

Identification and Molecular Characterization of Beta-Hemolytic Streptococci Isolated from Harbor Porpoises (*Phocoena phocoena*) of the North and Baltic Seas

M. SWENSHON,¹ C. LÄMMLER,^{1*} AND U. SIEBERT²

Institut für Bakteriologie und Immunologie der Justus-Liebig-Universität Gießen, Gießen,¹ and Forschungs- und Technologiezentrum Westküste, Christian-Albrechts Universität Kiel, Büsum,² Germany

Received 23 October 1997/Returned for modification 30 January 1998/Accepted 26 March 1998

The present study was designed to identify and comparatively investigate 35 beta-hemolytic streptococci isolated from stranded harbor porpoises or from animals caught in fishing nets of the North and Baltic seas. According to biochemical and serological data and to lectin agglutination tests with the lectin of *Arachis hypogaea*, all 35 isolates could be classified in Lancefield's serological group L and could be identified as *Streptococcus dysgalactiae* subsp. *dysgalactiae*. All 35 group L streptococci were uniformly sensitive to most of the antibiotics tested. To further analyze the epidemiological relationship, the isolates were subjected to macrorestriction analysis of their chromosomal DNA by pulsed-field gel electrophoresis. Digestion of the chromosomal DNA with the restriction enzymes *Sma*I and *Apa*I revealed that most of the group L streptococci seemed to be apparently identical or related. These results indicate that one clone or at least related group L streptococcal clones play an important role for infections of harbor porpoises of the North and Baltic seas. This might possibly be caused by a direct transfer of the bacteria from animal to animal.

The harbor porpoise (*Phocoena phocoena*) is the most-seen representative of the small cetaceans in the North and Baltic seas (3, 8). In connection with the seal epidemic in the North and Baltic seas in the years 1988 and 1989, an increased number of small cetacean carcasses on the German coast was registered. The collected data showed a dramatic decrease in the harbor porpoise population since the beginning of this century. This could be seen especially in the Baltic Sea (3, 16).

Bacteriological investigations of animals caught in fishing nets and stranded small cetaceans of the North and Baltic seas resulted in the isolation of various bacteria and frequently in the isolation of alpha- and beta-hemolytic streptococci. The beta-hemolytic streptococci could be isolated, partly in pure culture, from abscesses, from bronchopneumonia patients, and often from patients with septicemia causing suppurative leptomeningitis, pyelonephritis, myocarditis, and osteomyelitis (17). The isolated beta-hemolytic streptococci seemed to be one of the major causative agents of these diseases. However, in these studies the beta-hemolytic streptococci had not been identified to species level. In addition, nothing is known about the properties and epidemiological relationship of these bacteria. The present study was designed to identify and further characterize beta-hemolytic streptococci isolated from stranded harbor porpoises and from harbor porpoises caught in fishing nets in the North and Baltic seas.

MATERIALS AND METHODS

Bacterial isolates. A total of 35 beta-hemolytic streptococci isolated from various organs of 16 harbor porpoises were investigated in this study. Among the 35 cultured streptococci 33 were isolated from 14 harbor porpoises of the North

Sea and 2 were from two harbor porpoises from the Baltic Sea. The animal designations, the tissues from which the beta-hemolytic streptococci were isolated, and the places and dates of discovery of the harbor porpoises are summarized in Table 1. The bacteria were cultivated on Columbia sheep blood agar plates (Oxoid, Wesel, Germany) and in Todd-Hewitt broth (THB) (Gibco Europe, Karlsruhe, Germany) as fluid media. The growth intensity after cultivation on blood agar plates is shown in Table 1. None of the cultures required primary cultivation in THB to be recovered. Among the 35 isolates, 9 were isolated in pure culture and 16 predominated. For comparative purposes streptococci of serological group L isolated from pigs ($n = 2$), bovines ($n = 2$), and humans ($n = 2$) and the group L-streptococcal reference culture ATCC 9932 (MS 210) were included (18).

Biochemical properties. The biochemical properties of the beta-hemolytic streptococci were determined with a commercial identification system (rapid ID 32 Strep; bioMérieux, Nürtingen, Germany). The test kit, containing 32 different reactions (arginine dihydrolase, β -glucosidase, β -galactosidase, β -glucuronidase, α -galactosidase, alkaline phosphatase, ribose, mannitol, sorbitol, lactose, trehalose, raffinose, sucrose, arabinose, arabitol, cyclodextrin, acetoin production, alanine-phenylalanine-proline arylamidase, β -galactosidase, pyroglutamic acid arylamidase, *N*-acetyl- β -glucosaminidase, glycyl-tryptophane arylamidase, hydrolysis of hippurate, glycogen, pullulan, maltose, melibiose, melezitose, methyl- β -D-glucopyranoside, tagatose, β -mannosidase, and urease) was used according to the instructions of the manufacturer.

Serogrouping. With the commercial grouping kit Streptex (Murex, Burgwedel, Germany) the β -hemolytic cultures could be investigated for serogroup A, B, C, D, F, and G. In addition, the group-specific antigen was extracted by autoclaving the bacteria and the extracts were subsequently tested by agar gel diffusion with specific antiserum prepared against a group L streptococcal reference culture (18).

Lectin agglutination. The lectin from *Arachis hypogaea* (Sigma, Deisenhofen, Germany) was suspended in phosphate-buffered saline (PBS) at a final concentration of 0.5 g/ml. The streptococci were cultivated in 5 ml of THB for 24 h at 37°C, centrifuged, washed in PBS, and suspended in 200 μ l of PBS. To prevent self-agglutination the bacteria were pretreated with trypsin (5 μ g/200 μ l of bacterial suspension) for 1 h at 37°C, washed, and resuspended in PBS. For lectin agglutination tests 20 μ l of the lectin preparation was mixed with 20 μ l of streptococcal suspension on microscopic slides (13). The slides were rotated and examined for agglutination reaction.

Antibiotic susceptibility. The determination of antibiotic susceptibilities was performed according to the recommendations of the Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin, Berlin, Germany. According to the instructions, four to five colonies of the bacteria were inoculated in 3 ml of THB for 2 h at 37°C. Approximately 0.1 ml of this suspension was transferred onto Müller-Hinton agar (Oxoid) containing 5% sheep blood. The

* Corresponding author. Mailing address: Institut für Bakteriologie und Immunologie, Justus-Liebig-Universität Gießen, Frankfurter Straße 107, D-35392 Gießen, Germany. Phone: 49-641-99-38383. Fax: 49-641-99-38389. E-mail: Christoph.Laemmler@vetmed.uni-giessen.de.

TABLE 1. Summary of 35 beta-hemolytic streptococci isolated from 16 harbor porpoises from the North and Baltic seas^a

Animal	Place of discovery ^b	Date of discovery (day.mo.yr)	Nature of animal when found ^c	Streptococcus		Restriction pattern after digestion with:	
				Tissue of origin	Growth ^d	<i>SmaI</i>	<i>ApaI</i>
A	List on Sylt	24.02.1991	S	Lung	+	S I	A II
B	List on Sylt	09.08.1991	S	Lung	+	S I	A II
F	List on Sylt	24.02.1992	S	Kidney	+++	S II	A III
J	Kampen on Sylt	16.07.1994	N	Intestine	+	S I ^e	A I ^e
				Liver	+	S I	A I
				Lung	+	S I	A I
				Intestine	+	S I	A I
K	Kampen on Sylt	15.07.1994	S	Kidney	++	S I	A I
				Lung	+++	S I	A I
				Spleen	++	S I	A I
				Kidney	++	S I	A I
I	Wenningstedt on Sylt	19.06.1994	S	Lung	+++	S I	A I
				Liver	+	S I	A I
				Lung	+++	S I	A I
				Lymph node	+++	S I	A I
				Lung	+	S III	A IV
				Lung	++	S IV	A V
M	Westerland on Sylt	18.07.1995	S	Lung	++	S I	A VI
				Kidney	+++	S I	A VI
N	Westerland on Sylt	04.03.1995	S	Lung	++	S I	A VI
D	Baakdeel on Sylt	06.01.1992	S	Kidney	+++	S I	A VI
E	Rantum on Sylt	23.02.1992	N	Kidney	+	S II	A III
G	Hörnum on Sylt	18.04.1993	S	Kidney	+++	S I	A I
				Lung	+++	S I	A I
L	Nieblum on Föhr	08.09.1994	S	Kidney	++	S V	A VII
				Lung	+	S V	A VII
P	Amrum	18.07.1995	S	Intestine	+++	S I	A VI
				Intestine (lymph node)	+++	S I	A VI
				Kidney	+++	S I	A VI
				Liver	+++	S I	A VI
				Lung	+++	S I	A VI
				Spleen	+++	S I	A VI
				Kidney	+++	S I	A VI
O	Friedrichskoog	01.06.1995	S	Lung	+++	S I	A VI
				Lung	+++	S I	A VI
				Spleen	+++	S I	A VI
				Muscle	+++	S I	A VI
C	Laboe	05.12.1991	N	Lung	++	S VI	A VIII
				Kidney	+	S VII	A IX
H	Lübeck-Travemünde	01.06.1994	S	Kidney	+	S VII	A IX
				Lung	+	S VII	A IX

^a DNA restriction patterns of chromosomal DNA after digestion with endonucleases *SmaI* and *ApaI* are also shown.

^b List, Kampen, Wenningstedt, Westerland, Baakdeel, Rantum, and Hörnum (all on the island of Sylt) as well as Nieblum on Föhr, Amrum, and Friedrichskoog are all on the North Sea. Laboe and Lübeck-Travemünde are on the Baltic Sea.

^c S, stranded; N, caught in fishing nets.

^d Growth intensity after cultivation on blood agar (+, slight; ++, moderate; +++, excessive).

^e For differences, see Results section.

paper discs used contained bacitracin (0.04 IU), bacitracin (10 IU), cefoxitin (30 µg), chloramphenicol (30 µg), clindamycin (2 µg), erythromycin (15 µg), gentamicin (10 µg), minocycline (30 µg), neomycin (30 µg), nitrofurantoin (100 µg), penicillin G (10 IU), sulfamethoxazole-trimethoprim (23.75 and 1.25 µg, respectively), and tetracycline (30 µg).

Pulsed-field gel electrophoresis. The preparation and digestion of genomic DNA were performed according to the methods of Maslow et al. (11) and Thiele et al. (25). As described previously (21) the bacteria were cultivated in 40 ml of THB, centrifuged, washed in TE buffer (10 mM Tris-HCl, 1 mM EDTA [pH 8.0]), resuspended in TE buffer (5% transmission at 620 nm), and mixed with the same volume of 1% low-melting-point and low-gelling-point InCert agarose (FMC Bio Products, Rockland, Maine). This mixture was solidified for 10 min on ice in special molds. The cells were lysed by subsequent incubation of the blocks in 200 µl of ES buffer (0.5 M EDTA [pH 8.0], 1% *N*-lauroylsarcosine [Sigma]) for 30 min at room temperature followed by an incubation for 1 h at 37°C in the same buffer containing 10 µg of mutanolysin (10 U/µl; Sigma). After overnight treatment with proteinase K (final concentration, 0.5 µg/ml; Boehringer, Mannheim, Germany) in 200 µl of TE buffer at 56°C, the agarose blocks were washed twice for 30 min each time in TE buffer, incubated with phenylmethylsulfonyl fluoride (final concentration, 1.0 mM in 200 µl of TE buffer; Sigma) twice for 1 h each time at 56°C, and washed in TE buffer twice for 30 min each time. The genomic DNA of the isolates was digested with the endonucleases *SmaI* and *ApaI* (Stratagene, Heidelberg, Germany). For digestion with *SmaI* each agarose block was incubated with 4 µl of *SmaI* (20 U) suspended in 20 µl of 10× universal buffer (Stratagene) and 176 µl of twice-distilled water for 5 h at 25°C. For digestion with *ApaI* each agarose block was incubated with 1.5 µl of *ApaI* (15

U) suspended in 20 µl of 10× universal buffer (Stratagene) and 178.5 µl of twice-distilled water overnight at 30°C. Samples were electrophoresed at 14°C through 1% chromosomal-grade agarose (Bio-Rad Laboratories, Munich, Germany) (in a 13-by-14-by-0.55-cm gel) containing 0.5 µg of ethidium bromide (Sigma) per ml in 0.5× TBE buffer (45 mM Tris, 45 mM borate, 1.0 mM EDTA [pH 8.3]) in a CHEF-DR II PFGE cell (Bio-Rad). Lambda DNA/*HindIII* fragments at 0.1 to 200 kb (Sigma) and lambda DNA concatemers at 50 to 1,000 kb (Sigma) served as standards. The running conditions were as follows. Switch time ramping for *SmaI*-digested agarose blocks was 0.1 to 11 s for 8 h at 5 V/cm and then 9 to 34 s for 17 h at 6 V/cm. For *ApaI*-digested agarose blocks, switch time ramping was 0.1 to 10 s for 13 h at 6 V/cm and then 5 to 8 s for 7 h at 6 V/cm.

Statistics. The BMDP Statistical Software package (5) was used for the estimation of the genetic relationship of the beta-hemolytic streptococci, and the program PlotIt-Graphics and Statistics (6) was used for the presentation of the genetic relationship in a dendrogram.

RESULTS

All 35 isolates investigated in the present study were beta-hemolytic streptococci and could be classified biochemically and serologically into Lancefield's serological group L.

The biochemical properties of all 35 isolates determined with the rapid ID 32 Strep system appeared to be identical in most of the reactions. All cultures were positive for arginine

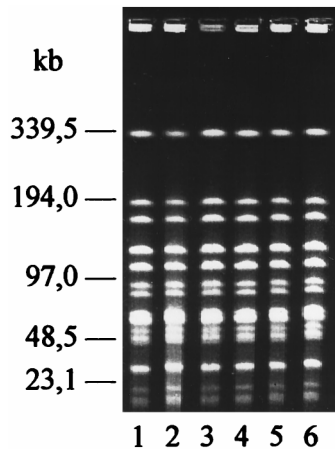


FIG. 1. PFGE restriction patterns of chromosomal DNA of group L streptococci isolated from six different organs of harbor porpoise P. Patterns were obtained after digestion of the DNA with endonuclease *Sma*I.

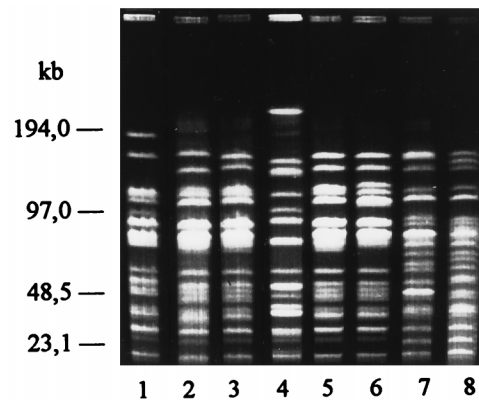


FIG. 2. Restriction patterns of group L streptococci isolated from harbor porpoises as follows: A VI, harbor porpoises P, O, and D (lane 3, 5, and 6); A IV, harbor porpoise M (lane 1); A V, harbor porpoise N (lane 2); A VII, harbor porpoise L (lane 4); A VIII, harbor porpoise C (lane 7); and A IX, harbor porpoise H (lane 8).

dihydrolase, β -glucuronidase, alkaline phosphatase, ribose, trehalose, sucrose, glycogen, pullulan, and maltose; were negative for β -glucosidase, β -galactosidase, α -galactosidase, pyroglutamic acid arylamidase, *N*-acetyl- β -glucosaminidase, glycyl-tryptophane arylamidase, β -mannosidase, urease, acetoin production, and hydrolysis of hippurate; and did not produce acid from mannitol, sorbitol, raffinose, *L*-arabinose, *D*-arabitol, melibiose, melezitose, methyl- β -*D*-glucopyranoside, and tagatose. Acid production from lactose could be observed for 33 cultures, and a positive cyclodextrin and a positive alanine-phenylalanine-proline arylamidase reaction could be observed for 14 and 33 cultures, respectively. Autoclaved extracts of all 35 isolates reacted with group L-specific antiserum. No reaction could be observed with the commercial grouping kit, including serogroups A, B, C, D, F, and G. In lectin agglutination tests all 35 isolates agglutinated with the lectin from *A. hypogaea*.

The determination of antibiotic susceptibility revealed that all 35 beta-hemolytic streptococci were sensitive to bacitracin (10 IU), cefoxitin, chloramphenicol, clindamycin, erythromycin, minocycline, nitrofurantoin, penicillin G, sulfamethoxazole-trimethoprim, and tetracycline and that most of the strains were sensitive to gentamicin (83%) and bacitracin (0.04 IU) (94%); all strains were resistant to neomycin.

To further analyze the epidemiological relationship, the isolates were subjected to DNA fingerprinting. The digestion of the chromosomal DNA of the isolates with the endonucleases *Sma*I and *Apa*I and the separation of the fragments by pulsed-field gel electrophoresis (PFGE) revealed that the beta-hemolytic streptococci isolated from a single harbor porpoise had identical restriction patterns (Fig. 1) with one exception. The isolate from the intestine of harbor porpoise J differed in one and two fragments after digestion with *Sma*I and *Apa*I, respectively. To compare the DNA pattern of the group L streptococci obtained from the 16 harbor porpoises, one isolate from each animal was subjected to comparative PFGE. The digestion of the chromosomal DNA with the endonucleases *Sma*I and *Apa*I revealed seven (SI to SVII) (not shown data) and nine (AI to AIX) different restriction patterns, respectively (Fig. 2). The origin of the animals and their DNA pattern are summarized in Table 1.

A dendrogram of similarity of the nine DNA restriction groups revealed after digestion of the chromosomal DNA with the endonuclease *Apa*I is presented in Fig. 3. According to this dendrogram analysis, the DNA profiles of all but one isolate

(harbor porpoise L) from the harbor porpoises of the North Sea were related to each other and differed from DNA profiles of both strains isolated from harbor porpoises of the Baltic Sea (harbor porpoises C and H). The DNA restriction patterns of the group L streptococcal control strains appeared with unrelated DNA profiles (data not shown).

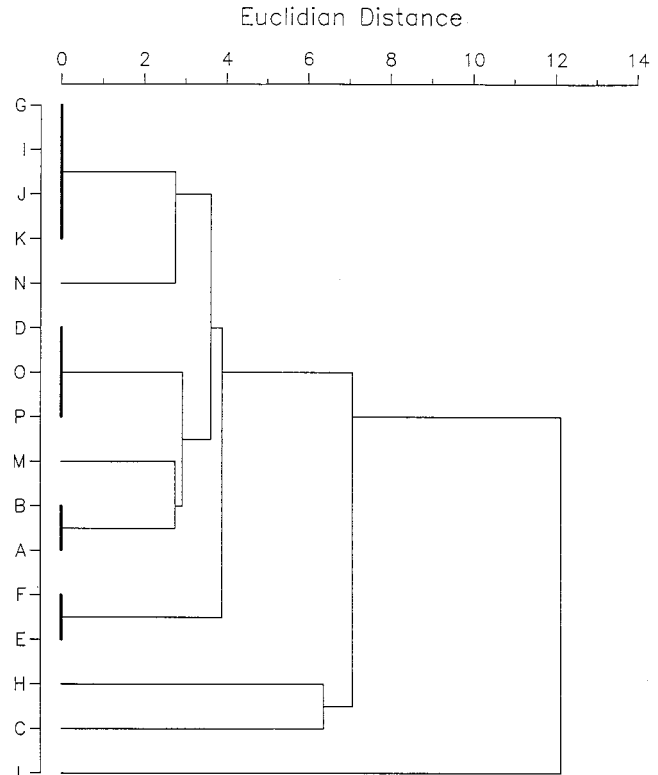


FIG. 3. Dendrogram demonstrating genetic relationship of different DNA patterns of group L streptococci isolated from 16 harbor porpoises (listed on the left). Patterns were obtained after digestion of the chromosomal DNA with the restriction enzyme *Apa*I.

DISCUSSION

All beta-hemolytic streptococci investigated in the present study were isolated from stranded harbor porpoises or harbor porpoises caught in fishing nets in the North and Baltic seas. On the basis of their biochemical and serological properties, all 35 isolates belonged to Lancefield's serological group L and could be identified as *S. dysgalactiae* subsp. *dysgalactiae* serovar L. The classification of group L as well as streptococci of serological group C of animal origin as *S. dysgalactiae* subsp. *dysgalactiae* was proposed by Vandamme et al. (26).

The group L streptococci could additionally be identified by lectin agglutination tests with the lectin from *A. hypogaea*. This has already been shown to be a useful additional test for presumptive identification of this species (13).

All group L streptococci seemed to be uniformly sensitive to most of the antibiotics tested in this study. According to previous studies group L streptococci isolated from various origins showed resistance to antibiotics such as tetracycline, minocycline, erythromycin, chloramphenicol, and gentamicin (18). Tetracycline- and minocycline-resistant group L streptococci seemed to possess the resistance genes *tet(M)* and *tet(O)* (22).

Streptococci of serological group L are well known as causative agents of bovine mastitis and various infections of pigs, dogs, poultry, and sheep, but they rarely cause infections in humans (2, 7, 9, 10, 19, 24). However, little is known about the isolation of streptococci of serological group L from harbor porpoises. Baker and Martin (1) investigated 41 harbor porpoises from British waters and could find group L streptococci as the cause of bacterial pneumonia. To our knowledge there are no further reports about the isolation of group L streptococci from sea animals.

The harbor porpoises of the present study were examined post mortem, and it was obvious that most of the animals, especially their respiratory systems, were infected by parasites. The bacteriological investigations revealed that besides beta-hemolytic streptococci other potentially pathogenic bacteria, such as *Clostridium perfringens*, *Erysipelothrix rhusiopathiae*, and *Staphylococcus aureus*, could be isolated from the organs of the animals. However, according to previous studies beta-hemolytic streptococci seemed to be the major pathogens causing septicemia, abscesses, or bronchopneumonia. These beta-hemolytic streptococci were isolated partly in pure culture (4, 15, 17).

To study their genetic relationship, the streptococci of the present study were further investigated by macrorestriction analysis of their chromosomal DNA after digestion with the restriction enzymes *SmaI* and *ApaI* by PFGE. This technique proved to be useful for molecular typing of animal-pathogenic *Streptococcus equi* subsp. *zooepidemicus* and *Rhodococcus equi* (20, 21).

The macrorestriction analysis of the present investigation was performed with two restriction enzymes (*SmaI* and *ApaI*) to avoid epidemiologically misleading results. The present study's investigation of group L streptococci revealed that DNA fragments obtained from isolates from different organs of one animal and from some of the isolates obtained from different animals produced apparently identical profiles, indicating that a single bacterial clone seems to be the causative agent. It was of interest that bacterial clones with identical profiles were mostly found among isolates from the same region (harbor porpoises A and B had identical profiles, as did harbor porpoises J and K) and/or among bacteria isolated at the same time (harbor porpoises E and F had identical profiles, as did harbor porpoises J and K). These findings can lead to the conclusion that direct transmission of the group L strep-

tococci between the various animals occurred. However, group L streptococci with identical DNA profiles could also be found among isolates from animals which were found and investigated 3 1/2 years apart (harbor porpoises D, O, and P). The stability of the DNA profiles over a long period has already been reported by other authors (12, 14). According to the PFGE analysis criteria of Tenover et al. (23), most of the nonidentical group L streptococci isolates from harbor porpoises of the North Sea appeared to be closely related or at least possibly related; the two isolates from harbor porpoises from the Baltic Sea were closely related to each other but not to those from the North Sea. A comparison of the DNA profiles of group L streptococci isolated from harbor porpoises with DNA profiles of group L streptococci isolated from bovines, pigs, or humans revealed no genetic relationship. These results indicate that one group L streptococcal clone or at least closely related clones contribute to the infections of the harbor porpoises in the North and Baltic seas and that direct transfer from animal to animal seems to be a reasonable means of transmission.

ACKNOWLEDGMENTS

The collection of carcasses and samples as well as the pathological investigations were supported by grants from the Bundesministerium für Umwelt, Naturschutz und Reaktorsicherheit and the Bundesministerium für Bildung, Wissenschaft, Forschung und Technologie.

REFERENCES

1. Baker, J. R., and A. R. Martin. 1992. Causes of mortality and parasites and incidental lesions in harbour porpoises (*Phocoena phocoena*) from British waters. *Vet. Rec.* **130**:554-558.
2. Barnham, M., and D. J. Neilson. 1987. Group L beta-haemolytic streptococcal infection in meat handlers: Another streptococcal zoonosis? *Epidemiol. Infect.* **99**:257-264.
3. Bohlken, H., H. Benke, and J. Wulf (ed.). 1993. Untersuchungen über Bestand, Gesundheitszustand und Wanderungen der Kleinwalpopulationen (*Cetacea*) in deutschen Gewässern. Endbericht zum FE-Vorhaben des BMU, Nr. 10805017/11. Institut für Haustierkunde, Universität Kiel, Kiel, Germany.
4. Benke, H., and U. Siebert. 1995. The current status of harbour porpoises (*Phocoena phocoena*) in German waters, p. 0-23. Internationale Walfangkommission, Dublin, SC/47/SM49.
5. Dixon, W. J. 1993. BMDP statistical software manual, vol. 1 and 2 University of California Press, Berkeley.
6. Eisensmith, S. P. 1993. PlotIt-Graphics and Statistics. Scientific Programming Enterprises, Haslett, Mich.
7. Hahn, G., and A. Tolle. 1979. Ergebnisse aus der Streptokokkenzentrale in Kiel von 1965-1977—ein Überblick. *Zentbl. Bakteriol. Hyg. Abt. 1* **244**:427-428.
8. Hammond, P. S., H. Benke, P. Berggren, D. L. Borchers, S. T. Buchland, A. Collet, M.-P. Heide-Jørgensen, S. Heimlich-Boran, A. R. Hiby, M. P. Leopold, and N. Oien. 1995. Distribution and abundance of the harbour porpoise and other small cetaceans in the North Sea and adjacent waters. *Life 92-2/UK/027*. Final report to the European Commission, October 1995. European Commission, Brussels, Belgium.
9. Hinterdorfer, F., J. Köfer, and G. Hahn. 1990. Streptokokkenisolate aus Untersuchungsmaterial von Schweinen in der Steiermark. *Wien. Tierarztl. Monshr.* **77**:161-163.
10. Mantovani, A., R. Restani, D. Sciarra, and P. Simonella. 1961. Streptococcus L infection in the dog. *J. Small Anim. Pract.* **2**:185-194.
11. Maslow, J. N., A. M. Slutsky, and R. D. Arbeit. 1993. Application of pulsed-field gel electrophoresis to molecular epidemiology, p. 563-572. *In* D. H. Persing, T. F. Smith, F. C. Tenover, and T. J. White (ed.), *Diagnostic molecular microbiology*. American Society for Microbiology, Washington, D.C.
12. Sanches, S., M. Ramirez, H. Troni, M. Abecassis, M. Padua, A. Tomasz, and H. de Lencastre. 1995. Evidence for the geographic spread of a methicillin-resistant *Staphylococcus aureus* clone between Portugal and Spain. *J. Clin. Microbiol.* **17**:1243-1246.
13. Schaufuß, P., C. Lämmle, B. Niewerth, and H. Blobel. 1987. Properties of L-streptococci in comparison with those of A-streptococci. *Med. Microbiol. Immunol.* **176**:169-173.
14. Schwarz, S., and B. Liebisch. 1994. Pulsed field gel electrophoretic identification of *Salmonella enterica* serovar *typhimurium* live vaccine strain Zoosoral H. Lett. *Appl. Microbiol.* **19**:469-472.
15. Siebert, U., and K. Frese. 1993. Endbericht über das Teilprojekt „Patholo-

- gisch-anatomische und histopathologische Untersuchungen an Kleinwalen“, p. 1–23. In H. Bohlken, H. Benke, and J. Wulf (ed.), Untersuchungen über Bestand, Gesundheitszustand und Wanderungen der Kleinwalpopulationen (*Cetacea*) in deutschen Gewässern. Endbericht zum FE-Vorhaben des BMU, Nr. 10805017/11. Institut für Haustierkunde der Universität Kiel, Kiel, Germany.
16. Siebert, U., H. Benke, E. Schulze, and R. P. Sonntag. 1996. Über den Zustand der Kleinwale, p. 242–248. In J. L. Lozen, R. Lampe, W. Mattäus, E. Rachor, H. Rumohr, and H. von Westernhagen (ed.), Warnsignale aus der Ostsee. Parey Buchverlag, Berlin, Germany.
 17. Siebert, U., R. Lick, R. Weiss, H. Frank, H. Benke, and K. Frese. 1996. Post-mortem findings in small cetaceans from German waters of the North and Baltic seas, p. 1–7. In Proceedings of the First Scientific Meeting of the European Association of Zoo- and Wildlife Veterinarians (EAZWV), Rostock, Germany.
 18. Sippel, K., B. Dülffer-Schneitzer, and C. Lämmler. 1995. Characteristic properties of streptococci of serological group L. J. Vet. Med. B. **42**:42–50.
 19. Skovgaard, N. 1967. Undersogelse vedrorende forekomst og oprindelse af Gr. L-streptokokker og stafylokokker i levnedsmidler. Nord. Veterinaermed. **6**:240–248.
 20. Soedarmanto, I., R. Oliveira, C. Lämmler, and H. Dürrling. 1997. Identification and epidemiological relationship of *Rhodococcus equi* isolated from cases of lymphadenitis in cattle. Zentbl. Bakteriol. **286**:457–467.
 21. Soedarmanto, I., F. H. Pasaribu, I. W. T. Wibawan, and C. Lämmler. 1996. Identification and molecular characterization of serological group C streptococci isolated from diseased pigs and monkeys in Indonesia. J. Clin. Microbiol. **34**:2201–2204.
 22. Soedarmanto, I., S. Schwarz, B. Liebisch, and C. Lämmler. 1995. Tetracycline resistance determinants among streptococci of serological group G and L. Vet. Microbiol. **45**:331–337.
 23. Tenover, F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelsen, B. E. Murray, D. H. Persing, and B. Swaminathan. 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. **33**:2233–2239.
 24. Thal, E., and K. Moberg. 1953. Serologische Gruppenbestimmung der bei Tieren vorkommenden β -hämolyisierenden Streptokokken. Nord. Veterinaermed. **5**:835–846.
 25. Thiele, D., H. Willems, G. Köpf, and H. Krauss. 1993. Polymorphism in DNA restriction patterns of *Coxiella burnetii* isolates investigated by pulsed-field gel electrophoresis and image analysis. Eur. J. Epidemiol. **29**:419–425.
 26. Vandamme, P., B. Pot, E. Falsen, K. Kersters, and L. A. Devriese. 1996. Taxonomic study of Lancefield streptococcal groups C, G and L (*Streptococcus dysgalactiae*) and proposal of *S. dysgalactiae* subsp. *equisimilis* subsp. nov. Int. J. Syst. Bacteriol. **46**:774–781.