

# **Brucella** surveillance in stranded marine mammals from the North Sea



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#### **SURVEILLANCE PROGRAMME**

Brucellosis in marine mammals is recognized as a zoonotic disease. Two different species of *Brucella*, *B. pinnipedialis* and *B.ceti*, have been described in pinnipeds and cetaceans, respectively<sup>1</sup>. Transmission of *Brucella* in marine mammals is poorly understood, but both vertical and food chain transmission have been suggested<sup>2</sup>.

The aim of this study was to evaluate the prevalence of *Brucella* infection among marine mammals, in order to assess the potential zoonotic risk of marine mammal Brucellae in the North Sea.

A *Brucella* surveillance program of stranded marine mammals on the coast of Belgium, France and Netherlands has been implemented since 1999. A total of 523 organ samples, from 207 marine mammals, were recovered between 1999 and 2013.

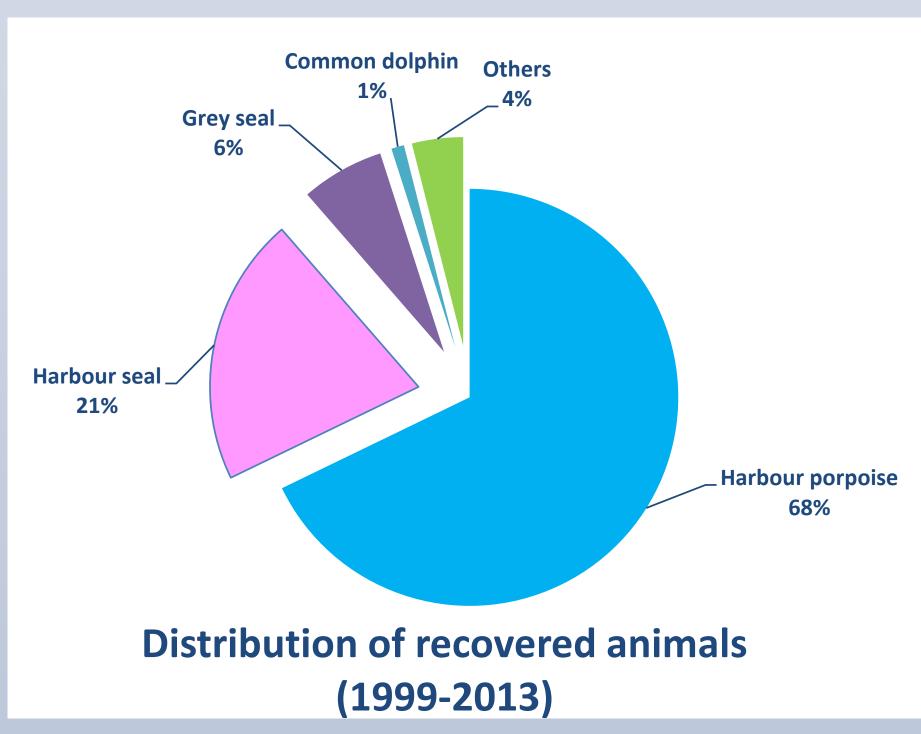
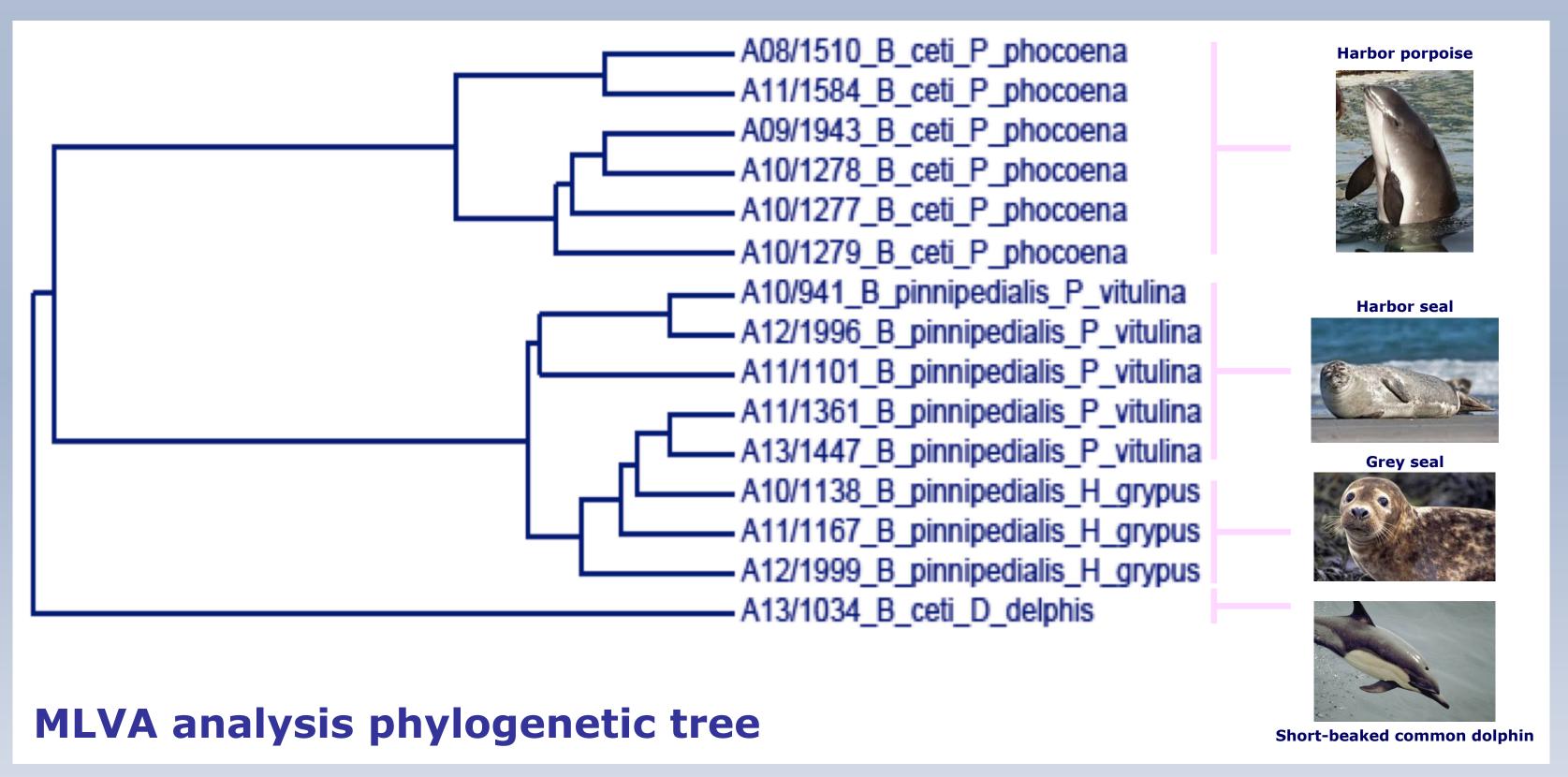


Figure 1: Distrubution of recoverd animals.

## **MLVA ANALYSIS**

Isolates were typed by MLVA (Multi Locus Variable Tandem Repeats Analysis) on the basis of 16 different markers<sup>3,4,6</sup>. Graphical representation and edition of the phylogenetic tree were performed with TreeDyn.



**Figure 2**: MLVA analysis phylogenetic tree performed with the TreeDyn programme from the MLVA bank website University Paris Sud.

Three cluster were found, and two corresponded to separated groups of *B. ceti*. One group was composed exclusively of harbor porpoises strains, while the second group only included the dolphin isolate. The third cluster was constituted of *B. pinnipedialis* strains from seals.

#### **BRUCELLA SCREENING**

Organs were cultured in Farrell medium for bacteriology detection. First, *Brucella* colonies were identified by colony morphology, microscopy and biochemical probes. A real-time PCR based in the *Bcsp31* sequence was made in order to confirm de genus<sup>5</sup>.

Brucella spp. was isolated in 7,2%, (15/207), of the stranded animals. The isolates were recovered from harbor porpoises (Phocoena phocoena) (n=6), harbor seals (Phoca vitulina) (n=5), grey seals (Phoca phocoena) phocoena phoc

Infected animals recoverded (1999-2013)									
Species	<b>Total animals</b>	Infected animals	Percentage %						
Harbour porpoise	137	6	4,4						
Harbour seal	42	5	11,9 23,1						
Grey seal	13	3							
Short-beaked common dolphin	2	1	50,0						
Others	6	0	0,0						
Total recovered	207	15	7,2						

**Table 1**: Infected animals recoverd from 1999 to 2013.

As expected, B. pinnipedialis and B. ceti were detected in pinnipeds and cetaceans, respectively. Brucella was mainly found in lungs (n=9), bronchial lymph nodes (n=6) and prescapular lymph nodes (n=5) among the positive animals, showing the importance of these organs as targets for Brucella detection (Table 2).

	Distribution of positive samples															
	Bronquial			Lung		Mesenteric Prescapular		Tronchal								
	Isolate	Species	LN	Lung	LN	Encephalon	LN	LN	LN	Spleen	Epididymis	Hepatic LN	Gastric LN	Skin ulcer	Mucous plug	Maxillary LN
	1 A08/1510	P. phocoena	+	+		-				-						
	2 A09/1943	P. phocoena				+	+	+								
	3 A10/1277	P. phocoena						-		-	+			-		
4	4 A10/1278	P. phocoena		+	-					-						
	*	P. phocoena	+	+						1						
	6 A11/1584	P. phocoena		+						-						
	7 A13/1034			+	-			-								
	8 A10/1138			-		-		+		+		+				
	9 A11/1167		+	+			+	+		+			+			
1	.0 A12/1999	H. grypus	+	-				-								
1	.1 A10/941	P. vitulina		-		-	-	+							-	-
1	.2 A11/1101	P. vitulina		+					-	-						
1	.3 A11/1361	P. vitulina	+	+			-			+						-
1	.4 A12/1996	P. vitulina	-	-				+								
1	.5 A13/1447	P. vitulina	+	+												

**Table 2**: Distribution of positive (+) and negative (-) samples. Boxes in white were samples not provided. LN= lymph node

## CONCLUSIONS

As a zoonotic disease, the presence of *Brucella* in marine mammals constitutes a biohazard for human health.

Different genetic profiles were identified by MLVA, demonstrating the strain variability of *Brucella* spp. circulating in marine mammals in the North Sea.

Further studies will be needed to study the spread of clonal strains among the marine mammal population.

In order to establish control actions, more studies on epidemiology and risk factors should be done.

## REFERENCES

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