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Original article

Marine mammal *Brucella* isolates with different genomic characteristics display a differential response when infecting human macrophages in culture

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Abstract

Marine mammal *Brucella* strains with different genomic characteristics according to distribution of IS*711* elements in their genomes were analysed for their intracellular behaviour in human THP-1 macrophage-like cells. Seven different groups of marine mammal strains were identified including a human isolate from New Zealand presumably from marine origin. Entry and intracellular survival of strains representative of these groups in THP-1 human macrophage-like cells were analysed at several times of infection. Three patterns of infection were identified. The *Brucella* strain isolated from the human case from New Zealand, and two other groups of strains belonging to *B. ceti* or *B. pinnipedialis* were able to infect THP-1 macrophage cells to the same extent as the virulent strains *B. suis* 1330 or *B. melitensis* 16M. Three other groups of strains belonging to *B. ceti* or *B. pinnipedialis* were able to enter the cells as classical virulent strains but were eliminated after 48 h. The last group was composed only of strains isolated from hooded seals (*Cystophora cristata*) and was even unable to enter and infect THP-1 macrophage cells. Thus, several groups of marine mammal *Brucella* strains appear to be non-infectious for human macrophages.

Keywords: *Brucella*; Marine mammal; Hooded seal; Macrophages; Infection; Southern blot; IS*711*

1. Introduction

Brucellae are Gram-negative, facultative, intracellular bacteria that can infect many species of animals and man. Six species were initially recognized within the genus *Brucella*: *B. abortus*, *B. melitensis*, *B. suis*, *B. ovis*, *B. canis*, and *B. neotomae* [1,2]. This classification is mainly based on differences in pathogenicity, host preference, and phenotypic characteristics [1–3]. Three additional species have recently been included in the genus *Brucella*, i.e. *B. ceti* and *B. pinnipedialis* isolated from marine mammals, with cetaceans (dolphin, porpoise (*Phocoena phocoena*), and whale species) and pinnipeds (various seal species) as preferred host respectively [4], and *B. microti* isolated from the common vole [5].

Since 1994, *Brucella* strains have been isolated from a wide variety of marine mammals [6]. DNA–DNA hybridization and other phenotypic characteristics revealed that they were part of the genus *Brucella*, but they have distinctive characteristics from strains isolated from terrestrial mammals. At the molecular level, evidence for two marine mammal *Brucella* species, i.e. *B. ceti* and *B. pinnipedialis*, has been firstly provided by study of DNA polymorphism at the porin-encoding *omp2* locus consisting of two highly related (about 85% DNA identity) genes, i.e. *omp2a* and *omp2b* [7]. Variation within these genes observed in *Brucella* species and biovars is mainly due to genetic conversion phenomena between the two gene copies [8,9]. Specific gene conversion variants are found for strains isolated from marine mammals [7]. Other distinctive characteristics at the molecular and genomic level have been provided by IS*711* DNA fingerprinting, which showed that a higher number of IS*711* copies occurs in the genome of *Brucella* strains isolated from marine

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mammals than in that of terrestrial mammal isolates [10,11]. Infrequent restriction site-PCR (IRS-PCR) methods, taking into account the higher number of IS*711* elements in their genome, confirmed the classification into two marine mammal *Brucella* species and specific DNA fragments were identified for each species [12,13]. Both IS*711* and IRS-PCR profiles indicated that subgroups exist within the *B. ceti* and *B. pinnipedialis* species. Variable number of tandem repeats (VNTR) typing and multilocus sequence analysis (MLSA) also confirmed this [14]. Macrorestriction analysis to determine the genomic structures of 24 isolates from diverse marine mammal species also identified subgroups within the pinniped and cetacean isolates [15]. For example, a 62 kb fragment was found only in pinniped isolates except hooded seal isolates.

Brucella spp. in marine mammals have been associated with various pathological expressions such as subcutaneous lesions, epididymitis, splenic and hepatic necrosis or meningoencephalitis [6]. They have been isolated from a wide variety of tissues and from reproductive organs of both sexes and also in aborted foetuses and placentas [6]. *Brucella* spp. have also been isolated from apparently healthy hooded seals in the North Atlantic Ocean [16]. Three human cases with naturally acquired infection by *Brucella* strains presumably of marine origin have been reported, one case of spinal osteomyelitis from a patient in New Zealand [17], and two neurobrucellosis cases from Peruvian patients [18]. Another case of laboratory-acquired infection has also been reported [19].

The key aspect of *Brucella* virulence is its ability to survive and multiply in macrophage cells via known virulence factors such as the VirB type IV secretion system, smooth-lipopolysaccharide (S-LPS), cyclic β-1,2 glucan, and the two-component regulatory system BvrR–BvrS [20–23].

Little is known about the putative virulence of marine mammal *Brucella* strains in man and their ability to multiply in human macrophages. In this study, the genomic background of a wide range of *Brucella* strains isolated from several marine mammal species was assessed by molecular analysis (Southern blot with an IS*711* probe). Representative strains with different genomic characteristics were further tested for their ability to infect human macrophage cells.

2. Materials and methods

2.1. Bacterial strains

Eighteen terrestrial mammal *Brucella* reference strains, 77 marine mammal strains, and one human isolate from New Zealand likely of marine origin were used in this study. The *Brucella* strains selected for macrophage infection tests are listed in Table 1. Culture conditions of the strains were those described previously [12]. DNA was prepared as described previously [24].

2.2. IS711 Southern blot

Southern blot hybridization with an IS*711* probe was performed on all strains described above. The IS*711* probe was obtained by PCR on the *B. pinnipedialis* reference strain B2/94 using the primers ISA: 5′-GGA TCG AAG CAT ATC TTC CG-3′ and ISB: 5′-TGT CTG CAT TCA ACG CAA CC-3′. Briefly, amplification reaction mixtures were prepared in volumes of 25 µl containing 1 × PCR buffer (Promega, Madison, WI), a 200 mM concentration of each deoxynucleoside triphosphate, a 1 µM concentration of each primer, 100 ng of genomic DNA, and 5 U of Go*Taq* DNA polymerase (Promega). The temperature cycling for the amplification was performed in an iCycler thermocycler (BioRad) as follows: cycle 1 was 94 °C for 5 min (denaturation); the next 30 cycles were 59 °C for 30 s (annealing), 70 °C for 1 min (extension), and 94 °C for 30 s (denaturation). The PCR product of 729 bp was purified using the QIAquick PCR purification Kit (Qiagen™). This probe was then labelled with the NE Blot-Phototope kit (Biolabs™), following the manufacturer's protocol.

Table 1
Characteristics of the strains used in this study.

Species	Strain	Host or source	Latin name	Geographic origin	IS*711* pattern (*n*)[a]	Infection pattern[b]
B. melitensis	16M (ATCC 23456; BCCN R1)[c]	Goat	*Capra aegagrus*	United States	A (6)	1
B. suis	1330 (ATCC 23444; BCCN R12)[c]	Swine	*Sus scrofa*	United States	B (6)	1
B. ceti	M13/05/1	Striped dolphin	*Stenella coeruleoalba*	Scotland	H (26)	1
B. pinnipedialis	B2/94 (NCTC 12890; BCCN 94-73)[c]	Common seal	*Phoca vitulina*	Scotland	D (23)	1
Brucella spp.	02/611	Human	*Homo sapiens*	New Zealand	I (23)	1
B. ceti	B1/94 (NCTC 12891; BCCN 94-74)[c]	Porpoise	*Phocoena phocoena*	Scotland	G (29)	2
B. ceti	M452/97/2	Common dolphin	*Delphinus delphis*	Scotland	F (30)	2
B. pinnipedialis	M2533/93/1	Common seal	*Phoca vitulina*	Scotland	E (24)	2
B. pinnipedialis	17a-1	Hooded seal	*Cystophora cristata*	Norway	C (19)	3
B. pinnipedialis	22a-2	Hooded seal	*Cystophora cristata*	Norway	C (19)	3
B. pinnipedialis	23a-1	Hooded seal	*Cystophora cristata*	Norway	C (19)	3
B. pinnipedialis	37a-1	Hooded seal	*Cystophora cristata*	Norway	C (19)	3
B. pinnipedialis	38g-1	Hooded seal	*Cystophora cristata*	Norway	C (19)	3
B. pinnipedialis	53c-1	Hooded seal	*Cystophora cristata*	Norway	C (19)	3

[a] Lane presented in Fig 1. n, number of IS*711* copies according to the number of IS*711* carrying EcoRI fragments detected by Southern blot.
[b] Obtained with THP-1 human macrophage-like cells infection by several marine mammal isolates.
[c] Reference strain.