ADDITIONAL DATA ON ANTI-BRUCELLA ANTIBODIES IN ARCTOCEPHALUS GAZELLA FROM CAPE SHIRREFF, LIVINGSTON ISLAND, ANTARCTICA

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Abstract

The first instance of anti-Brucella antibodies in the Southern Hemisphere was recorded in two pinniped species from Antarctica: Antarctic fur seal (Arctocephalus gazella) and Weddell seal (Leptonychotes weddellii). Taking into account these first records, this paper describes additional data on Brucella antibodies in A. gazella obtained at Cape Shirreff, Livingston Island, Antarctica. Eighty-six A. gazella body fluid samples were collected from 77 live animals and nine from dead animals. Body fluid samples (blood, pleural fluid or pericardic fluid) were tested using the Rose Bengal (RB) test, the Competitive Enzymoimmunoassay (c-ELISA) test and the Competitive Enzymoimmunoassay ‘COMPELISA®’ test. Antibodies against Brucella spp. were detected in one sample with the RB test, and in five of the 86 samples (5.8%) using the c-ELISA test. These results present serological evidence of Brucella infection in A. gazella. We suggest the utility of extra-vascular fluid for this serological research. We recognise the c-ELISA test as the best option in this regard, and suggest that Brucella spp. has an extended distribution and is probably found worldwide in marine mammals. We highlight the importance of evaluating in the CCAMLR Working Group on Ecosystem Monitoring and Management (WG-EMM) the incidence of infectious diseases, since diseases like brucellosis may produce reproductive failure in predatory species. However, it is not possible to relate evidence of Brucella infection to any anthropogenic event in Antarctica since the bacteria have not yet been isolated and no studies on this subject have been conducted there previously.

Résumé

 être présent sur des mammifères marins du monde entier. Nous soulignons l'importance de l'évaluation, au sein du Groupe de travail de la CCAMLR sur le contrôle et la gestion de l'écosystème (WG-EMM), de l'incidence des maladies infectieuses, du fait que des maladies telles que la brucellose peuvent provoquer un arrêt de la reproduction des espèces prédatrices. Il n'est toutefois pas possible de rapprocher l'infection de Brucella d'un événement anthropogénique en Antarctique car les bactéries n'ont pas encore été isolées et il n'a encore été procédé à aucune étude de cette question.

**Résumé**

En Antarctique, les antitèlas de Brucella ont été observés pour la première fois dans deux espèces de pinnipèdes antarctiques: Arctocephalus gazella et le loup de Weddell (Leptonychotes weddelli). Les résultats soulignent l'importance de l'évaluation de l'incidence des maladies infectieuses sur l'écosystème de la CCRVMA (WG-EMM) et la gestion de la reproduction des espèces prédatrices. Il n'est toutefois pas possible de rapprocher l'infection de Brucella d'un événement anthropogénique en Antarctique car les bactéries n'ont pas encore été isolées et il n'a encore été procédé à aucune étude de cette question.

**Resumen**

Por primera vez se detectó la presencia de anticuerpos anti-Brucella en el hemisferio sur en dos especies de pinnípedos antárticos: lobo antártico (Arctocephalus gazella) y la foca de Weddell (Leptonychotes weddelli). Teniendo en cuenta esta observación inicial, este trabajo presenta datos adicionales sobre los anticuerpos anti-Brucella en A. gazella del Cabo Shirreff, isla Livingston, en la Antártica. Se tomaron muestras de fluidos corporales de 77 ejemplares vivos de A. gazella y muestras de animales muertos. Las muestras de fluidos corporales fueron analizadas mediante la prueba de Rosa de Bengala (RB), inmunoensayo enzimático de competencia (c-ELISA) y inmunoensayo de competencia 'COMPELISA@'. Se detectaron anticuerpos anti-Brucella spp. en una muestra mediante la prueba de RB, y en cinco de las 86 muestras (5,8%) mediante la prueba c-ELISA. Estos resultados muestran que existe evidencia serológica de infección de Brucella en A. gazella, la utilidad de las muestras de fluidos corporales (sangre, líquido de la pleura o del pericardio) fueron analizadas mediante la prueba de Rosa de Bengala (RB), inmunoensayo enzimático de competencia (c-ELISA) y el inmunoensayo de competencia 'COMPELISA@'. Se detectaron anticuerpos anti-Brucella spp. en una muestra mediante la prueba de RB, y en cinco de las 86 muestras (5,8%) mediante la prueba c-ELISA. Estos resultados demuestran que existe evidencia serológica de infección de Brucella en A. gazella, la utilidad de las muestras de fluidos corporales para la investigación de Brucella en A. gazella fue demostrada. Los resultados sugieren que Brucella spp. está ampliamente distribuida, y posiblemente se encuentra presente en los mamíferos marinos a nivel mundial. Se subraya la importancia que tiene para el grupo de trabajo sobre el seguimiento y ordenación del ecosistema de la CCRVMA (WG-EMM) la evaluación de la incidencia de las enfermedades infecciosas, ya que este tipo de infecciones puede afectar gravemente la reproducción de las especies depredadoras. Sin embargo, debido a que el agente bacteriano no ha sido aislado todavía y a la falta de trabajos sobre este tema en la región, no es posible relacionar los indicios de brucelosis con eventos antropogénicos en la Antártica.
INTRODUCTION

Brucellosis is an infectious disease that mainly affects the reproductive system of animals, causing abortion and sterility. It is also a zoonotic disease. Instances of bacterial infection by the genus *Brucella* in wild marine mammals from the Northern Hemisphere have been recorded along the coasts of Scotland (Ross et al., 1994; Ross et al., 1996), Arctic Canada (Nielsen et al., 1996b), England (Foster et al., 1996; Jepson et al., 1997) and 10 locations of the North Atlantic Ocean, Russia and Norway, including Svalbard Islands (Tryland et al., 1999). It has also been reported in captive marine mammals in the USA (Ewalt et al., 1994).

Brucellosis has been recorded in aquatic mammal wildlife from four families: Mustelidae (Foster et al., 1996; Phocoenidae (Ross et al., 1994; Foster et al., 1996; Ross et al., 1996; Nielsen et al., 1996b; Jepson et al., 1997; Tryland et al., 1999); Delphinidae (Ewalt et al., 1994; Ross et al., 1994; Foster et al., 1996; Ross et al., 1996; Jepson et al., 1997; Miller et al., 1999) and Balaenopteridae (Clavareau et al., 1998; Tryland et al., 1999).

Some studies only describe serological evidence of *Brucella* infection (humoral response) (Nielsen et al., 1996b; Jepson et al., 1997), while others have cultured a very similar bacterium (Ross et al., 1994; Ewalt et al., 1994; Foster et al., 1996; Ross et al., 1996; Clavareau et al., 1998; Tryland et al., 1999; Bricker et al., 2000). This isolated pathogen shows a close relationship with bacteria from the genus *Brucella* (Ewalt et al., 1994; Foster et al., 1996; Jahans et al., 1997; Clavareau et al., 1998; Bricker et al., 2000) and has been proposed as a new species (Jahans et al., 1997; Bricker et al., 2000) named *Brucella maris*, with three biovars (Jahans et al., 1997).

There are possible similarities in the pathogenesis and transmission of *Brucella* spp. that affect marine and terrestrial mammals. The isolation of *Brucella* spp. from a *Tursiops truncatus* foetus suggests that these bacteria may have an affinity for the uterus and may cause abortion in marine mammals (Ewalt et al., 1994; Miller et al., 1999). The same idea is suggested in relation to other tissues from which these organisms have been isolated in marine mammals, according to Foster et al. (1996) and Jahans et al., (1997).

The first instance of anti-*Brucella* antibodies in the Southern Hemisphere was recorded in two pinniped species from Antarctica: Antarctic fur seal (*Arctocephalus gazella*) and Weddell seal (*Leptonychotes weddellii*) (Blank et al., 1999; Retamal et al., 2000). However, epidemiological aspects such as other affected species, virulence, prevalence, pathogenesis, geographic distribution and zoonotic potential are still unknown.

Taking into account these first records, the present serological study was aimed at obtaining a larger sample size in order to evaluate the extent of the infection and contribute to the epidemiological knowledge of *Brucella* infection in *A. gazella* in the study area.

MATERIAL AND METHODS

Fieldwork was carried out at the Site of Special Scientific Interest (SSSI) No. 32 and CCAMLR Ecosystem Monitoring Program (CEMP) site No. 2 'Cape Shirreff and San Telmo Islands' (62°47'S; 60°27'W), located on the northeastern coast of Livingston Island (South Shetland Islands), Antarctica, as a complementary activity of Project 018 'Ecological studies on the Antarctic fur seal, *Arctocephalus gazella*', being carried out by the Instituto Antártico Chileno (INACH).

SSSI No. 32 and CEMP Site No. 2 contain one of the southernmost breeding populations of *A. gazella*, with a current population size of 20 139 individuals (Vallejos et al., 2000).

A total of 79 blood samples, 1 pleural fluid, 1 peritoneal fluid and 5 pericardic fluid samples were obtained from 86 specimens of *A. gazella* between December 1999 and February 2000.

Nine samples were taken from dead *A. gazella*: two juvenile females, one adult female, one juvenile male, three sub-adult males and two adult males. All the specimens were necropsied and blood samples were obtained from the jugular vein or the cardiac cavity. The extravascular body fluid samples were obtained from body cavities when it was not possible to obtain blood samples due to clotting.

Blood samples (77) were obtained from live *A. gazella*: four female pups, 10 male pups, one yearling female, one yearling male and 61 lactating adult females. Blood samples were obtained by puncturing the interdigital vein in the rear flippers. All the live-sampled specimens were tagged after sampling, as in the previous studies, and all of them were apparently healthy animals. However, some pathologic signs, possibly indicating brucellosis (e.g. oorquitis, pups born dead, pups dying shortly after birth and non-pregnant females), have been observed in animals from the same breeding
Table 1: Individual characteristics and sample types from the positive serological test results, of anti-
Brucella antibodies in A. rctepehuls gazella. * - dead animal; RB - Rose Bengal test; COMPELISA® - Competitive Enzymoimmunoassay ‘COMPELISA®’ test; c-ELISA - Competitive Enzymoimmunoassay (c-ELISA) test.

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Sex</th>
<th>Age</th>
<th>RB</th>
<th>COMPELISA®</th>
<th>c-ELISA (% I)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>Female</td>
<td>Adult</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive (33)</td>
</tr>
<tr>
<td>Blood</td>
<td>Female</td>
<td>Adult</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive (35)</td>
</tr>
<tr>
<td>Pleural fluid*</td>
<td>Male</td>
<td>Sub-adult</td>
<td>Positive</td>
<td>Negative</td>
<td>Positive (65)</td>
</tr>
<tr>
<td>Pericardic fluid*</td>
<td>Male</td>
<td>Adult</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive (46)</td>
</tr>
</tbody>
</table>

Phenotype, morphometric characteristics and fur colour determined age categories. A. gazella pups (belonging to the same cohort) were identified as pups by their size and black fur. Tags attached during the previous season identified male and female yearlings. The juvenile dead female and male were recorded as juveniles due to their phenotype and body size. The sub-adult dead males were identified as non-breeding males due to their phenotype and body size. The body size, phenotype and maturity of its reproductive system observed during the necropsy identified the dead adult female. The dead adult males were identified as having the ‘bull phenotype’. Adult lactating females were identified by observing them suckling pups.

All body fluid samples were centrifuged at 700 x g for 7 minutes to obtain sera, which were kept at freezing temperature in the field (-4°C to 0°C) for three months and then sent to the laboratory where they were kept at -25°C until analysis. The following serological tests were used in the laboratory to detect anti-Brucella antibodies in sera:

- Rose Bengal (RB) test*, according to the Manual of Standards for Diagnostic Tests and Vaccines from the International Organization of Epizooties (IOE, 1996);

- Competitive Enzymoimmunoassay (c-ELISA) test*, according to FAO/IAEA (1994). The reaction was determined as a percentage of inhibition on colour development (% I) with respect to the conjugate control that had 0% I; and the sera ranging from 30% I to 100% I were considered positives; and

- Competitive Enzymoimmunoassay ‘COMPELISA®’ test* (Veterinary Laboratories Agency, UK), according to the test instructions.

Bovine sera collected from a brucellosis-free area and from cows with positive bacteriological culture respectively (Abalos et al., 1996) were used as positive and negative controls.

RESULTS

The positive results of the serological tests are shown in Table 1. Five of the 86 sampled animals (5.8%) were positive using the c-ELISA test. When examining the five positive results from the c-ELISA test, the detectable anti-Brucella antibodies ranged between 33 and 65% I (Table 1). Negative and positive bovine sera controls ranged near to 0 and 1% respectively.

Three positive results were obtained from blood samples taken from live animals, and the two positive results were obtained from dead animals (one pleural fluid sample and one pericardic fluid sample).

In relation to the tests applied in this study, one out of five positive c-ELISA sera was also positive to the RB test (Table 1). Table 1 also shows that no positive results were obtained with the COMPELISA® test.

DISCUSSION AND CONCLUSION

In relation to the tests applied in this study, one out of five positive c-ELISA sera was also positive to the RB test. Nevertheless we would expect that sera with high % I for the c-ELISA test would have a similar reaction in the RB test. On the other hand, no positive results were shown.

* Tests performed at the Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Santiago, Chile.
with the COMPELISA® test, suggesting that it could be less sensitive for detecting anti-Brucella antibodies in A. gazella and confirming the c-ELISA test as the best option for serological studies.

The positive results of the c-ELISA test in two dead animals obtained through one pleural fluid sample and one pericardic fluid sample suggest the utility of extra-vascular fluid collection for serological research and make the sampling procedure easier during the post mortem analyses.

If the 31.3% of antibody prevalence reported for the previous season (Retamal et al., 2000) is compared directly with the results observed in this study (5.8%), a decrease in antibody prevalence is observed. If we only consider the antibody prevalence of the dead animals (25%) recorded in Retamal et al., (2000), no significant differences are shown with the 22.2% antibody prevalence obtained in this study. Hence, the decrease in antibody prevalence observed in the two studies is due to samples obtained from live animals (33.3% antibody prevalence recorded in Retamal et al., 2000 and 3.9% observed in this study) and is probably related to the size of the sample (12 and 77 live tested animals included in the previous and present study respectively) rather than there being a real decrease in prevalence.

Serological test limitations refer to the absence of a special cut-off to be applied to Pinnipedia tested; there is a lack of positive A. gazella controls, since sera from animals where Brucella have been isolated are required and serologic cross-reactions against Brucella could be caused by other microorganisms (MacMillan, 1990). Hence, negative and positive status could be modified in the future. However, the culture evidence of a very similar bacterial species affecting marine mammals of the Northern Hemisphere (Ross et al., 1994; Ewalt et al., 1994; Foster et al., 1996; Ross et al., 1996; Clavareau et al., 1998; Tryland et al., 1999; Bricker et al., 2000), the recent record of the presence of anti-Brucella antibodies in A. gazella from Antarctica (Blank et al., 1999; Retamal et al., 2000) and the high accuracy and precision of the c-ELISA test (Nielsen et al., 1996a), provide strong evidence of Brucella infection in the A. gazella population from Cape Shirreff and suggest that this bacterium has a wide distribution and is probably found worldwide in marine mammals. Since Cape Shirreff is the only place where this study has been performed, we cannot establish the extent of the infection in the Southern Ocean.

Infectious diseases that affect Antarctic marine mammals are poorly understood and only a few records are known, most of them included in Kerry and Riddle, 1999; Laws and Taylor, 1957; Stirling, 1969; Tierney, 1977; Panagis et al., 1982; Bengtson and Boveng, 1991; Junin and Castello, 1995; McFarlane, 1996). The serological evidence of Brucella infection in A. gazella underscores the importance of evaluating the incidence of infectious diseases in Southern Ocean fauna. All infectious diseases are of fundamental importance, threatening the survival of populations. Such a problem could also influence the conservation risk status of the species affected, possibly having an impact on the population dynamics. These are important questions that should be considered by the CCAMLR Working Group on Ecosystem Monitoring and Management (WG-EMM). Brucella spp. may lead to the reproductive failure of predatory species in a way different from that caused by a reduction in the food supply due to fishing or the influence of environmental phenomena such as El Niño.

From the point of view of the protection of the Antarctic environment, it is important to differentiate diseases which may come from outside the Southern Ocean ecosystem (potential biological contamination due to human activity; or non-described natural transmission, like overlapping distribution range of the affected species) from those that originate from within this environment. However, it is still not possible to relate Brucella spp. infection to any anthropic event in Antarctica since Brucella have yet to be isolated. Key information on their epidemiology is still unknown and there have been no previous studies on this subject in these latitudes.

The transmission of Brucella in terrestrial mammals occurs by direct contact among animals. Therefore, important factors for the dissemination of these bacteria in marine mammals could be related to the distribution and seasonal migratory habits of the affected species. In this respect, A. gazella is a species with a sub-Antarctic distribution (Aguyay and Torres, 1967; Aguyay et al., 1992; Bonner, 1994). However, some records have been made for southern Australian islands (Bonner, 1994), southern (Texera, 1974) and central Chile (Torres et al., 1984; Bonner, 1994).

On the other hand, the distribution range of marine mammal species infected with Brucella spp. in the Northern Hemisphere (Ewalt et al., 1994; Ross et al., 1996; Foster et al., 1996; Jahans et al., 1997; Jepson et al., 1997; Clavareau et al., 1998) sometimes extends to both hemispheres. However,
this does not provide a clear pattern of natural contact among individuals that can explain the dissemination of Brucella into the Southern Ocean since the affected species are described as different populations (Evans et al., 1982) or subspecies according to the geographical area that they occupy (Pastene et al., 1994; Carwardine and Camm, 1995). In relation to their migratory habits, most of the affected species do not carry out large-scale migratory movements and some of them only make small seasonal movements (Carwardine and Camm, 1995).

In the future, other studies will be necessary to determine the effectiveness of the tests applied, the isolation of Brucella and to determine their eventual zoonotic potential to be considered by researchers who work with marine mammals. We also suggest that similar studies in intermediate geographic regions, as well as in other aquatic mammal species be carried out; these could be a biological bridge between both geographically extreme latitudes.

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