

albumin to 1:750 and 1:1500. Overnight incubation at 4°C was the best condition for the different primary antibodies employed. Swine anti-rabbit immunoglobulins 1:20 for use as a secondary antibody and PAP complex 1:100 were both made up in phosphate buffered saline with 1 per cent bovine serum albumin and incubated for 30 minutes at room temperature. To detect the immune complexes, the sections were incubated in a solution of 60 mg of 3,3-diamino-benzidine tetrahydrochloride, 100 µl hydrogen peroxide and 100 ml of phosphate buffered saline for eight minutes and counterstained with Mayer's haematoxylin.

Ganglia from all horses with either mal seco or EGS showed numerous affected neurons with their cytoplasm positively stained for anti-whole horse serum (Fig 1). No differences between immunostaining with anti-whole horse serum preincubated with bovine serum albumin or horse serum albumin were detected. Fewer neuronal perikarya stained positively for anti-horse IgG. The highest dilutions of anti-whole horse serum and anti-horse IgG causing the strongest staining with the lowest background were 1:1500 and 1:750, respectively. PAP staining of affected neurons was not observed when normal serum replaced the anti-whole horse serum or the anti-horse IgG. Ganglia from the horse destroyed due to volvulus showed few neurons positively stained for both primary antibodies. Neurons in similar sections from normal horses failed to immunostain with either primary antibody (Fig 2).

The degenerative changes of the nerve cell body observed in the haematoxylin and eosin-stained ganglia sections from the affected horses consisted mainly of chromatolysis and vacuolation in the damaged neuron perikarya, neuronophagia, intercellular and intracytoplasmic eosinophilic bodies and pyknotic and eccentric nuclei. As they are considered pathognomonic lesions (Pollin and Griffiths 1992, Griffiths and others 1993) they confirmed a clinical diagnosis of mal seco or EGS.

This study presents the first evidence of intraneuronal serum proteins in cases of mal seco. These results have not been reported before and are consistent with those described for EGS (Griffiths and others 1994b).

The putative neurotoxin suspected of being the aetiological agent in EGS may reach neurons by retrograde axonal transport (Griffiths and others 1994a). If this is true for mal seco, a cell-mediated and/or a humoral immune response, either to the unknown aetiological agent or to some component of the nervous system, could explain the presence of serum-derived molecules in the nerve cell body as was considered for EGS (Griffiths and others 1994a). It is also possible that, as in other species (Olsson 1984), the blood vessels of autonomic ganglia of horses have no blood-nerve barrier. If this were the case, the serum proteins could be non-specifically absorbed by the affected neurons. Why this only occurs in damaged neurons, what stimulates it to occur and what the significance is of these serum proteins in the neuronal perikarya remain to be elucidated.

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## Brucella species infection in North Sea seal and cetacean populations

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AS part of a programme of investigation into strandings of marine mammals, post mortem examinations of stranded and by-caught animals from around the Scottish coast were undertaken by Scottish Agricultural College (SAC) Veterinary Services. In the course of these investigations, evidence of infection with bacteria belonging to the genus *Brucella* was found in three species of marine mammal. Following an initial report (Ross and others 1994), this communication presents the findings in greater detail and discusses the significance of *Brucella* infection in sea mammals (see also Foster and others 1996).

Tissues selected in the course of post mortem examinations were cultured on to Columbia agar (Difco) supplemented with 5 per cent citrated sheep blood and incubated at 37°C in an atmosphere of 10 per cent carbon dioxide for four days. Organisms suspected of being brucellae on the basis of colonial appearance, morphology and slide agglutination with *Brucella abortus* antiserum (Murex Diagnostics) were submitted to the OIE Brucella Reference Laboratory, Weybridge, where they were further examined using standard techniques of identification (Corbel and others 1983).

Small Gram-negative coccobacilli were recovered in culture from four common seals (*Phoca vitulina*), a harbour porpoise (*Phocoena phocoena*) and a common dolphin (*Delphinus delphis*). All of these animals were collected from the Moray Firth area although the majority of animals which were examined post mortem in the course of this study originated from the same area. One strain (M1068/91) was isolated from a subcutaneous lesion on a porpoise; all other tissues which yielded growths of *Brucella* species showed no significant pathological changes. Details of all sources of isolation are presented in Table 1.

Each of the eight isolates exhibited morphological and staining characteristics consistent with the bacterial genus *Brucella* and was susceptible to lysis by genus-specific phages. All isolates from the seals proved to be carbon dioxide dependent for growth in primary culture whereas the isolates from the cetaceans showed no such dependency. Further characterisation of the isolates is in progress to help establish if they are species adapted or peculiar to the marine environment. Studies to determine their pathogenicity for other mammalian species are also being undertaken.

To determine whether brucellae might be more widely distributed among marine mammal populations, cetacean and seal serum samples from a wildlife serum bank at SAC Veterinary

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**TABLE 1: Sites of isolation of *Brucella* species from seals and cetaceans**

Reference number	Date of stranding	Species	Site of isolation of <i>Brucella</i> species
M1068/91	06.06.91	<i>Phocoena phocoena</i>	Subcutaneous lesion
M0039/94	09.01.94	<i>Phocoena phocoena</i>	Spleen, mammary gland
M0644/93	29.03.93	<i>Delphinus delphis</i>	Subcutaneous lesion
M0339/94	17.02.94	<i>Phoca vitulina</i>	Gastric lymph node
M2357/93	08.10.93	<i>Phoca vitulina</i>	Spleen
M0292/94	14.02.94	<i>Phoca vitulina</i>	Spleen
M0336/95	17.02.94	<i>Phoca vitulina</i>	Internal iliac lymph node

Services, Inverness, were screened using standard serological techniques. Cetacean sera had been collected from fresh carcasses found stranded between 1991 and 1993. Sera from common seals and grey seals (*Halichoerus grypus*) had been similarly derived but included sera obtained from apparently healthy free-living animals sampled during a capture-release programme (Thompson and others 1992). Forty-five sera from Lake Baikal seals (*P. sibirica*) were also included as, being a landlocked species, they are unlikely to have been exposed in the recent past to infections of the marine environment and might thereby serve as negative controls. A total of 235 sera representing five marine mammal species was screened using standard serological techniques – the Rose Bengal plate test (RBPT) (Brinley Morgan and others 1978), serum agglutination test (SAT) (Brinley Morgan and others 1978) and indirect enzyme-linked immunosorbent assay (iELISA) (Nielsen and others 1992). Positive thresholds in the SAT were taken as greater than 60 iu and in the iELISA test as an optical density greater than 30 per cent of the optical density of the Second International Standard anti-*B. abortus* serum, representing those which, in domestic animals, would be regarded as significant.

The number of positive results obtained in the panel of serological tests (Table 2) provided, for each species, strong evidence of *Brucella* infection. The exception was *P. sibirica*, in which exposure to infection did not appear to have occurred. Seronegativity in *P. sibirica* helps to exclude the possibility of host-related incompatibility with the serological tests employed.

In an individual animal, serology alone provides insufficient evidence of infection, particularly where it is known from experience in domestic animals that positive reactions in serological tests may occasionally be caused by infection with organisms sharing antigenic determinants on their O-polysaccharides (Corbel 1985). In particular, *Yersinia enterocolitica* serotype O9 has been shown to cause cross-reactions in serological tests for evidence of *B. abortus* infection in cattle (Corbel 1985), but has not been isolated from sea mammals in the authors' study to date.

The serological results and the bacterial isolations together indicate that brucella infections occur in harbour porpoise, common dolphin and common seal populations. They further suggest that infection may be present in a wider range of mammalian species in the marine and littoral environments. This appears to be the first published report of the isolation of brucellae from free-living seals and cetaceans. The isolation of a brucella from a bottlenose dolphin (*Tursiops truncatus*) fetus has been reported (Ewalt and others 1994) but this fetus was derived from a captive individual (W. G. Miller, personal communication). Brucellosis was included in the differential diagnosis of an antibiotic-responsive illness described in 1982 in a captive killer whale on the basis of a positive RBPT and SAT titre of 1/320 (Taylor 1982).

Concern over declines in the abundance of certain marine animals has tended to concentrate on the effects which anthropogenic factors may have on reproductive success and survival (Reijnders 1986a, b). Less attention has been paid to the potential effects which enzootic disease may have in producing natural variations in population parameters. Indeed, logistic problems have severely constrained progress toward an understanding of the range and effects of micro- and macro-parasites present in marine mammal hosts. Among terrestrial mammals, the natural hosts of brucellae include cattle, goats, sheep and pigs, among which infection of the reproductive tract can lead to abortion or an orchitis which results in male sterility (Nielsen and Duncan 1990). The possible

**TABLE 2: Results of *Brucella* serology for seal and cetacean serum samples**

Species	Number tested	RBPT +ve	SAT +ve	iELISA +ve
<i>Phoca vitulina</i>	140	69 (49)*	25 (18)	45 (32)
<i>Phoca sibirica</i>	45	0	0	0
<i>Halichoerus grypus</i>	31	10 (32)	4 (13)	7 (23)
<i>Phocoena phocoena</i>	18	5 (28)	2 (11)	4 (22)
<i>Delphinus delphis</i>	1	1	1	1

\* Per cent positive results

RBPT Rose Bengal plate test

SAT Serum agglutination test

iELISA Indirect enzyme-linked immunosorbent assay

occurrence of brucellosis among several North Sea populations of marine mammals creates the hypothesis that reproduction in seals and cetaceans may be similarly compromised, potentially leading to natural spatial and temporal variations in reproductive success in these species. The recovery of a brucella from a dolphin fetus (Ewalt and others 1994) adds weight to this hypothesis.

Further study of brucella infection in marine animals offers a tool for addressing epizootiological questions raised during the recent morbillivirus outbreaks (Domingo and others 1990, Kennedy and others 1992), particularly those relating to the mechanisms for the transfer of such diseases among and between widely dispersed marine mammal populations (Grenfell and others 1992, Heide-Jorgensen and others 1992). Further work to determine the prevalence and significance of brucella infection both in other marine species and in other geographical areas is planned. Brucellosis is recognised as an important zoonotic infection of man (Nielsen and Duncan 1990), infection occurring via the oral or ocular route following the ingestion of infected milk or the handling of infected discharges and fetuses. The health implications of this potential zoonotic infection should now be taken into account by all those involved in marine mammal research or rehabilitation.

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