CCAMLR Science, Vol. 8 (2001): 147–154

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

O. Blank ✉
Departamento Científico
Instituto Antártico Chileno (INACH)
Rómulo Correa 375, Punta Arenas, Chile
Email – oblankh@entelchile.net

P. Retamal and P. Abalos
Departamento de Medicina Preventiva Animal
Facultad de Ciencias Veterinarias y Pecuarias
Universidad de Chile
Casilla 2-Correo 15, Santiago, Chile

D. Torres
Departamento Científico
Instituto Antártico Chileno (INACH)
Av. Luis Thayer Ojeda 814, Correo 9
Providencia, Santiago, Chile

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

Le premier cas d'anti-corps anti-*Brucella* dans l'hémisphère sud a été relevé sur deux espèces de pinnipèdes de l'Antarctique : l'otarie de Kerguelen (*Arctocephalus gazella*) et le phoque de Weddell (*Leptonychotes weddellii*). Compte tenu de ces premiers enregistrements, ce document fournit des données complémentaires sur les anti-corps de *Brucella* trouvés sur *A. gazella* au cap Shirreff, île Livingston, Antarctique. Quatre-vingt six échantillons de liquides biologiques de *A. gazella* ont été collectés : 77 sur des animaux vivants, les neuf autres sur des animaux morts. Les échantillons de liquides biologiques (sang, liquide pleural ou liquide péricardique) ont été soumis à l'épreuve Rose Bengal (RB), à l'épreuve compétitive de l'immuno-essai enzymatique (c-ELISA) et à l'épreuve compétitive de l'immuno-essai enzymatique "COMPELISA®". Des anti-corps de *Brucella* spp. ont été trouvés dans un échantillon soumis à l'épreuve RB, et dans cinq des 86 échantillons (5,8%) soumis à l'épreuve c-ELISA. Ces résultats sont la preuve sérologique d'une infection de *Brucella* chez *A. gazella*. Nous suggérons d'utiliser le liquide extra-vasculaire pour effectuer cette recherche sérologique. Nous reconnaissons qu'à cet effet, l'épreuve c-ELISA offre la meilleure solution et présumons que *Brucella* spp. est largement répandu et qu'il pourrait

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'on pas encore été isolées et il n'a encore été procédé à aucune étude de cette question.

Резюме

В Южном полушарии противобруцеллезные антитела были впервые зарегистрированы в двух видах ластоногих из Антарктики: Южном морском котике (*Arctocephalus gazella*) и тюлене Уэдделла (*Leptonychotes weddellii*). В статье описываются дополнительные данные по антителам к *Brucella* в *A. gazella*, полученные на мысе Ширрефф (о-в Ливингстон, Антарктика). Было получено 86 проб жидкости: из 77 живых и 9 из мертвых животных. Анализ образцов жидкости (крови, плеврального и перекардиального экссудата) проводился с использованием теста Rose Bengal (RB), конкурентного иммуноферментного анализа (c-ELISA) и конкурентного иммуноферментного анализа 'COMPELISA®'. Антитела к видам *Brucella* были обнаружены в одном образце, используя тест RB, и в 5 из 86 образцов (5.8%), используя тест c-ELISA. Эти результаты серологических исследований подтверждают наличие инфекции *Brucella* в *A. gazella*. Мы предлагаем для таких серологических исследований использовать экстраваскулярную жидкость и считаем, что в этом отношении тест c-ELISA является лучшим. Возможно, виды *Brucella* имеют широкое распространение и могут быть найдены в морских млекопитающих по всему миру. Мы подчеркиваем важность оценки Рабочей группой АНТКОМа по экосистемному мониторингу и управлению (WG-EMM) распространенности инфекционных заболеваний, т. к. такие болезни, как бруцеллез, могут приводить к неразмножению хищников. Так как бактерия пока выделена не была, и других исследований по этому вопросу в Антарктике не проводилось, связать бруцеллез с антропогенным воздействием там невозможно.

Resumen

La primera vez que se detectó la presencia de anticuerpos anti-*Brucella* en el hemisferio sur fue en dos especies de pinnípedos antárticos: el lobo fino antártico (*Arctocephalus gazella*) y la foca de Weddell (*Leptonychotes weddellii*). 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 86 muestras de fluidos corporales de 77 ejemplares vivos de *A. gazella* y 9 muestras de animales muertos. 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 líquido extra-vascular para las investigaciones serológicas y se reconocen a la prueba de c-ELISA como la mejor opción. 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.

Keywords: pathology, *Brucella*, Pinnipedia, Antarctic fur seal, *Arctocephalus gazella*, CEMP Site No. 2, Antarctica, CCAMLR

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); Phocidae (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, pathogenia, 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.rctocephalus gazella*. * – dead animal; RB – Rose Bengal test; COMPELISA® – Competitive Enzymoimmunoassay 'COMPELISA®' test; c-ELISA – Competitive Enzymoimmunoassay (c-ELISA) test.

Sample Type	Sex	Age	RB	COMPELISA®	c-ELISA (% I)
Blood	Female	Adult	Negative	Negative	Positive (33)
Blood	Female	Adult	Negative	Negative	Positive (35)
Blood	Female	Adult	Negative	Negative	Positive (45)
Pleural fluid*	Male	Sub-adult	Positive	Negative	Positive (65)
Pericardic fluid*	Male	Adult	Negative	Negative	Positive (46)

colony. These were not sampled due to lesser sampling opportunities, the desire to prevent disturbance, and also logistic problems.

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° 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 (Aguayo and Torres, 1967; Aguayo 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.

ACKNOWLEDGEMENTS

The authors would like to thank the authorities of INACH who made the fieldwork possible. We would also like to thank the Chilean Navy for its logistic support. We especially want to acknowledge the support given by the field team, Layla Osman (Universidad Austral de Chile), Verónica Vallejos (Departamento Científico INACH), Jorge Acevedo (Universidad Arturo Prat) and Mario Briones (INACH), without whose enthusiastic help this study could not have been completed. Thanks also to the US AMLR Program, especially Michael Goebel, Rennie Holt, Matt Rutishauser and Brian Parker. Finally, special thanks to Anelio Aguayo, Michael Goebel, Dan Costa, Valentina Ramirez, Francisco Sales and Rodrigo Hucke-Gaete for their critique of the manuscripts, to Ricardo Matus for his great help and patience, and to Juan Ríos for his help in the English text.

REFERENCES

Abalos, P., L. Ibarra, L. Pinochet, F. Navia and X. Bosier. 1996. Residual anti-*Brucella abortus* srain 19 antibodies detected in adult cattle by two indirect-ELISA tests. *Vet. Rec.*: 138–140.

Aguayo, A. and D. Torres. 1967. Observaciones sobre mamíferos marinos durante la vigésima Comisión Antártica Chilena. Primer censo de Pinipedios en las Islas Shetland del Sur. *Rev. Biol. Mar.* (Montemar), 13 (1): 1–57.

Aguayo, A., J. Capella, H. Torres, R. Jaña and D. Torres. 1992. Progreso en el estudio ecológico del lobo fino antártico, *Arctocephalus gazella*, en Cabo Shirreff, Isla Livingston, Antártica. *Bol. Antart. Chileno*, 11 (1): 12–14.

Bengtson, J.L. and P. Boveng. 1991. Antibodies to canine distemper virus in Antarctic seals. *Mar. Mamm. Sci.*, 7: 85–87.

Blank, O., P. Retamal, D. Torres and P. Abalos. 1999. First record of *Brucella* spp. antibodies in *Arctocephalus gazella* and *Leptonychotes weddellii* from Cape Shirreff, Livingston Island, Antarctica (SC-CAMLR-XVIII/BG/17). *CCAMLR Scientific Abstracts*: p. 5.

Bonner, N. 1994. The fur seals. In: *Seals and Sea Lions of the World*, Chapter 2. London: 36–58.

Bricker, B., D. Ewalt, A. MacMillan, G. Foster and S. Brew. 2000. Molecular characterization of *Brucella* strain isolated from marine mammals. *J. Clin. Microbiol.*: 1258–1262.

Carwardine, M. and M. Camm. 1995. *Whales, Dolphins and Porpoises: the Visual Guide of All the World's Cetaceans*. Dorling Kindersley Limited, London: 225 pp.

Clavareau, C., V. Wellemans, K. Walravens, M. Tryland, J.M. Verger, M. Grayon, A. Cloeckaert, J.J. Letesson and J. Godfroid. 1998. Phenotypic and molecular characterization of a *Brucella* strain isolated from a minke whale *(Balaenoptera acutorostrata)*. *Microbiol.-UK*, 144 (12): 3267–3273.

Evans, W.E., A.V. Yablokov and A.E. Bowles. 1982. Geographic variation in the color pattern of killer whales (*Orcinus orca*). *Rep. Int. Whal. Commn.*, 32: 687–694.

Ewalt, D.R., J.B. Payeur, B.M. Martin, D.R. Cummins and W.G. Miller. 1994. Characteristics of a *Brucella* species from a bottlenose dolphin (*Tursiops truncatus*). *J. Vet. Diagn. Invest.*, 6: 448–452.

FAO/IAEA. 1994. *Brucellosis ELISA Kit Manual. Competitive Enzymeimmunoassay for Detection of Antibody to Brucella abortus*. Joint FAO/IAEA Programme. Animal Production and Health Unit. Seibersdorf, Austria.

Foster, G., K.L. Jahans, R.J. Reid and H.M. Ross. 1996. Isolation of *Brucella* species from cetaceans, aeals and otters. *Vet. Rec.*, 138: 583–586.

IOE. 1996. *Manual of Standards for Diagnostic Tests and Vaccines*. Third Edition. ISBN 92-9044-423-1.

Jahans, K.L., G. Foster and E.S. Brougthon. 1997. The characterization of *Brucella* strains isolated from marine mammals. *Vet. Microbiol.*, 57: 373–382.

Jepson, P.D., S. Brew, A.P. MacMillan, J.R. Baker, J. Barnett, J.K. Kirkwood, T. Kuiken, I.R. Robinson and V.R. Simpson. 1997. Antibodies to *Brucella* in marine mammals around the coast of England and Wales. *Vet. Rec.*, 141: 513–515.

Junin, M. and H.P. Castello. 1995. Osteomyelitis of the skull in a leopard seal, *Hydrurga leptonyx*. *Mar. Mamm. Sci.*, 11: 403–406.

Kerry, K. , M. Riddle and J. Clarke. 1999. Diseases of Antarctic wildlife. A report for the Scientific Committee on Antarctic Research (SCAR) and the Council of Managers of National Antarctic Programs (COMNAP). Australian Antarctic Division: 104 pp.

Laws, R.M. and R.J.F. Taylor. 1957. A mass of dying crabeater seals, *Lobodon carcinophagus* (Gray). *Proceed. Zool. Soc. London*, 129: 315–324.

MacMillan, A. 1990. Conventional serological test. In: Nielsen K. and J.R. Duncan (Eds). *Animal Brucellosis*. CRC Press, Boca Raton, Florida, USA: 153–197.

McFarlane, R.A. 1996. Gross pathology of the Weddell seal (*Leptonychotes weddellii*) in the Vestfold Hills, East Antarctica. *Aq. Mamm.*, 22: 27–33.

Miller, G., G. Adams, T. Fichi, N. Cheville, J. Payeur, D. Harley, C. House and S. Ridgway. 1999. Brucella-induced abortion and infection in bottlenose dolphins (*Tursiops truncatus*). *J. Zoo Wild. Med.*, 30 (1): 100–110.

Nielsen, K.H., D. Gall, W. Kelly, A. Vigliocco, D. Henning and M. García. 1996a. *Immunoassay Development: Application to Enzyme Immunoassay for the Diagnosis of Brucellosis*. Agriculture and Agri-Food Canada Monograph. ISBN 0-662-24163-0.

Nielsen O., K. Nielsen and R. Stewart. 1996b. Serologic evidence of *Brucella* spp. exposure in Atlantic walruses (*Odobenus rosmarus rosmarus*) and ringed seals (*Phoca hispida*) of Arctic Canada. *Arctic.*, 49 (4): 383–386.

Panagis, K., P. Apps and M. H. Knight. 1982. Seal finger: occurrence in Antarctica. *S. Afr. J. Antarc. Res.*, 12: p. 49.

Pastene, L.A., Y. Fujise and K. Numachi. 1994. Differentiation of mitochondrial DNA between ordinary and dwarf forms of southern minke whale. *Rep. Int. Whal. Commn*: p. 44.

Retamal, P., O. Blank, P. Abalos and D. Torres. 2000. Detection of anti-*Brucella* antibodies in pinnipeds from the Antarctic Territory. *Vet. Rec.*, 146: 166–167.

Ross, H.M., G. Foster, R.J. Reid, K.L. Jahans and A.P. MacMillan. 1994. *Brucella* species infection in sea-mammals. *Vet. Rec.*: p. 359 (letter).

Ross, H.M., K.L. Jahans, A.P. MacMillan, R.J. Reid, P.M. Thompson and G. Foster. 1996. *Brucella* species infection in North Sea and cetacean populations. *Vet. Rec.*, 138: 647–648.

Stirling, I. 1969. Tooth wear as a mortality factor in the Weddell seal, *Leptonychotes weddellii*. *J. Mamm.*, 50: 559–565.

Texera, W.A. 1974. Nuevos antecedentes sobre mamíferos de Magallanes. *Ans. Inst. Pat.* (Chile), 4: 335–357.

Tierney, T.J. 1977. Disease and injury in the southern elephant seal. *Austr. Vet. Jour.*, 53: 91–92.

Torres, D., C. Guerra and J.C. Cardenas. 1984. Primeros registros de *Arctocephalus gazella* y nuevos hallazgos de *Arctocephalus tropicalis* y *Leptonychotes weddellii* en el Archipiélago de Juan Fernández. *Ser. Cient. INACH.*, 31: 115–148.

Tryland M., L. Kleivane, A. Alfredsson, M. Kjeld, A. Arnason, S. Stuen and J. Godfroid. 1999. Evidence of *Brucella* in marine mammals in the North Atlantic Ocean. *Vet. Rec.*, 144: 588–592.

Vallejos, V., J. Acevedo, O. Blank, L. Osman and D. Torres. 2000. Informe de terreno, Proyecto 018-INACH, 'Ecología del lobo fino antártico, *Arctocephalus gazella*'. Instituto Antártico Chileno: 70 pp. (unpublished field report).

Liste des tableaux

Tableau 1: Caractéristiques individuelles et types d'échantillons des résultats positifs de l'épreuve sérologique d'anti-corps de *Brucella* chez *Arctocephalus gazella*. * – animal mort; RB – épreuve Rose Bengal; COMPELISA® – épreuve compétitive de l'immuno-essai enzymatique 'COMPELISA®; c-ELISA – épreuve compétitive de l'immuno-essai enzymatique (c-ELISA).

Список таблиц

Табл. 1: Индивидуальные характеристики и типы образцов – положительные результаты серологических исследований на антитела к *Brucella* в *Arctocephalus gazella*. * – мертвые особи; RB – тест Rose Bengal; COMPELISA® – конкурентный иммуноферментный анализ 'COMPELISA®'; c-ELISA – конкурентный иммуноферментный (c-ELISA) анализ.

Lista de las tablas

Tabla 1: Características individuales y tipo de muestra para los resultados positivos de las pruebas serológicas de anticuerpos anti-*Brucella* en *Arctocephalus gazella*. * – animal muerto; RB – prueba de Rosa de Bengala; COMPELISA® – inmunoensayo enzimático de competencia 'COMPELISA®'; c-ELISA – inmunoensayo enzimático competencia (c-ELISA).