Detection of anti-*Brucella* antibodies in pinnipeds from the Antarctic territory

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SEVERAL reports of infection by a non-defined *Brucella* species have been recorded in wild and captive marine mammals from Scotland, England and the USA (Ewalt and others 1994, Ross and others 1996, Jahans and others 1997). The animal species involved in these studies were seals (*Halichoerus grypus*, *Phoca vitulina*, *Cystophora cristata*), otters (*Lutra lutra*) and cetaceans from the families Phocoenidae (porpoises) (*Phocoena phocoena*), Delphinidae (*Orcinus orca*, *Globicephala melas*, *Delphinus delphis*, *Stenella coeruleoalba*, *Tursiops truncatus*, *Lagenorhynchus acutus*) and Balaenopteridae (*Balaenoptera acutorostrata*), from which very similar bacteria have been cultured or a humoral response against *Brucella* has been detected (Ewalt and others 1994, Foster and others 1996, Ross and others 1996, Jahans and others 1997, Jepson and others 1997, Clavareau and others 1998).

As a result of laboratory identification procedures, a close relationship with bacteria from the genus *Brucella* has been well established. Their particular characteristics led to the proposal of a new species named *Brucella maris*, presenting three serovars (Ewalt and others 1994, Foster and others 1996, Jahans and others 1997). Data concerning epidemiological aspects, such as affected species range, prevalence and geographic distribution or its pathogenicity, virulence and zoonotic potential, are still limited. In order to contribute to this knowledge, this short communication reports the presence of anti-*Brucella* antibodies in pinnipeds from the Antarctic territory detected by different serological tests.

The sampling procedure was carried out during the activities programmed for Project 018 of the Chilean Antarctic Institute (INACH). The study was conducted on 16 Antarctic fur seals (*Arctocephalus gazella*) and one Weddell seal (*Leptonychotes weddellii*). These animals were found at the Antarctic fur seal reproductive colony that exists at the site of special scientific interest (SSSI) number 32 and the Convention for Conservation of the Antarctic Marine Living Resources (CCAMLR) ecosystem monitoring programme (CEMP) site, Cape Shirreff and San Telmo islets (62° 47′ S; 60° 27′ W), on Livingston Island (South Shetland Islands), Antarctica. The population of this colony was 13,715 specimens during the summer census (Torres and others 1999). Blood samples were collected during December 1998 and January 1999.

Five samples were taken from dead animals, corresponding to one *L weddellii* specimen, plus three pups and one adult male *A gazella*. Postmortem examinations were performed on all specimens and blood was obtained by jugular or cardiac puncture. Twelve live *A gazella* were sampled by puncture of an interdigital vein of the rear fins. These animals were a yearling male and 11 adult females in lactation.

The age of the seals was determined by postmortem examination of characteristics in the dead animals, and by the phenotypic and external morphometric characteristics and fur colour in the live animals. In the case of *A gazella*, the three pups born during the season were identified basically by their size and black fur; the adult male was characterised as an animal matching a 'sultan phenotype' and was catalogued as an old adult according to postmortem criteria. The adult females in lactation were catalogued as adult females of reproductive age, and the yearling male was an animal of known age that had been tagged during the previous season. The *L weddellii* specimen was catalogued as a juvenile male due to his phenotype and intermediate size between a pup and an adult male.

After collection, clotted blood samples were centrifuged at 700 *g* for seven minutes and sera were kept at environmental temperature in the field (0 to 5°C) for five weeks before arrival at the laboratory, and they were then aliquoted and kept at –25°C until use.

The sera were tested using the following serological tests to detect anti-*Brucella* antibodies: rose Bengal test (RBT) performed at the Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, and complement fixation test (CFT) performed at the Laboratorio Pecuario Central, Servicio Agrícola y Ganadero (SAG), Ministerio de Agricultura, Chile, as described by the OIE (1996); agar gel immunodiffusion (AGID) test as described by Pinochet and others (1990), using a polysaccharide antigen obtained from *Brucella abortus* 1119-3 strain by hot water extraction and ethanolic precipitation; and competitive ELISA (cELISA) performed at the Laboratorio Pecuario Central, SAG, Ministerio de Agricultura, Chile, as described by Nielsen and others (1996). Bovine sera were used as negative and positive controls, which were collected from a brucellosis-free area and from cows with bacteriological culture, respectively (Abalos and others 1996). Monoclonal antibody M84 and goat anti-mouse immunoglobulin G (IgG) horseradish peroxidase conjugate were also used. Competition was determined as the percentage of inhibition of colour development, and sera ranging from 30 to 100 per cent were considered as having antibodies against *Brucella*.

The results of the serological tests and the characteristics of the sampled animals are shown in Table 1. Six of the 17 animals (35 per cent) were positive by at least one of the tests performed. The Weddell seal was positive by cELISA with 73 per cent inhibition, as well as five Antarctic fur seals, two of which were also positive by CFT, with one weakly positive by RBT. According to age and sex, the positive animals were a juvenile male, a yearling male, a pup and three adult females.

The results suggest that at least two species of pinnipeds, *A gazella* and *L weddellii*, from the Antarctic territory, have been exposed to antigens of a *Brucella* species. To confirm any infection, it is necessary to isolate the bacteria, since cross-reactions against *Brucella* could be caused by other microorganisms (MacMillan 1990). However, the reported presence of a related bacterial species (Ewalt and others 1994, Foster and others 1996) in marine mammals of the northern hemisphere, and the high accuracy and precision of the cELISA (Nielsen and others 1996), are strong evidence of the possible presence of the infection in Antarctica.

Only the AGID test did not detect any antibodies, but it is known that this technique is less sensitive than classical diagnostic tests used in brucellosis (Wright and Nielsen 1990). The RBT reacted weakly with one serum which, however, showed the presence of antibodies in the cELISA. It would be expected that sera with a similar or higher percentage inhibition would have a similar reaction in the RBT, so it was likely that these results were caused by differences in antibody classes (immunoglobulin M [IgM], IgG) or quality (avidity and affinity). The CFT results were coincident in two positive sera with the cELISA but, unfortunately, the high anticomplementary activity (11/17) shown by these pinnipeds' sera makes this test unsuitable for use in these animals.

It is interesting to note that the only *L weddellii* individual sampled had a high percentage inhibition (73 per cent) in the cELISA, suggesting active or fairly recent infection. However, when examining the results in *A gazella*, five of 16 individuals had detectable antibodies. Regarding these cELISA results, it can be concluded that in six of the animals tested, antibodies against *Brucella* antigens were detected and they

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TABLE 1: Details of pinnipeds (*Leptonychotes weddellii* and *Arctocephalus gazella*) sampled in the Antarctic territory and the serological test results for the detection of anti-*Brucella* species antibodies

Species	Sex	Age group	Sampled (date)	RBT	AGID	CFT (titre)	cELISA (% I)
*L weddellii**	M	Juvenile	01/99	N	N	AC	P (73)
*A gazella**	M	Pup	01/99	N	N	N	N
*A gazella**	M	Pup	01/99	N	N	AC	P (32)
*A gazella**	M	Pup	01/99	N	N	N	N
*A gazella**	M	Adult	01/99	N	N	AC	N
A gazella	M	Yearling	01/99	WP	N	AC	P (34)
A gazella	F	Adult	12/98	N	N	AC	N
A gazella	F	Adult	12/98	N	N	AC	N
A gazella	F	Adult	12/98	N	N	AC	N
A gazella	F	Adult	12/98	N	N	AC	N
A gazella	F	Adult	12/98	N	N	N	N
A gazella	F	Adult	12/98	N	N	N	N
A gazella	F	Adult	12/98	N	N	P (1/20)	P (45)
A gazella	F	Adult	12/98	N	N	P (1/20)	P (37)
A gazella	F	Adult	01/99	N	N	AC	P (44)
A gazella	F	Adult	01/99	N	N	AC	N
A gazella	F	Adult	01/99	N	N	AC	N

* Dead animal, N negative, P positive, AC Anticomplementary activity, WP Weakly positive, RBT Rose Bengal test, AGID Agar gel immunodiffusion, CFT Complement fixation test, cELISA Competitive ELISA, M Male, F Female

were classified as positive in accordance with Nielsen and others (1996), who determined a cut off of 30 per cent inhibition. Even though there is no special cut off point recommeded when pinnipeds are tested, the presence of antibodies should be considered to be the more important fact. This indicates a real possibility of a *Brucella* infection in marine mammals occurring in the Antarctic territory which could also, considering previous reports, be widely distributed around the world.

Brucellosis is an infectious disease with profound reproductive implications in terrestrial mammals, and the presence of a similar disease in marine mammals could present a problem in species which are at risk conservationally. The geographical distribution of *A gazella* and *L weddellii* (Torres and others 1984, Bonner 1994) in the southern hemisphere and other species of marine mammal in the northern hemisphere (Bonner 1994, Carwardine and Camm 1995) which are possibly infected with a *Brucella* species (Ewalt and others 1994, Foster and others 1996, Ross and others 1996, Jahans and others 1997, Clavareau and others 1998), does not suggest a clear pattern of contact among individuals. For this reason, studies are required in intermediate geographical regions as well as in other marine mammal species which could be a bridge between areas.

Finally, more efforts should be directed towards the detection of antibodies in other species of marine mammals, and the isolation of the bacteria for comparison with those isolated in the northern hemisphere and with other *Brucella* species. These studies will increase the knowledge of the ecological, epidemiological and zoonotic potential of *Brucella* species in marine mammals around the world.

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