»

Pappas G, Papdimitriou P, Akritidis N, Christou L, Tsianos EV.

The new global map of human brucellosis.

The source of infection is almost always to be found in the animal reservoir
Animal brucellosis in the Arctic
Symptoms in chronic brucellosis - wildlife

*Brucella suis... biovar 4*
Symptoms in chronic brucellosis - wildlife

Brucella suis… biovar 4

Contagious Ecthyma, Rangiferine Brucellosis, and Lungworm Infection in a Muskox (Ovibos moschatus) from the Canadian Arctic, 2014

Author(s): Matilde Tomaselli, Chimoné Dalton, Pádraig J. Duignan, Susan Kutz, Frank van der Meer, Pratap Kafle, Om Surujballi, Claude Turcotte, and Sylvia Checkley

Published By: Wildlife Disease Association
https://doi.org/10.7589/2015-12-327
URL: http://www.bioone.org/doi/full/10.7589/2015-12-327
Symptoms in chronic brucellosis - wildlife

Brucella ceti

Foster et al., 2002, Gonzalez et al., 2002; Dagleish et al., 2007
Human brucellosis in the Arctic

*Brucella suis* biovar 4: Confirmed

Marine mammal brucellae: Not described (yet?)
Brucellosis: Understanding an Important Arctic Infectious Disease
Center for Climate and Health
Michael Brubaker MS, James Berner MD, Jay Butler MD, Michael Bradley DVM
CCH Bulletin No. 5, November 30, 2010

Human Brucellosis Cases in Alaska (1973 to 2010)

Source: State of Alaska Department of Health and Social Services
Isolates of *Brucella suis* biovar 4 from animals and humans in Canada, 1982–1990

Lorry B. Forbes


### Table 1. Summary of tissues from which *B. suis* biovar 4 was isolateda

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Gross lesion</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Suppurative</td>
<td>Nonsuppurative</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Not described</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>Carpal joint</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>2</td>
<td>28</td>
</tr>
<tr>
<td>Testicle</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>Joints other than carpus</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td><strong>Blood</strong></td>
<td><strong>10</strong></td>
<td><strong>10</strong></td>
</tr>
<tr>
<td>Subcutaneous abscess</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Mammary gland</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Epididymis</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Abscessed muscle</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Kidney</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Uterus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placenta</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abscess of rumen wall</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

*aOne hundred culture-positive cases of caribou, reindeer, muskox, and human origin. Some cases had more than one positive tissue*

*bClassed as suppurative if any of the following terms were used in describing the lesion: abscess, pus, purulent, suppurative*
Brucella spp. in Marine Mammals

Brucella ceti sp. nov. and Brucella pinnipedialis sp. nov. for Brucella strains with cetaceans and seals as their preferred hosts

Geoffrey Foster,1 Bjorn S. Osterman,2 Jacques Godfroid,3 Isabelle Jacques4,5 and Axel Cloeckaert4

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3Faculty of Veterinary Science, Department of Veterinary Tropical Diseases, University of Pretoria, Onderstepoort 0110, South Africa
4INRA, UR1282, Infectiologie Animaux et Santé Publique, IASP, Nauzilly, F-37380, France
5Institut Universitaire de Technologie, Université François Rabelais, 29 rue du Pont-Volant, 37002 Tours cedex 2, France

Small Gram-negative coccobacilli resembling Brucella strains have been reported from marine mammals since the mid-1990s. Their placement in the genus Brucella has been supported by the following characteristics: they are aerobic, non-motile and catalase-positive, do not produce acid from carbohydrates and have a DNA–DNA relatedness value of >77% with the six established members of the genus. Twenty-eight European isolates of the genus Brucella from marine mammals were distinguished from the six recognized species by their pattern of utilization of eleven substrates in oxidative metabolism tests and phage lysis. The 28 strains could be further separated into two groups with cetaceans and seals as their respective preferred hosts on the basis of molecular methods and on differences in the metabolism of L-arabinose, D-galactose and D-xylene. The names Brucella ceti sp. nov. and Brucella pinnipedialis sp. nov. are proposed for the isolates from cetaceans and seals, respectively. The type strain of Brucella ceti sp. nov. is NCTC 12991T (=BCCN 94-74T) and the type strain of Brucella pinnipedialis sp. nov. is NCTC 12896T (=BCCN 94-73T).
Novel isolation from marine mammals in 1994
Isolated only on the northern hemisphere
Isolation of marine mammal brucellae from humans

McDonald et al. 2006, Sohn et al. 2003:
- Masses in brain and vertebrae
- All three patents had been in contact with raw products from the sea. No contact with marine mammals.
- **ST27** (Previously isolated from an aborted bottlenose dolphin and placentas from aborting California sea lions)

Brew et al. 1999:
- Laboratory acquired infection in the UK
- **ST23** (Previously isolated from porpoises)

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Sohn et al. 2003

McDonald et al. 2006
Brucella infections in cetaceans

Brucellosis!
Neurobrucellosis in Stranded Dolphins, Costa Rica

Gabriela Hernández-Mora, Rocío González-Barrientos, Juan-Alberto Morales, Esteban Chaves-Olarte, Caterina Guzmán-Verri, Eliás Baquero-Calvo, María-Jesús De-Miguel, Clara-Maria Marin, José-Maria Blasco, and Edgardo Moreno

Ten striped dolphins, *Stenella coeruleoalba*, stranded along the Costa Rican Pacific coast, had meningoencephalitis and antibodies against *Brucella* spp. *Brucella ceti* was isolated from cerebrospinal fluid of 6 dolphins and 1 fetus. *S. coeruleoalba* constitutes a highly susceptible host and a potential reservoir for *B. ceti* transmission.

Figure 1. Clinical, pathologic, and immunofluorescence findings in stranded striped dolphin, *Stenella coeruleoalba*. A) Striped dolphin displaying swimming disorders being assisted by local persons; B) dolphin fetus within placenta; C) punctuated placental abscesses (arrows); D) immunofluorescent *brucellae* in impressions of placenta tissues; E) congested and hyperemic brain and cerebellum; F) Wright-Giemsa–stained mononuclear cell infiltrate in cerebrospinal fluid; G) immunofluorescent green *Brucella* spp. and *Brucella* debris within phagocytic cells infiltrating cerebrospinal fluid; the inset corresponds to an amplified phagocytic cell with fluorescent *Brucella* spp. and debris.
Brucella infections in True Seals

Brucellosis?
Brucellosis in hooded seal (?)

Prevalence of *Brucella pinnipediae* in healthy hooded seals (*Cystophora cristata*) from the North Atlantic Ocean and ringed seals (*Phoca hispida*) from Svalbard

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Table 2

Correlation between isolation of *Brucella pinnipediae* from organ samples (*n* = 174) and the presence of anti-*Brucella* antibodies in serum from 29 hooded seals (*Cystophora cristata*) caught between Svalbard and Greenland, autumn 2002 (only seropositive and/or culture positive individuals are presented; open spaces indicate no growth of *Brucella* or seronegative results)

<table>
<thead>
<tr>
<th>Animal number</th>
<th>Age (months)</th>
<th>Sex</th>
<th>Tonsil</th>
<th>Lung</th>
<th>Lung lymph node</th>
<th>Spleen</th>
<th>Liver</th>
<th>Kidney</th>
<th>Testicle</th>
<th>Serology</th>
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<tr>
<td>1</td>
<td>6</td>
<td>M</td>
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<td>30</td>
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<tbody>
<tr>
<td>(%)</td>
<td>15</td>
<td>14</td>
<td>38</td>
<td>31</td>
<td>21</td>
<td>10</td>
<td>22</td>
<td>31</td>
</tr>
</tbody>
</table>

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* Since birth takes place in March and the animals were caught in September, ages registered as <1 year corresponds to 6 months, 1–2 years to 18 months, 2–3 years to 30 months, and 3–4 years to 42 months.

* Not investigated.
Hooded seal populations

- The Northwest Atlantic population
  - Increased since the 1980s
  - Estimated population size in 2005: 593,500

- The Northeast Atlantic population
  - Estimated population size prior to 1940: 575,000
  - Estimated population size in 2011: 85,000

Sealing - Harvest of hooded seals

Large difference in harvest between the two populations

Exploitation of the Northwest Atlantic population

Exploitation of the Northeast Atlantic population

ICES 2011.
Does Brucella infection contribute to the Northeast population crash?

Seroprevalence 5 % (n = 10/204)

Seroprevalence 35 % (n = 38/137)
- 31 % (n = 9/29)

Bacteriology - 38 % (n = 11/29)

Bohlin et al., 2010
Age-dependent prevalence of anti-\textit{Brucella} antibodies in hooded seals \textit{Cystophora cristata}

Ingebjørg H. Nymo$^{1,**}$, Morten Tryland$^{1,**}$, Anne Kirstine Frie$^{2,**}$, Tore Haug$^{2,**}$, Geoffrey Foster$^3$, Rolf Rødven$^4,**$, Jacques Godfroid$^{1,**}$

$^1$Section of Arctic Veterinary Medicine, Norwegian School of Veterinary Science, Stakkevollveten 23, 9010 Tromsø, Norway
$^2$Institute of Marine Research, PO Box 6404, 9294 Tromsø, Norway
$^3$SAC Consulting Veterinary Services, Drummondhill, Stratherrick Road, Inverness IV2 4JZ, UK
$^4$Bioforsk — Norwegian Institute of Agricultural and Environmental Research, PO Box 2284, 9269 Tromsø, Norway
Seroprevalence in hooded seal

- **Age:**
  - Pups: 2.5 %
  - Yearlings: 35.3 %
  - The mean probability of being seropositive decreased with age for hooded seals > one year
  - All seropositive ≥ one year were 1-5 years old

- No relation between *Brucella*-serostatus in pups or yearlings and weight, length or dorsal blubber thickness
- No relation between *Brucella*-serostatus and the presence of corpus luteum or corpus albicans, or the number of corpus albicans
- Isolation has never been achieved from a hooded seal > 18 months

**Sampling from the declining Northeast Atlantic population**

**Sampling from the increasing Northwest Atlantic population**
Are hooded seals the preferred hosts for *B. pinnipedialis* HS?

The hallmark of brucellosis is chronicity

*Assessed in gentamycin protection assays in macrophages*
BAL performed on sacrificed hooded seals, 1 - 3 h post mortem
Alveolar macrophages identified by:

- Culture morphology
- Expression of membrane markers assessed by flow cytometry and immunocytochemistry
  - CD14
  - CD18
  - (MHC II, CD11c)
- Phagocytosis
Alveolar macrophages

Entry and Elimination of Marine Mammal Brucella spp. by Hooded Seal (Cystophora cristata) Alveolar Macrophages In Vitro

Anett K. Larsen1,2, Ingebjørg H. Hyno1, Preben Boysen1, Morten Tryland1, Jacques Godfrid1,2

1 Section for Arctic Veterinary Medicine, Department of Food Safety and Infectious Biology, Norwegian School of Veterinary Science, Tromsø, Norway; 2 Member of the Fram Centre, High North Research Centre for Climate and the Environment, Tromsø, Norway, 3 Section for Microbiology, Immunology, and Parasitology, Department of Food Safety and Infectious Biology, Norwegian School of Veterinary Science, Oslo, Norway
Conclusions

Seals are not the preferred host for *B. pinnipedialis*, but rather a “dead-end” or spillover host being susceptible to infection derived from other sources in the marine environment.
Reservoir in the marine environment
In experimental challenge, Atlantic Cod (*Gadus morhua*) can sustain a *B. pinnipedialis* infection
Brucella infections in True and Eared Seals in North America
Assay dependence of *Brucella* antibody prevalence in a declining Alaskan harbor seal (*Phoca vitulina*) population

Karsten Hueffer¹, Scott M Gende² and Todd M O’Hara¹

Seroprevalence of *Brucella* antibodies in harbor seals in Alaska, USA, with age, regional, and reproductive comparisons

A. Hoover-Miller¹,²,*, J. L. Dunn³, C. L. Field³, G. Blundell⁴, S. Atkinson²

¹Alaska SeaLife Center, 301 Railway Ave, PO Box 1329, Seward, AK 99664, USA
²University of Alaska Fairbanks, College of Fisheries and Ocean Sciences, 17101 Pt. Lena Loop, Juneau, AK 99801, USA
³Department of Research and Veterinary Services, Mystic Aquarium, 55 Coogan Blvd, Mystic, CT 06355, USA
⁴Alaska Department of Fish and Game, Division of Wildlife Conservation, PO Box 110024, Juneau, AK 99811, USA
Brucella placentitis and seroprevalence in northern fur seals (Callorhinus ursinus) of the Pribilof Islands, Alaska

Colleen G. Duncan, Rebekah Tiller, Demetrius Mathis, Robyn Stoddard, Gilbert J. Kersh, Bobette Dickerson, and Tom Gelatt

Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, CO (Duncan); Bacterial Special Pathogens (Tiller, Mathis, Stoddard) and Rickettsial Zoonoses (Kersh) Branches of the Centers for Disease Control and Prevention, Atlanta, GA; and the National Marine Fisheries Service, Alaska Fisheries Science Center, National Marine Mammal Lab, Seattle, WA (Dickerson, Gelatt)

Figure 1.
Northern fur seal (Callorhinus ursinus) placentas with histologic lesions associated with Brucella infection. A, there is a regionally extensive area of inflammation and necrosis. Hematoxylin and eosin (HE). Bar = 500 μm. B, centrally within the affected region is a large artery with arteritis. HE. Bar = 20 μm. C, rare organisms are identified within the cytoplasm of trophoblasts at the periphery of the lesion. HE. Bar = 40 μm. D, Brucella immunostaining is present within the cytoplasm of trophoblasts as well as within the necrotic cellular debris. Immunohistochemical staining. Bar = 20 μm.

Figure 2.
Clustering analysis of multilocus variable number tandem repeat analysis profiles using the unweighted pair group method with arithmetic averages analysis obtained from northern fur seal (Callorhinus ursinus) placentas and other Brucella pinnipedialis isolates from the United States and Canada.
SEROLOGIC SURVEY OF BRUCELLA SPP. ANTIBODIES IN SOME MARINE MAMMALS OF NORTH AMERICA

Ole Nielsen, Robert E. A. Stewart, Klaus Nielsen, Lena Measures, and Padraig Duignan

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2 Animal Diseases Research Institute, Canadian Food Inspection Agency, 3051 Fellowfield Road, Nepean, Ontario K2G 8Y9, Canada
3 Department of Fisheries and Oceans, Maurice Lamontagne Institute, P.O. Box 1000 Mont-Joli, Quebec G0H 3Z4, Canada
4 Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston North, New Zealand
5 Corresponding Author (e-mail: nielsen@cfi-npa.gc.ca)

Table 3. Prevalence of Brucella spp. binding antibodies in fourteen species of North American marine mammals.

<table>
<thead>
<tr>
<th>Species</th>
<th>Number tested</th>
<th>O-chain</th>
<th>M84</th>
<th>Number positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Halichoerus grypus</td>
<td>255</td>
<td>1 (0.3)a</td>
<td>10 (3.9)</td>
<td>10 (3.9)</td>
</tr>
<tr>
<td>Phoca vitulina</td>
<td>163</td>
<td>9 (5.5)</td>
<td>21 (12.9)</td>
<td>21 (12.9)</td>
</tr>
<tr>
<td>Cystophora cristata</td>
<td>204</td>
<td>5 (2.4)</td>
<td>9 (4.4)</td>
<td>10 (4.9)</td>
</tr>
<tr>
<td>Phoca groenlandica</td>
<td>453</td>
<td>6 (1.3)</td>
<td>7 (1.5)</td>
<td>8 (1.8)</td>
</tr>
<tr>
<td>Phoca hispida</td>
<td>628</td>
<td>1 (0.2)</td>
<td>7 (1.1)</td>
<td>7 (1.1)</td>
</tr>
<tr>
<td>Odobenus rosmarus</td>
<td>170</td>
<td>5 (2.9)</td>
<td>3 (1.8)</td>
<td>5 (2.9)</td>
</tr>
<tr>
<td>Delphinapterus leucas</td>
<td>488</td>
<td>25 (5.1)</td>
<td>26 (5.3)</td>
<td>28 (5.7)</td>
</tr>
<tr>
<td>Monodon monoceros</td>
<td>77</td>
<td>5 (6.5)</td>
<td>5 (6.5)</td>
<td>5 (6.5)</td>
</tr>
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<td>Balaena mysticetus</td>
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<td>0</td>
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<td>Phocoena phocoena</td>
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<td>Globicephala melas</td>
<td>19</td>
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<td>0</td>
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<tr>
<td>Balaenoptera acutorostrata</td>
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<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lagenorhynchus acutus</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hyperoodon ampullatus</td>
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<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>2,470</strong></td>
<td><strong>57 (2.3)</strong></td>
<td><strong>88 (3.6)</strong></td>
<td><strong>94 (3.8)</strong></td>
</tr>
</tbody>
</table>

a Number positive (percent positive).
• The majority of isolates represent genotypes previously described in Europe although novel genotypes were identified in both *B. ceti* clades.

• Harp seals were found to carry *B. pinnipedialis* genotypes previously confined to hooded seals.

• Isolates were characterized from beluga whales and found to represent a number of distinct *B. pinnipedialis* genotypes.

• The known host range of ST27 was extended with the identification of this ST from California sea lion samples.
In eared seals, *Brucella* antibodies were found in two Steller sea lions (*Eumetopias jubatus*) (2%) and none of the 107 Northern fur seals (*Callorhinus ursinus*).

- The low seroprevalence in eared seals indicate a low level of exposure or lack of susceptibility to infection. Alternatively, mortality due to the *Brucella* infection may remove seropositive animals from the population.

*Brucella* antibodies were detected in all true seal species investigated; harbor seals (*Phoca vitulina*) (25%), spotted seals (*Phoca largha*) (19%), ribbon seals (*Histriophoca fasciata*) (16%), and ringed seals (*Pusa hispida hispida*) (14%).

- There was a low seroprevalence among pups, a higher seroprevalence among juveniles, and a subsequent decreasing probability of seropositivity with age in harbor seals.
Transmission of Brucella spp. to polar bears

Results:

Bears are exposed to *B. suis* biovar 4 or marine mammal brucellae
Thank you for your attention

« Nothing is permanent, but change... »