



# Anti-Brucella antibodies in pinnipeds of Australia

## Introduction

*Brucella* are Gram-negative intracellular bacteria capable of infecting a range of species including man. Currently divided into six species based mainly on differences in pathogenicity and host preference, the infections produced are characteristically localised in the reproductive organs and may cause abortions in some species. Exposure occurs through contact with infected animals and animal products such as unpasteurised dairy products and meat. The disease is endemic throughout many areas of the world.

The first isolation of *Brucella* species from marine mammals was reported by Ross and others in 1994<sup>1</sup>. Since then, numerous incidence of further cases have been reported, occurring in a range of species inhabiting many of the world's oceans. Particularly well documented are cases providing evidence in the Northern hemisphere, specifically from the seas around Scotland and the North of England<sup>2</sup>, Finnmark in northern Norway<sup>3</sup>, Baffin Island and the Gulf of St Lawrence, Canada<sup>4</sup>, the Pacific coastline of the USA<sup>5</sup> and off the Cornish coast of the United Kingdom<sup>6</sup>. Additional serological evidence exists in cases arising in further geographical locations such as the coasts around England and Wales<sup>7</sup>, the North Atlantic Ocean, west of Iceland to the north of Norway and Russia<sup>8</sup>, the Mediterranean Sea<sup>1</sup>, Arctic Canada and the Atlantic coast of North America<sup>9</sup>. Less

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well documented are reports of cases arising in the southern hemisphere, although two accounts of serological evidence exist, in cetaceans off the coast of Peru<sup>10</sup> and in pinnipeds in the Antarctic territory<sup>11</sup>. Molecular techniques have demonstrated that isolates differ from those originating from terrestrial mammals and can distinguish strains whose preference are pinnipeds or cetaceans as hosts. Two new species names have therefore been proposed: *B. pinnipediae* (isolates originating from pinnipeds) and *B. cetaceae* (isolates originating from cetaceans)<sup>12</sup>. Species interactions whether at sea or on land, eg. seal haul-out sites could be considered as a potential method of direct transmission from animal to animal. Some species are highly social animals, often belonging to large groups. Breeding, maternal transmission or physical trauma should also be considered as potential routes of transmission and the transmission of brucellosis by infected lungworms is another possibility<sup>5,13</sup>.

## Materials and methods

Between 1992 and 2001 blood samples were taken from 15 captive and wild rehabilitated fur seals (*Arctocephalus pusillus doriferus*) and 3 leopard seals (*Hydrurga leptonyx*). Blood samples from 12 wild Australian sea lions (*Neophoca cinerea*) originating from Kangaroo Island located 110 km southwest of Adelaide were also obtained in 2002. All were screened for the presence of antibodies to *Brucella* spp.

A competitive and two indirect ELISAs (cELISA and iELISA) were used to detect the presence of *Brucella* antibodies in the marine mammal sera. The cELISA, described by MacMillan and others<sup>14</sup> uses a lipopolysaccharide (LPS) *B. melitensis* antigen and a monoclonal antibody conjugate. Of the two indirect ELISAs, *B. melitensis* 16M antigen was used for the first, as described for the (cELISA), and the other a *B. abortus* LPS antigen was used. The iELISAs require anti-globulin conjugate with specificity for the immunoglobulin isotypes of the species under test, however protein A has been shown by Sikkema<sup>15</sup> and Eliasson and others<sup>16</sup> to bind to the IgG of a range of marine mammals. Positive/negative thresholds for these assays were set with some uncertainty but are based on those used for testing a wide range of terrestrial mammals for brucellosis from Britain<sup>17</sup>. Currently cut-off thresholds are set at >10% for both iELISAs and <60% for cELISA are considered positive.

Prevalence of anti-Brucella antibodies in serum from captive and rehabilitated fur and leopard seals of Australia

Animal Ref	iELISA (AS3)	iELISA (16M)	cELISA (16M)	Interpretation seropositive (%)
1-7	<10	<10	>60	Negative
8	15	17	91	Weak reaction
9	<10	<10	>60	Negative
10	125	164	12	Positive
11-12	<10	<10	>60	Negative
13	18	32	54	Positive
14-18	<10	<10	>60	Negative



## Results and Discussion

One of 3 leopard seals (a female) and 1 of 15 fur seals (a female) presented high antibody titers and were considered positive by all three tests. Another female fur seal showed a weak positive reaction by the iELISA only. Although all 3 serologically positive animals were captive they were originally born in the wild. Two of the leopard seals were female and 1 male, and of the fur seals 3 were male and 12 female.

Of the 12 wild Australian sea lions, (2 of which were male and 10 female), 9 (all female) were considered positive (75%) by all tests and 2 (1 female and 1 male) showed weak positive reactions by the iELISA and iELISA (16M) respectively. Another male was considered negative.

Analysis of the results shows that 11 animals overall gave a strong serological reaction by all tests. This evidence suggests a likely exposure to *Brucella* species and is the first report of positive *Brucella* serology in these three pinniped species within and around the coast of Australia. It is the third report originating from the southern hemisphere.

Still little is known about *Brucella* infections in marine mammals and, as yet, no *Brucella* spp have been isolated from pinnipeds in the southern hemisphere. The report of *Brucella* spp abortion in a captive bottle nose dolphin in the USA<sup>18</sup>, together with cultural evidence and serological evidence from both hemispheres, poses the question of how



Prevalence of anti-*Brucella* antibodies in serum from wild Australian sea lions of Kangaroo Island

Animal Ref	iELISA (AS3)	iELISA (16M)	cELISA (16M)	Interpretation seropositive (%)
1	67	110	25	Positive
2	50	72	14	Positive
3	53	96	13	Positive
4	55	74	13	Positive
5	57	78	12	Positive
6	123	196	13	Positive
7	40	35	38	Positive
8	28	84	37	Positive
9	5	8	114	Negative
10	9	16	63	Weak reaction
11	19	21	104	Weak positive
12	34	27	14	Positive



this disease affects the population dynamics. The zoonotic potential has also been documented in the case of a laboratory worker exposed to a marine strain<sup>19</sup>. Two further cases of neurobrucellosis with intracerebral granuloma caused by a marine mammal strain confirm organisms can affect humans in various settings<sup>20</sup>.

Further efforts should be directed towards the detection of antibodies and the isolation of *Brucella* from southern hemisphere pinnipeds. Increased understanding may highlight the implications for some of the most endangered marine mammal species worldwide.

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