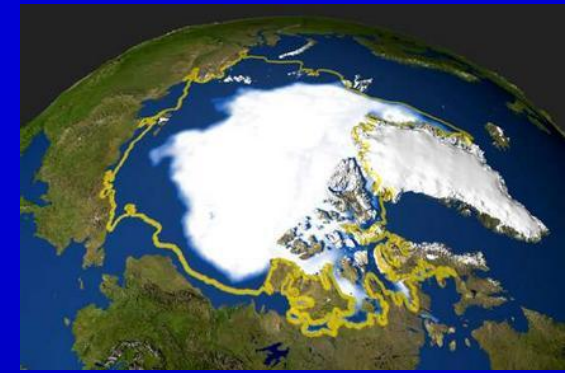




Brucellosis in the Arctic



Department of Food Safety and Infection Biology
Section of Arctic Veterinary Medicine

Jacques Godfroid



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Brucellosis

(*Brucella ceti* and *Brucella pinnipedialis*)
in marine mammals



Brucellosis (*Brucella suis* biovar 4) in reindeer





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2004-2007

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Norges veterinærhøgskole
Norwegian School of Veterinary Science

Since January 2008

Seksjon for arktisk veterinærmedisin
Section of Arctic Veterinary Medicine



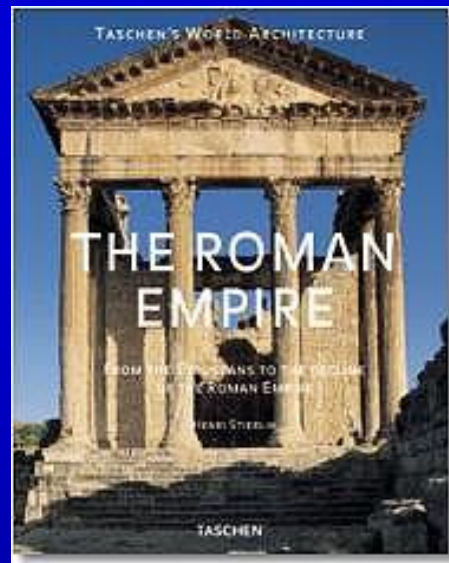
The slide features a large, light blue circular watermark in the background with the text "NORVEGIAN VETERINARIA" and a central figure. Below the main text, there are five small illustrations of arctic animals: a reindeer, a walrus, a wolf, a seal, and a polar bear.

“There is nothing permanent but change”

Anonym

« We regard brucellosis as the world's most widespread of all zoonoses and apart from its toll on people, it has an enormous impact on the animal industry »

WHO, 1998



Roman GOAT CHEESE

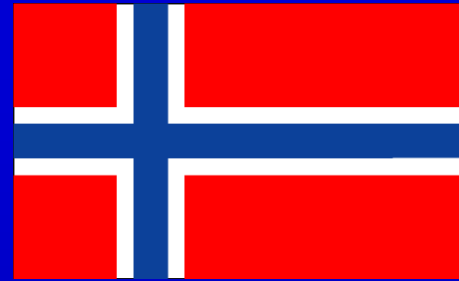


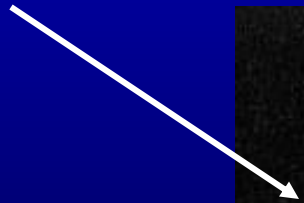
Figure 2. A Roman sculpture showing the milking of a goat (data).



What happened in Pompeii
and
Herculaneum
August 24, 79 before Christ ?







Bacteria in Two-millennia-old Cheese, and Related Epizoonoses in Roman Populations

L. Capasso

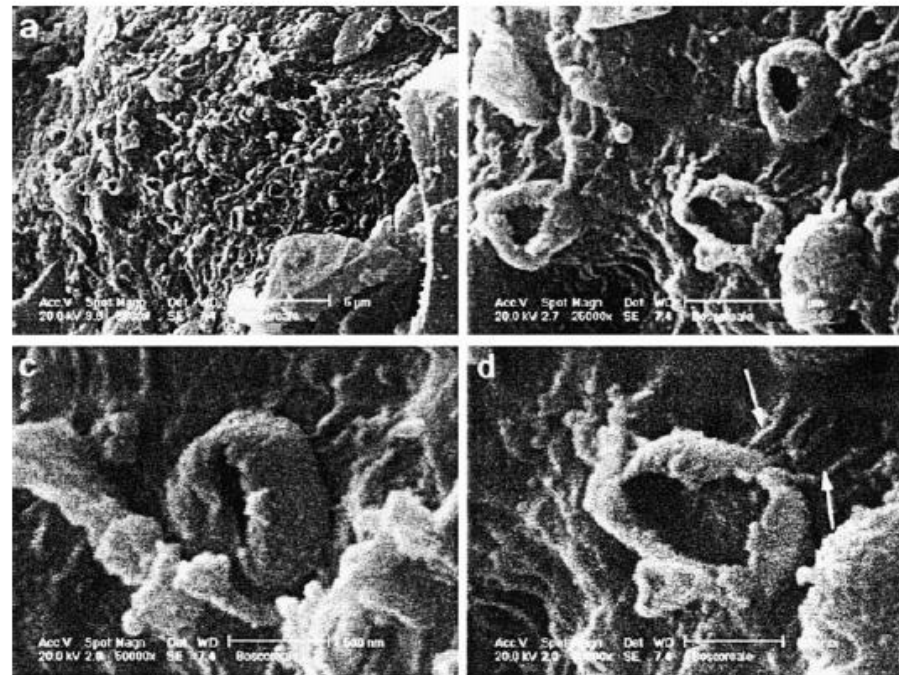
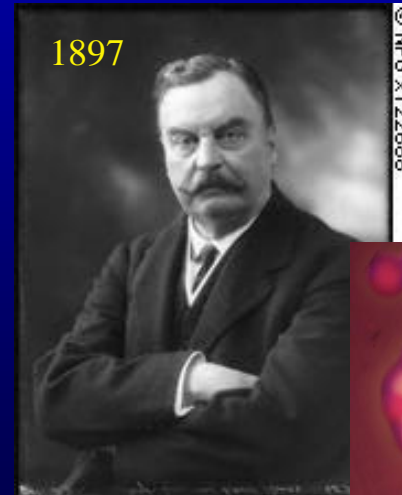
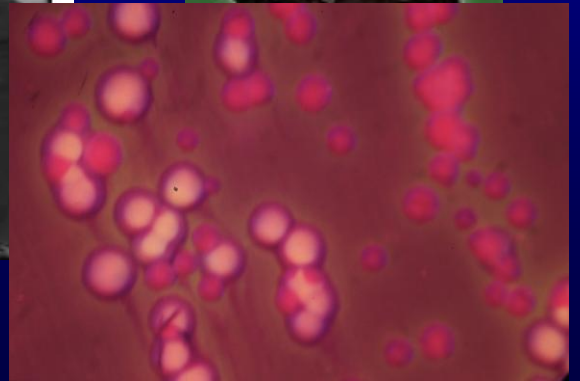


Figure 5. Cocci-like bacterial particles in a high-density colony (a: 5000 \times) in the Herculaneum carbonized cheese (79 AD); The monomorphic and monodimensional cocci (around 0.8 μ m) show large holes with invaginated borders (b: 25,000 \times). In some case we can estimate the thickness of bacterial-wall (about 10 nm) (c: 50 000 \times), and we can demonstrate the presence of proteinic bridges between the bacterial walls and the cheese mixture (arrows in d: 50,000 \times). These remain of bacterial walls are morphologically comparable with *Streptococci* or *Brucellae*.

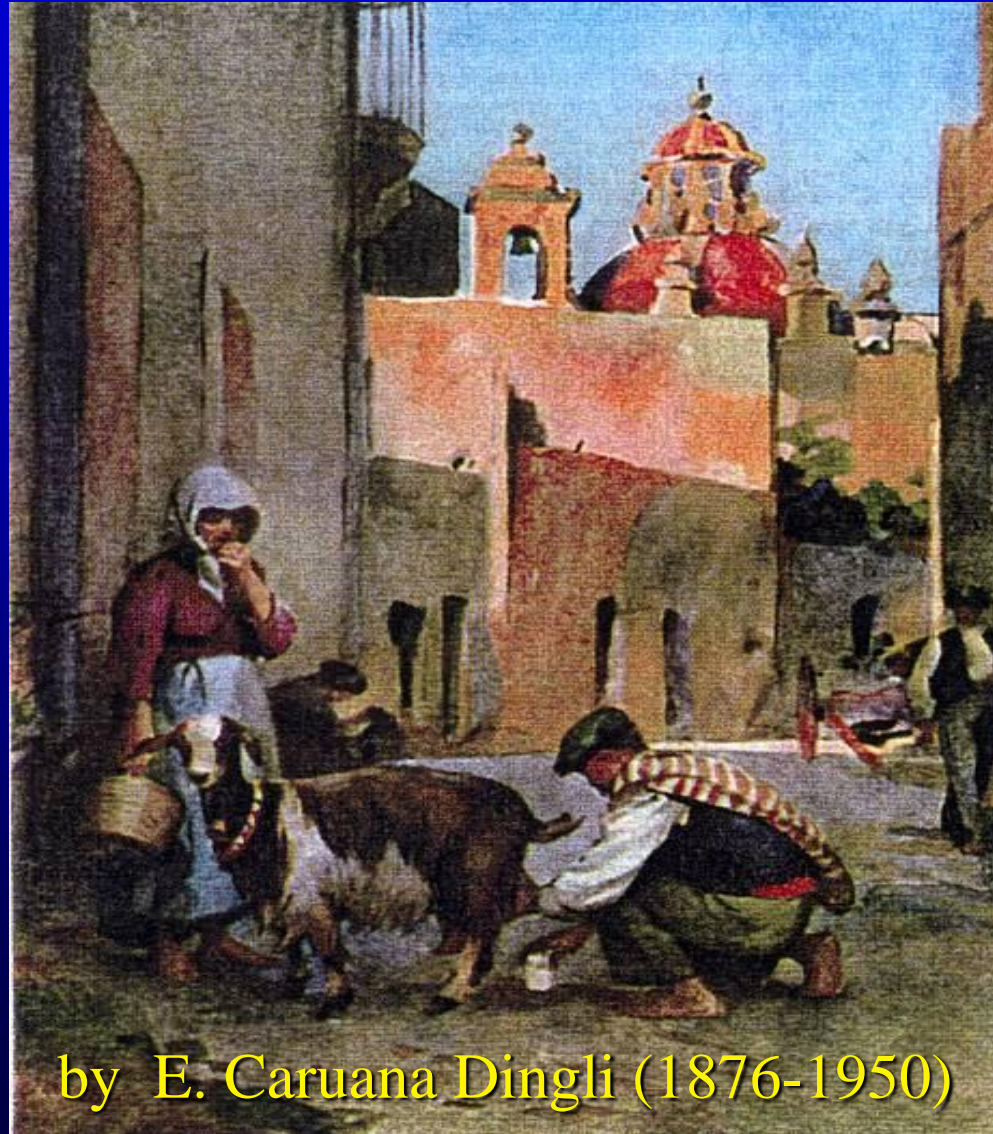
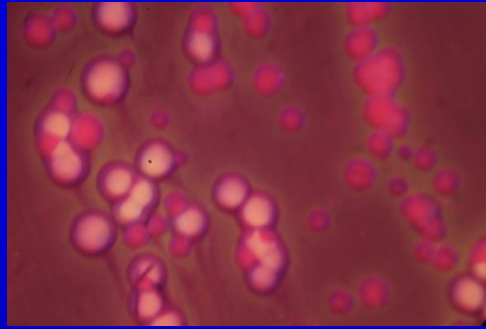


Sir David Bruce



Brucellosis

The Maltese goat



by E. Caruana Dingli (1876-1950)



Sir Temi Zammit

Brucella species and biovars, preferential hosts and pathogenicity for humans, before 1994

Species	Biovars	Preferential host(s)	Pathogenicity in humans
<i>B. melitensis</i>	1-3	Sheep, Goat	High
<i>B. abortus</i>	1-6, 9	Cattle	High
<i>B. suis</i>	1, 3	Pig	High
	2	Wild boar, Hare	No
	4	Reindeer, Caribou	High
	5	Rodents	?
<i>B. neotomae</i>	-	Desert rat	Moderate
<i>B. ovis</i>	-	Ram	No
<i>B. canis</i>	-	Dog	Moderate

Identification of an unknown wildlife reservoir of *Brucella suis* biovar 2 in Western Europe



Godfroid J., Michel P., Uytterhaegen L., De Smet K., Rasseneur F., Boelaert F., Saegerman C., Patigny X., 1994. Brucellose enzootique (*Brucella suis* biotype 2) chez le sanglier (*Sus scrofa*) en Belgique. Ann. Med. Vet., 138: 263-268.

Brucella species and biovars, preferential hosts and pathogenicity for humans in 2010

Species	Biovars	Preferential host(s)	Pathogenicity in humans
<i>B. melitensis</i>	1-3	Sheep, Goat	High
<i>B. abortus</i>	1-6, 9	Cattle	High
<i>B. suis</i>	1, 3	Pig	High
	2	Wild boar, Hare	No
	4	Reindeer, Caribou	High
	5	Rodents	?
	-	Desert rat	Moderate
<i>B. neotomae</i>	-	Desert rat	Moderate
<i>B. ovis</i>	-	Ram	No
<i>B. canis</i>	-	Dog	Moderate
<i>B. pinnipedialis</i>	-	Cetaceans	?
<i>B. ceti</i>	-	Seals	?
<i>B. microti</i>	-	Soil, Vole, Fox	?
<i>B. inopinata</i>	-	Human	?

?: Although human cases have been described, the actual pathogenicity remains unknown

Marine Mammal Brucellosis ... ?

- In 1994, the first description of a *Brucella* species isolated from an aborted fetus of a captive bottlenose dolphin (*Tursiops truncatus*) in San Diego, California was reported by Ewalt *et al.*
- In 1996, the first presumptive (serological) evidence of *Brucella* spp. exposure in Atlantic walrus (*Odobenus rosmarus rosmarus*) and ringed seals (*Phoca hispida*) of Arctic Canada was reported by Nielsen *et al.*
- In 1996, Foster *et al.*, and Ross *et al.*, reported the isolation of *Brucella* species in North Sea seal and cetacean (dolphins and porpoises) populations in stranded or by-caught animals, around the Scottish coast
- Questions:
 - New *Brucella* species ... ?
 - Presence of *Brucella* in a wide range of marine mammals ?
 - Significance of the presence of *Brucella* in marine mammals ?
 - Zoonotic potential ?

OLD BRUCE HWY

**HISTORICAL
VILLAGE**

**Veterinary
Brucellosis**

BRUCE HWY

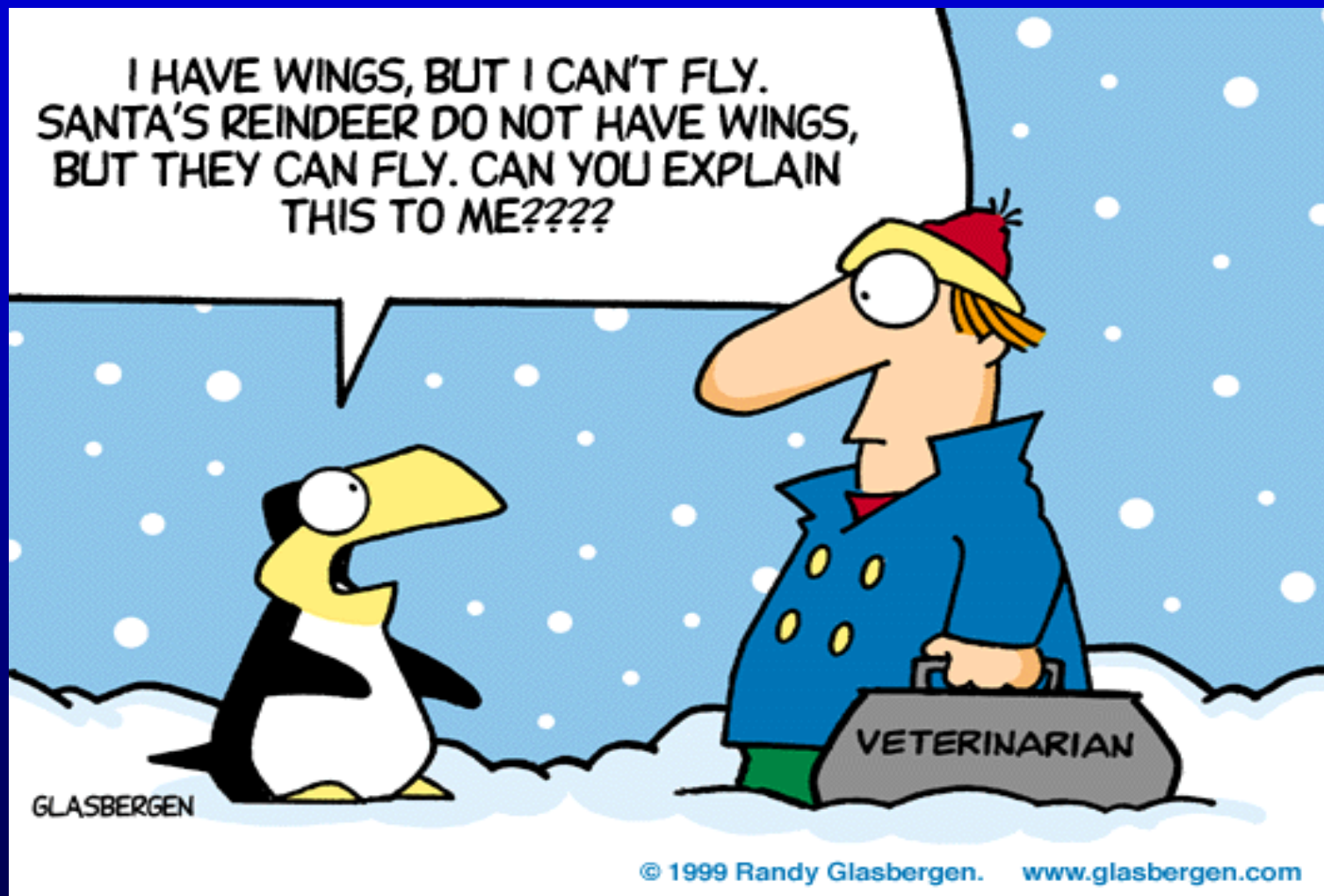


GENOMIC / PROTEOMIC

**Marine mammals Brucellosis and
Rangirefine Brucellosis**



I HAVE WINGS, BUT I CAN'T FLY.
SANTA'S REINDEER DO NOT HAVE WINGS,
BUT THEY CAN FLY. CAN YOU EXPLAIN
THIS TO ME?????



GLASBERGEN

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[Link ?](#)





Brucellose enzootique (*Brucella suis* biotype 2) chez le sanglier (*Sus scrofa*) en Belgique. Ann. Med. Vet., 138: 263-268. Godfroid J., Michel P., Uytterhaegen L., De Smet K., Rasseneur F., Boelaert F., Saegerman C., Patigny X., 1994.

Biovars 1 & 3

Biovar 2



*Brucella
suis*

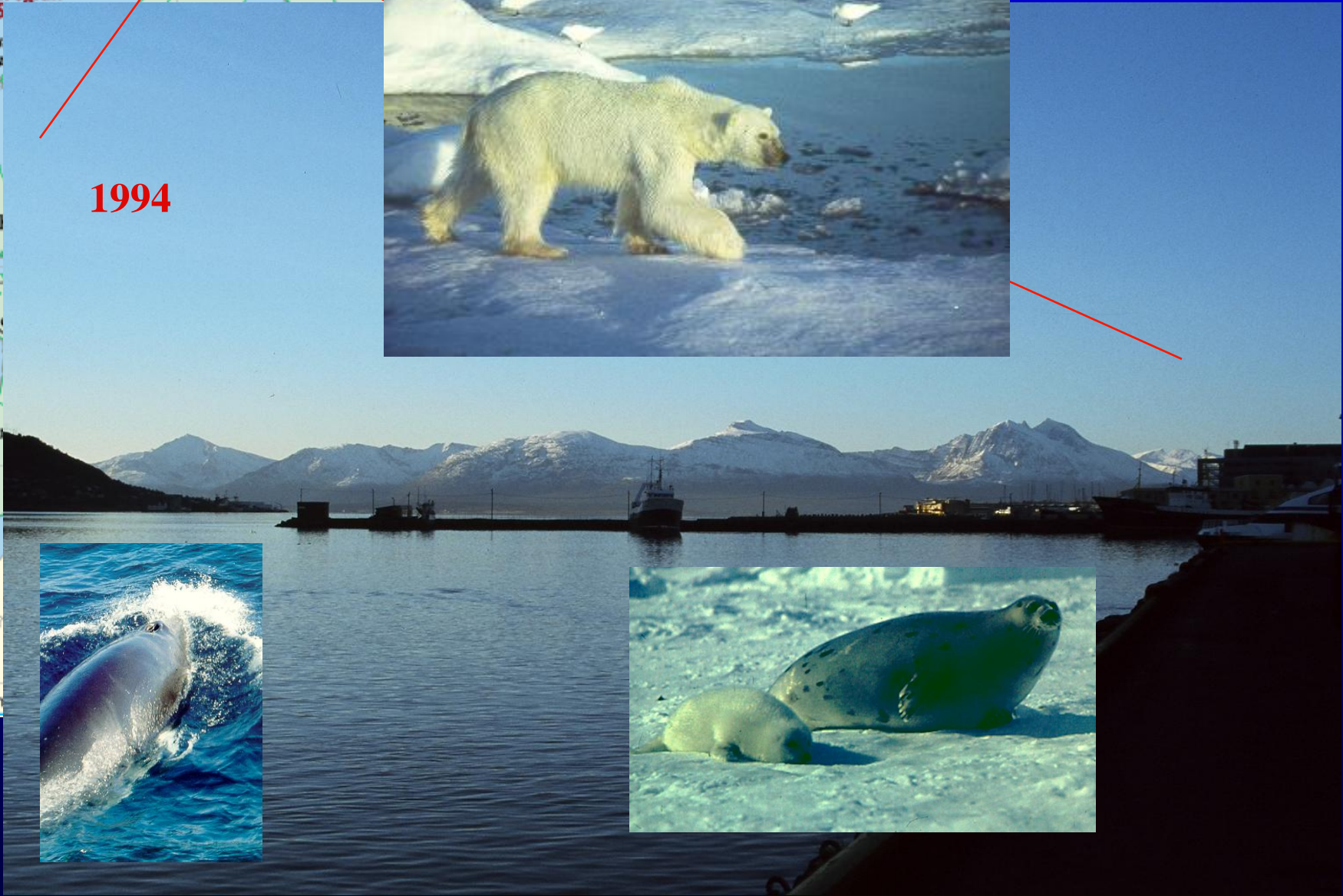
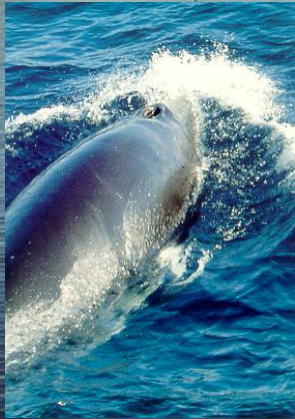


Biovar 4





1994



Greenland



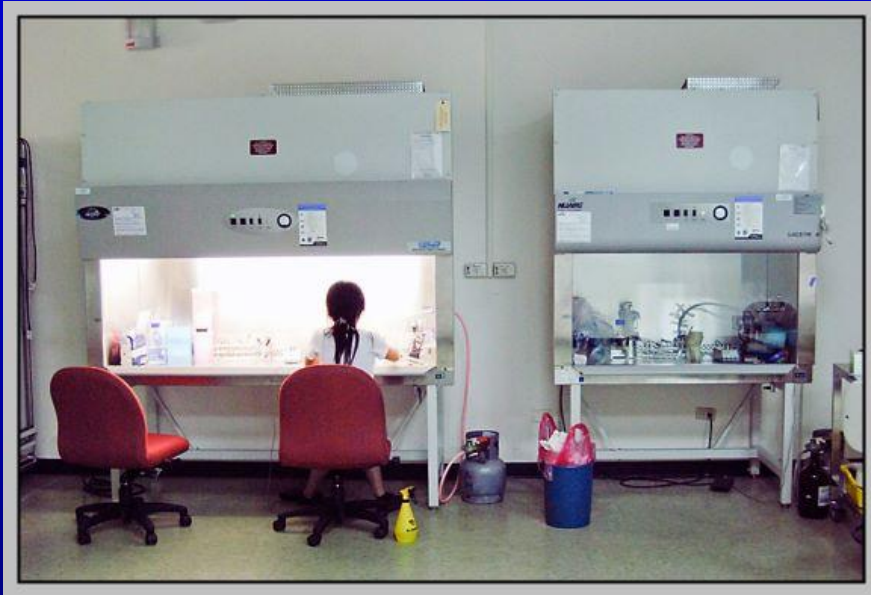
Field work







Now Laboratory work!



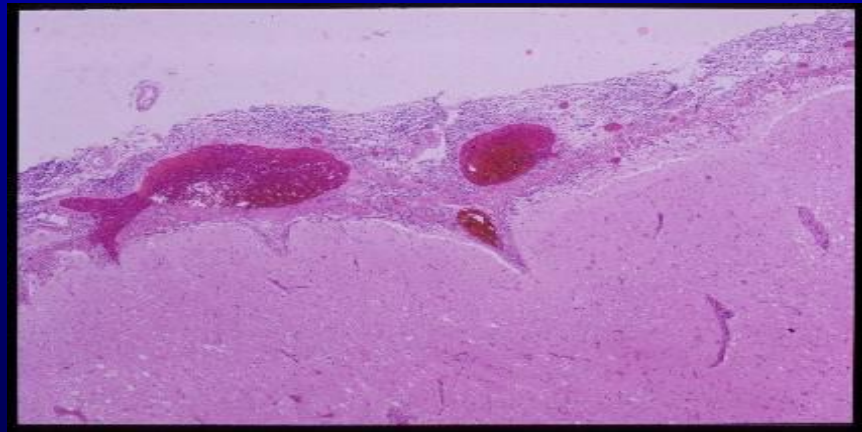
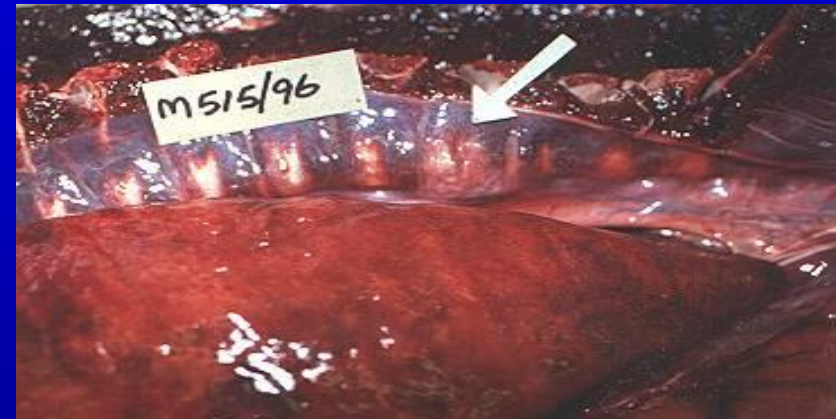
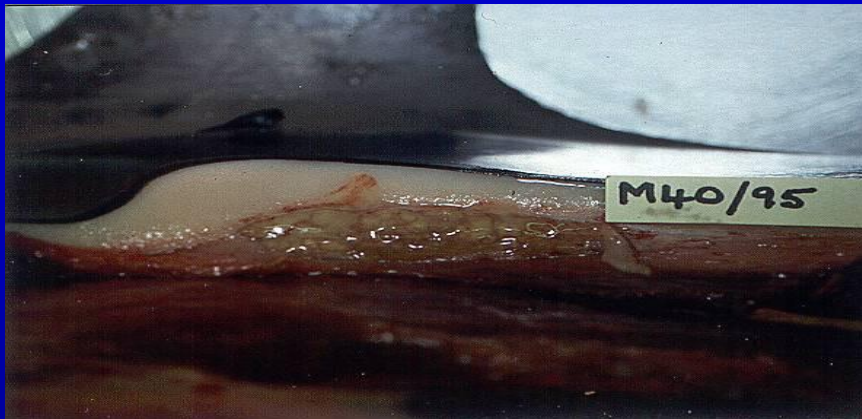
First, serology !

Evidence of *Brucella* infection in marine mammals in the North Atlantic Ocean

Tryland M, Kleivane L, Alfredsson A, Kjeld M, Arnason A, Stuen S, Godfroid J. 1999. Vet. Rec., 144: 588-592.

- **1370 Serum samples, from different sea mammals species caught during the 1983-1996 period**
- **Anti-*Brucella* antibodies were detected in all the species investigated, except for the bearded seal (*Erignathus barbatus*), with the following prevalences:**
 - ✓ hooded seals (*Cystophora cristata*) **35** per cent
 - ✓ harp seals (*Phoca groenlandica*) **2** per cent
 - ✓ ringed seals (*Phoca hispida*) **10** per cent
 - ✓ minke whales (*Balaenoptera acutorostrata*) **8** per cent
 - ✓ Fin whales (*Balaenoptera physalus*) **11** per cent
 - ✓ Sei whales (*Balaenoptera borealis*) **14** per cent

Then Pathology (photos: G. Foster & T. Patterson)



Gonzalez L., Patterson I. A., Reid R. J., Foster G., Barberan M., Blasco J. .M., Kennedy S., Howie F. E., Godfroid J., MacMillan A. P., Schock A., Buxton D., 2002. Chronic Meningoencephalitis Associated with *Brucella* sp. Infection in Live-stranded Striped Dolphins (*Stenella coeruleoalba*). *J. Comp. Pathol.*, 126: 147-52.

Then bacteriology !

- **An isolate belonging to the genus *Brucella* was obtained from the liver and spleen of one of the seropositive minke whale**

Clavareau C., Wellemans V., Walravens K., Tryland M., Verger J.-M., Cloeckaert A., Letesson J.-J., Godfroid J., 1998. Phenotypic and Molecular Characterization of a *Brucella* strain Isolated from a Minke Whale (*Balaenoptera acutorostrata*). *Microbiology*, 144: 3267-3273.

- **This suggests that antibodies against the surface lipopolysaccharide of *Brucella* species are widely distributed among marine mammals in the North Atlantic Ocean**

Brucella ceti sp. nov. and *Brucella pinnipedialis* sp. nov. for *Brucella* strains with cetaceans and seals as their preferred hosts

Geoffrey Foster,¹ Bjorn S. Osterman,² Jacques Godfroid,³ Isabelle Jacques^{4,5} and Axel Cloeckaert⁴

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¹SAC Veterinary Services, Inverness IV2 4JZ, UK

²Department of Clinical Microbiology, Karolinska University Hospital, MTC, Stockholm SE-17176, Sweden

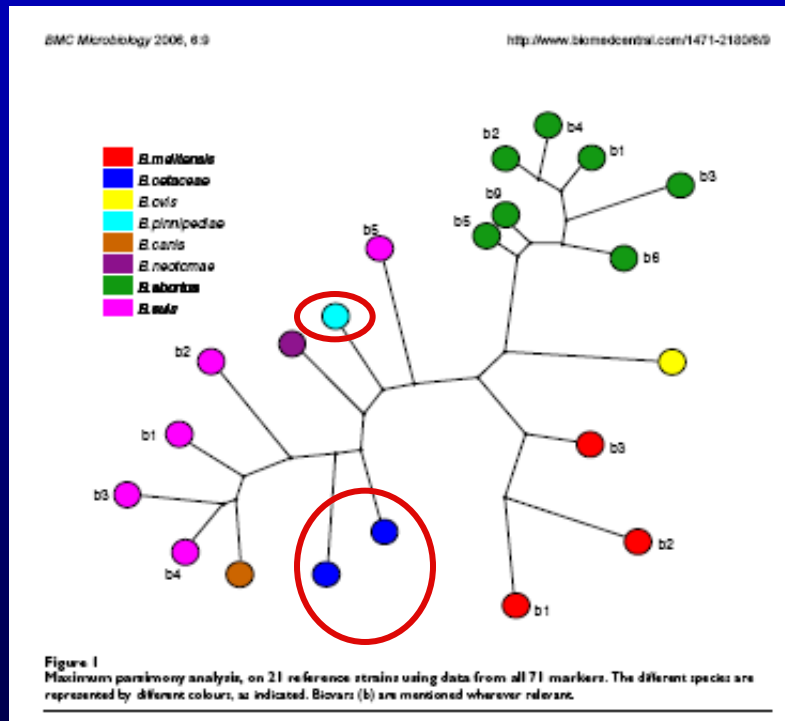
³Faculty of Veterinary Science, Department of Veterinary Tropical Diseases, University of Pretoria, Onderstepoort 0110, South Africa

⁴INRA, UR1282, Infectiologie Animale et Santé Publique, IASP, Nouzilly, F-37380, France

⁵Institut Universitaire de Technologie, Université François Rabelais, 29 rue du Pont-Volant, 37082 Tours cedex 2, France

Classification of *Brucella spp.* isolated from marine mammals by

MLVA



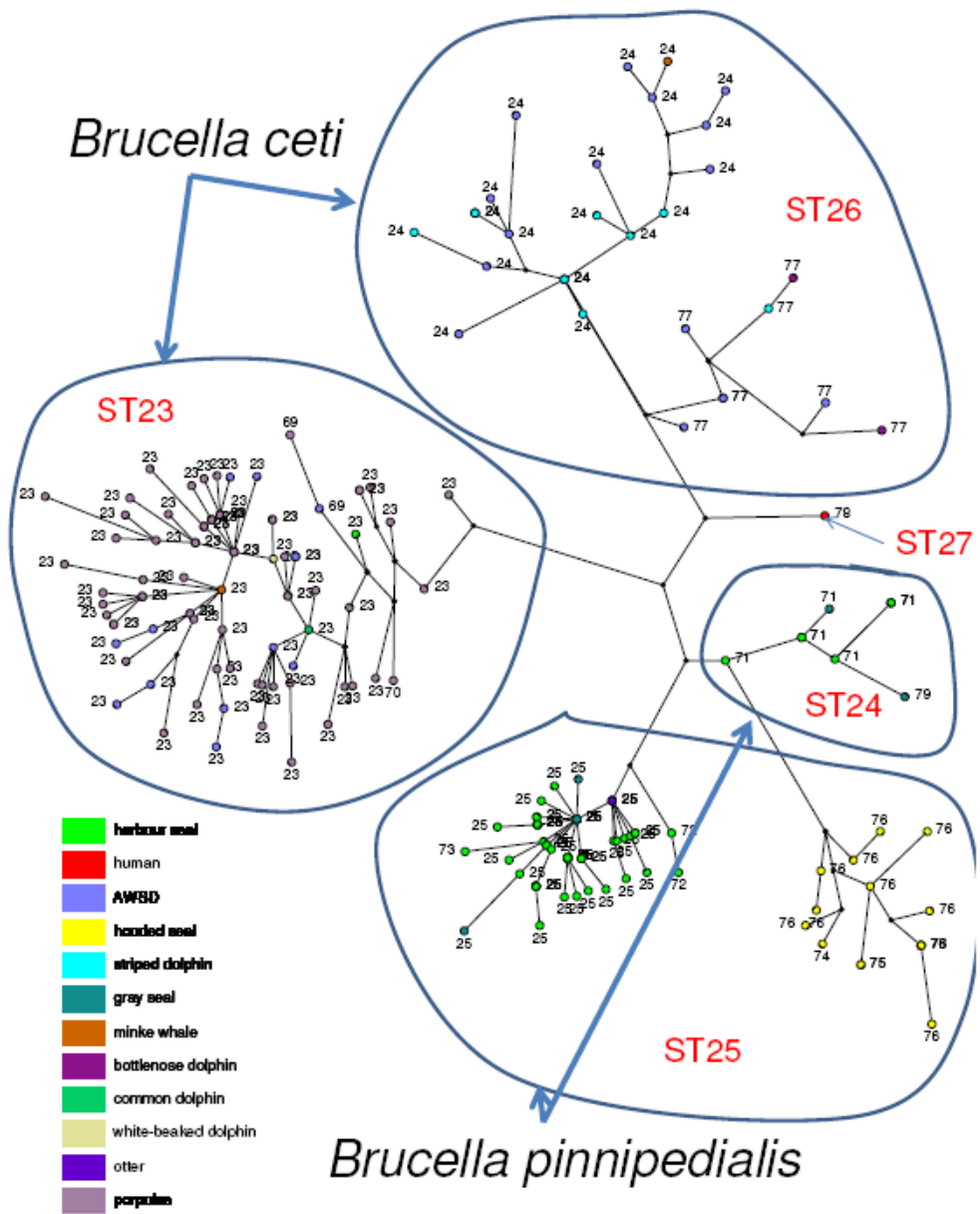
<u>MARKER</u>	<u>Number of repeat</u>
Bruce 04	6
Bruce 06	2
Bruce 07	6
Bruce 08	5
Bruce 09	3
Bruce 11	8
Bruce 12	7
Bruce 16	7
Bruce 18	4
Bruce 21	9
Bruce 30	6
Bruce 42	3
Bruce 43	2
Bruce 45	5
Bruce 55	1

MLVA typing of Marine Mammal *Brucella* spp.

MLVA-16 typing of 170 marine mammal *Brucella* isolates from different animal and geographic origins identifies 5 major groups within *Brucella ceti* and *Brucella pinnipedialis*

Maquart M., Le Flèche P., Foster G., Tryland M., Ramisse F., Djønne B., Al Dahouk S., Jacques I., Neubauer H., Walravens K., Godfroid J., Cloeckert A., Vergnaud G. 2009. MLVA-16 typing of 295 marine mammal *Brucella* isolates from different animal and geographic origins identifies 7 major groups within *Brucella ceti* and *Brucella pinnipedialis*. BMC Microbiol., 9:145.

Brucella ceti



Brucella pinnipedialis

First isolation of *Brucella ceti* in a stranded porpoise in Belgium in 2008



David Fretin, CERVA
Thierry Jauniaux, Ulg

Isolation of *Brucella* sp. from lung and brain

Purification of DNA → MLVA analysis

<u>MARKER</u>	<u>Number of repeat</u>
Bruce 04	6
Bruce 06	2
Bruce 07	6
Bruce 08	5
Bruce 09	3
Bruce 11	8
Bruce 12	7
Bruce 16	7
Bruce 18	4
Bruce 21	9
Bruce 30	6
Bruce 42	3
Bruce 43	2
Bruce 45	5
Bruce 55	1

B. ceti



**veterinary
microbiology**

www.elsevier.com/locate/vetmic

Prevalence of *Brucella pinnipediae* in healthy hooded seals (*Cystophora cristata*) from the North Atlantic Ocean and ringed seals (*Phoca hispida*) from Svalbard

Morten Tryland^{a,*}, Karen Kristine Sørensen^{b,1}, Jacques Godfroid^{c,2}

^aSection of Arctic Veterinary Medicine, Department of Food Safety and Infection Biology, The Norwegian School of Veterinary Science, P.O. Box 6204, NO-9292 Tromsø, Norway

^bNational Veterinary Institute Tromsø, NO-9292 Tromsø, Norway

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Received 6 May 2004; received in revised form 1 October 2004; accepted 22 October 2004

Table 2

Correlation between isolation of *Brucella pinnipediae* from organ samples ($n = 174$) and the presence of anti-*Brucella* antibodies in serum from 29 hooded seals (*Cystophora cristata*) caught between Svalbard and Greenland, autumn 2002 (only seropositive and/or culture positive individuals are presented; open spaces indicate no growth of *Brucella* or seronegative results)

Animal number	Age (months) ^a	Sex	Tonsil	Lung	Lung lymph node	Spleen	Liver	Kidney	Testicle	Serology
1	6	M			NI ^b					+
8	30	F								+
9	6	F			+	+				+
17	30	M	+	+	+	+	+	+	+	+
22	6	M		+	+	+	+			+
23	6	F	+	+	+	+	+			
24	18	M	+			+			+	
25	18	M			+	+				+
30	18	M			+	+	+			+
37	6	M	+	+	+	+	+	+	NI ^b	+
38	6	F						+		
39	18	F			+	+	+			+
53	6	M			+					
Positive/tested			4/26	4/29	9/24	9/29	6/29	3/29	2/9	9/29
(%)			15	14	38	31	21	10	22	31

^a Since birth takes place in March and the animals were caught in September, ages registered as <1 year corresponds to 6 months, 1–2 years to 18 months, 2–3 years to 30 months, and 3–4 years to 42 months.

^b Not investigated.

Abortion during the first gestation is a core clinical symptom of *Brucella* sp. infections in terrestrial mammalian species, resulting in an increased age at first viable parturition.

Abortion has also been described in bottlenose dolphins

Hooded seal population dynamics

Despite reduced hunting, model runs using recent pup production estimates as input suggest that the Greenland Sea hooded seal population has decreased substantially since the 1950s, and stabilized at a low level (10–15% of the 1946-level) since the 1970s (58, 98). Aerial surveys suggested a minimum pup production of 24 000 in 1997 and 15–16 000 in 2005 and 2007 (98, 138). Thus, current pup production in the Greenland Sea is considerably lower than in 1997 and no commercial hunting quotas have been issued for hooded seals in the Greenland Sea since 2006

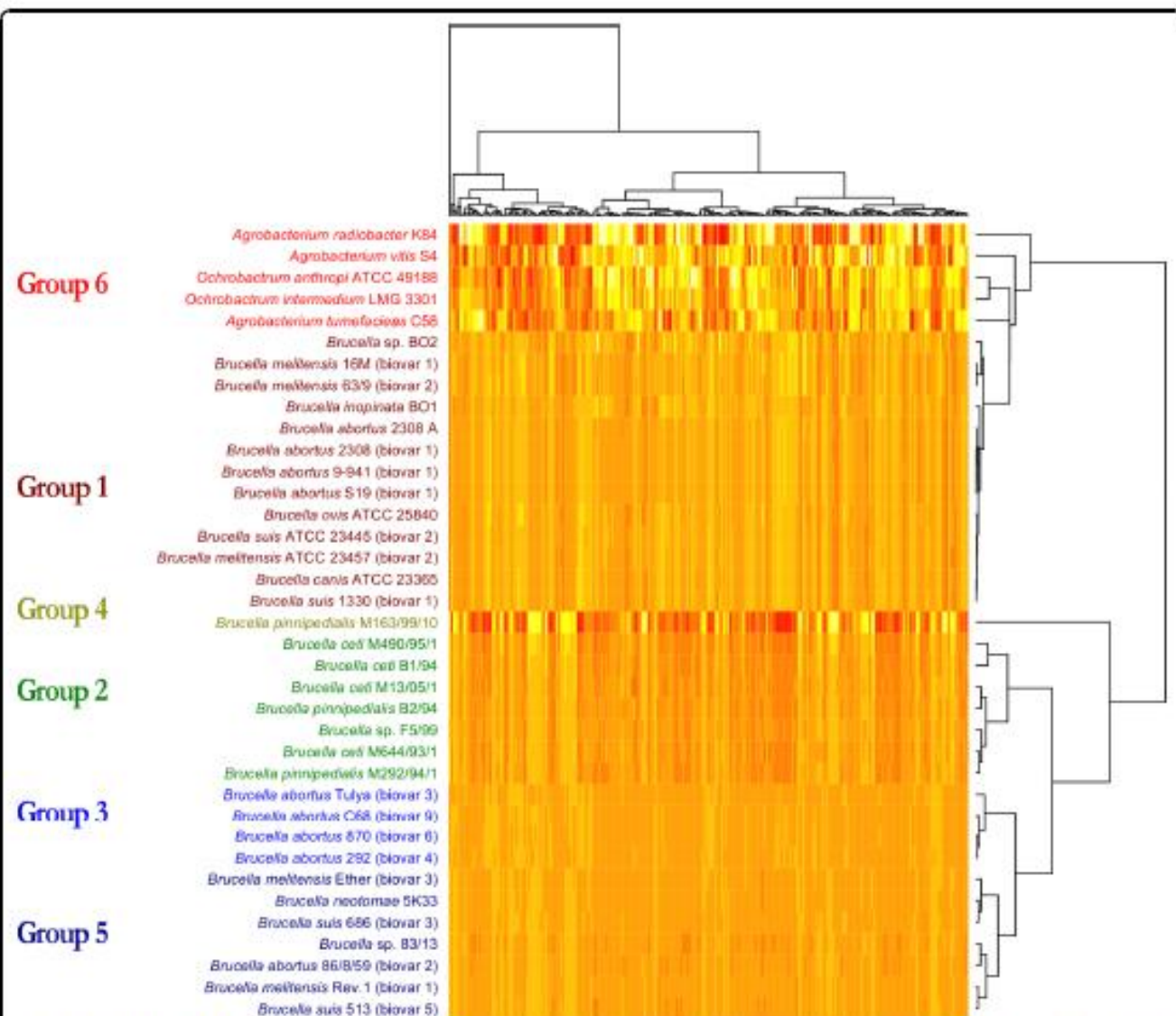


Figure 1 ZOM based heatmap. The ZOM Based heatmap shows all genomes compared using cluster analysis based on 4th order Markov chain model predicted tetranucleotide frequencies. It can be seen that the sequenced species from genus *Brucella* are very similar in terms of tetranucleotide usage patterns, with larger differences found in the more distantly related genera of *Agrobacterium* and *Ochrobactrum*. Although all species in genus *Brucella* are very similar in terms of base composition, as measured using the ZOM based method, several subgroups can be observed. For instance, marine associated (Groups 2 and 4) and terrestrial mammal associated (Groups 1, 3 and 5) species of genus *Brucella* are segregated into different groups.

Naturally-occurring Human *Brucella* Infections

- **Neurobrucellosis - California (Sohn *et al.*, 2002)**
 - 2 Peruvians - intracerebral granuloma
 - No link to marine mammals
- **Spinal osteomyelitis - New Zealand (McDonald *et al.*, 2006)**
 - No reported link to marine mammals
 - Exposure to uncooked fish bait
 - Consumed raw fish





Extended ecological niche

Tryland M., Derocher A. E., Wjg O., Godfroid J., 2001. *Brucella* antibodies in polar bears (*Ursus maritimus*) from Svalbard and the Barents sea. *Journal of Wildlife Disease*, 37: 523-531.



Photo: Morten Tryland

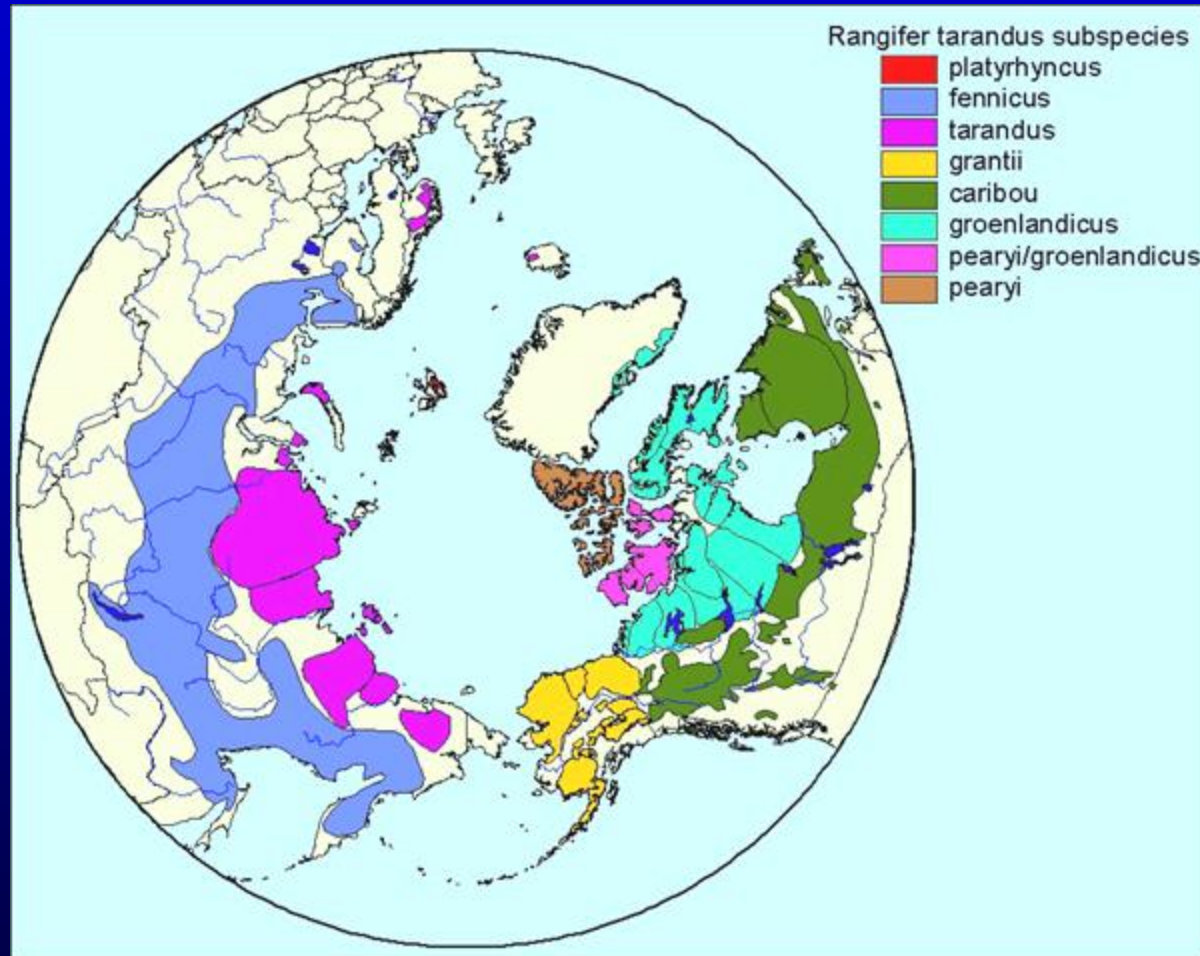
Brucellosis in polar bear: new data or info ?

- *Brucella* species survey in polar bears (*Ursus maritimus*) of northern Alaska. O'Hara TM, Holcomb D, Elzer P, Estep J, Perry Q, Hagius S, Kirk C. J Wildl Dis. 2010 Jul;46(3):687-94.
Wildlife Toxicology Laboratory, Institute of Arctic Biology and Department of Biology and Wildlife, University of Alaska Fairbanks, Fairbanks, Alaska 99775, USA.
- “ It appears the polar bear antibody does not react with the antigens used on the marine cELISA assay, potentially indicating a terrestrial (nonpinniped) source of *Brucella* spp. ”

Political Arctic



Rangifer tarandus subspecies distribution



Biovars 1 & 3

Biovar 2



*Brucella
suis*



Biovar 4



Short communication

Brucella suis identification and biovar typing by real-time PCR

David Fretin^a, Adrian M. Whatmore^b, Sascha Al Dahouk^{c,1}, Heinrich Neubauer^{c,2},
 Bruno Garin-Bastuji^d, David Albert^d, Mieke Van Hessche^a, Marie Ménart^a,
 Jacques Godfroid^{a,3}, Karl Walravens^a, Pierre Wattiau^{a,*}

**SNPs
signatures**

Pstp (1677)	PyrH (816/817)	MalG (954/963)	
A	GT	TC	→ <i>B. suis</i> 1
G	AG	TC	→ <i>B. suis</i> 2
A	AG	TC	→ <i>B. suis</i> 3,4
G	AG	CT	→ <i>B. suis</i> 5

Brucellosis (*Brucella suis* biovar 4) in reindeer



Kautokeino



A screening ELISA for brucellosis in reindeer.

Zentralbl Veterinarmed B. 1999 Nov;46(9):649-57.

Asbakk K, Gall D, Stuen S.

**Norwegian School of Veterinary Science, Department of Arctic Veterinary Medicine,
Tromsø, Norway.**

Abstract

An enzyme-linked immunosorbent assay (ELISA) for the screening of brucellosis in reindeer was developed. The assay, which utilizes s-LPS from *Brucella abortus* as antigen and biotin-labelled rabbit antibody to reindeer immunoglobulin as detecting antibody, has a high specificity and sensitivity, as indicated in a validation with sera from reindeer cultured positive for *Brucella suis* biovar 4 and sera from **reindeer free of brucellosis.**





« The basis of the Nenets way of life is reindeer herding. Reindeer mean EVERYTHING to Nenets -- food, clothing, transportation. We are nomads, and the reindeer are so important to our way of life that they are almost like part of our families! Groups of reindeer numbering up to several hundred are owned by each extended family group. »

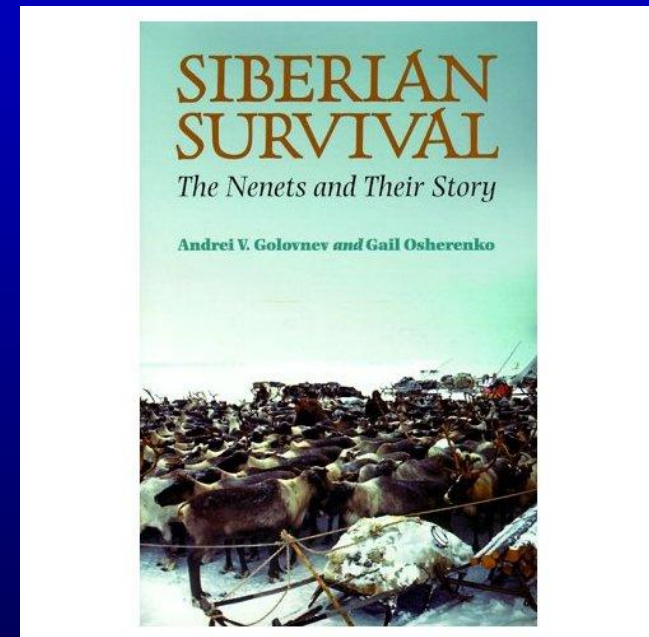


pooka.nunanet.com/~oxana/page2.html



Brucellosis a zoonotic disease of concern...

« The reindeer are vaccinated against diseases, especially against brucellosis. »



Brucellosis – Reindeer – Alaska / Canada

GEOGRAPHIC PATTERN OF SERUM ANTIBODY PREVALENCE FOR *BRUCELLA* SPP. IN CARIBOU, GRIZZLY BEARS, AND WOLVES FROM ALASKA, 1975–1998

Randall L. Zarnke,^{1,3} Jay M. Ver Hoef,^{1,2} and Robert A. DeLong¹

¹ Alaska Department of Fish and Game, 1300 College Road, Fairbanks, Alaska 99701-1599, USA

² Current address: NOAA National Marine Mammal Lab, 7600 Sand Point Way NE, Bldg 4, Seattle, Washington 98115-6349, USA

³ Corresponding author (email: ltrap2@acsalaska.net)

ABSTRACT: Blood samples were collected from 2,635 caribou (*Rangifer tarandus*), 1,238 grizzly bears (*Ursus arctos*), and 930 wolves (*Canis lupus*) from throughout mainland Alaska during 1975–98. Sera were tested for evidence of exposure to *Brucella* spp. Serum antibody prevalences were highest in the northwestern region of the state. In any specific area, antibody prevalences for caribou and wolves were of a similar magnitude, whereas antibody prevalence for bears in these same areas were two to three times higher.

Key words: Alaska, *Brucella* spp., caribou, grizzly bear, wolf.



RANGIFERINE BRUCELLOSIS ON BAFFIN ISLAND

Michael A. D. Ferguson

Department of Resources, Wildlife and Economic Development, Government of the Northwest Territories, Pond Inlet, Northwest Territories X0A 0S0, Canada

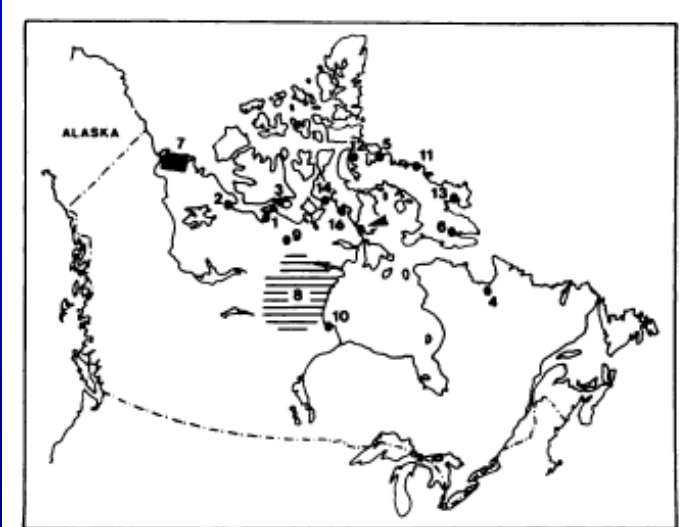


FIGURE 2. Location of reported instances of brucellosis in northern Canada. Numbers correspond to the cases listed in Table 1. The arrow indicates Repulse Bay, the location of the case of granulomatous nephritis in a barren ground caribou and of case number 15 in Table 1.

Climate change and infectious diseases of animals in the Arctic



010 KORTRAPPORT/BRIEF REPORT SERIES
NORSK POLARINSTITUTT 2009



Morten Tryland, Jacques Godfroid and Per Arneberg (eds.)

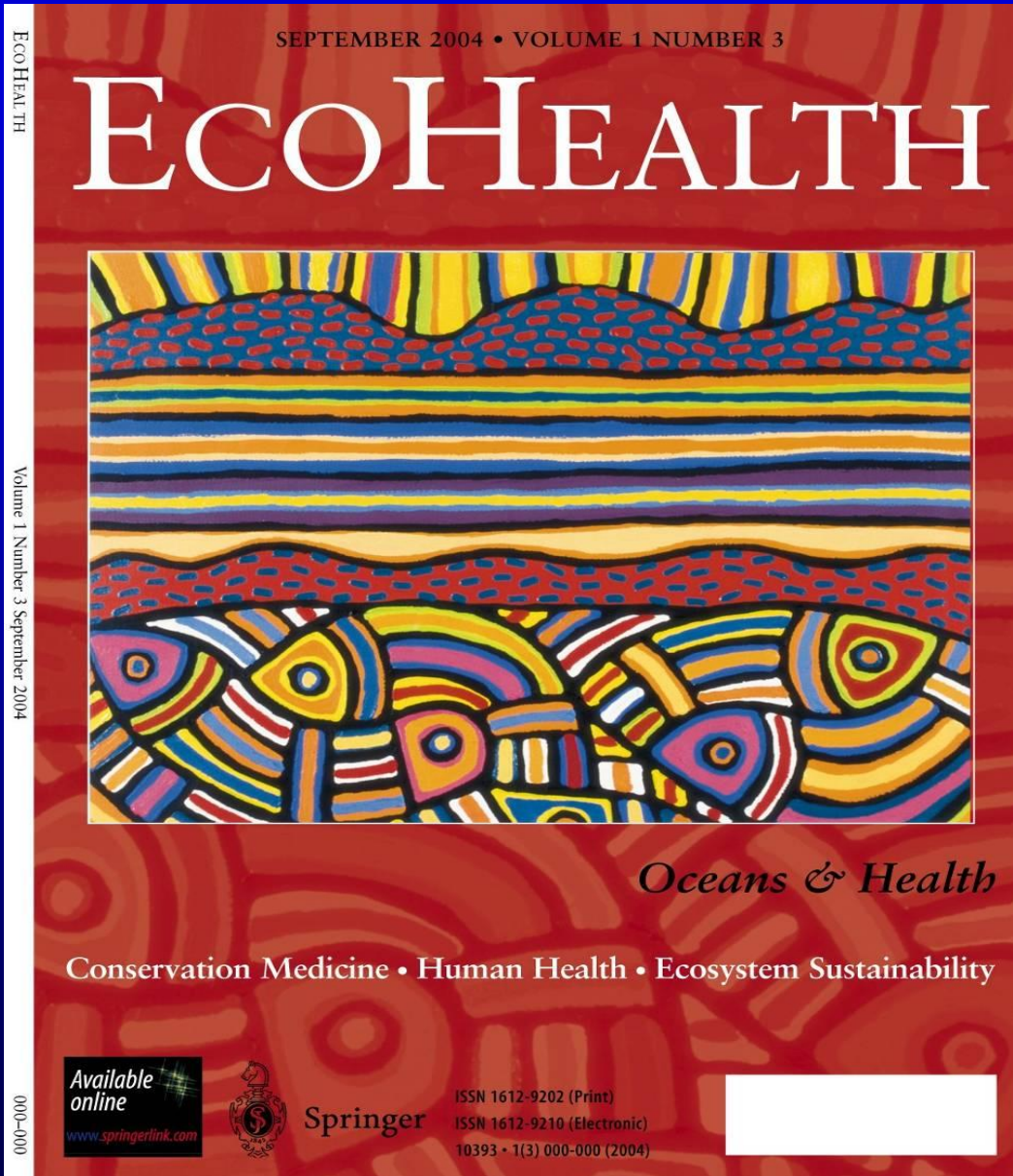
Impact of climate change on infectious diseases of animals in the Norwegian Arctic



Norwegian School of Veterinary Science

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Alonso Aguirre,
VP for Conservation Medicine



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The origin of brucellosis



In terrestrial mammals



Thank you for your attention