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Characterization of bovine and human group B streptococci isolated in Turkey

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In the study, group B streptococci (GBS) isolated from bovines and humans in and around Van, eastern Turkey, were serotyped, and their haemagglutination and lectin-agglutination properties were also determined. This study is the first epidemiological survey of GBS serotypes performed in Turkey. A total of 148 GBS isolates, 76 from bovine milk and 72 from women attending a maternity polyclinic, were examined by co-agglutination, slide haemagglutination and slide lectin-agglutination tests. By the co-agglutination test, 34 (44·7 %) of bovine isolates and 49 (68 %) of human isolates could be serotyped. In bovine isolates, type VII (11·8 %), III (10·5 %), Ic (6·5 %) and VIII (3·9 %) were the most frequently detected serotypes. The most frequent human serotypes were Ic (33·3 %), IV (8·3 %), VIII (6·9 %), V (5·5 %) and R (5·5 %). In the haemagglutination test using rabbit erythrocytes, 23 (33·3 %) bovine and 15 (23·4 %) human isolates were found to be positive. The bovine GBS isolates showed a significant positive agglutination reaction with *Dolichos biflorus* lectin (30·4 %), whereas the human GBS isolates were found to be positive for *Arachis hypogea* (18·8 %) and *Canavalia ensiformis* (37·5 %) lectins. The treatment of GBS with trypsin was also found to be important for the demonstration of the haemagglutination and lectin-agglutination properties of GBS. The results of the study provide data on serotype distribution and the formulation of a possible GBS vaccine in Turkey, and the lectin-agglutination tests may also be useful for differentiating bovine and human GBS strains.

INTRODUCTION

Group B streptococci (GBS) are a major cause of human neonatal infection and bovine contagious mastitis (Finch & Martin, 1984; Schuctat & Wenger, 1994). Although a wide variety of micro-organisms have been implicated as causative agents of bovine mastitis, the isolation rate of streptococci is high (13–34 % of all cases), and GBS (10–58 %) form the greatest proportion of the streptococcal isolates. The prevalence of infection with GBS can reach 44 % in infected herds (Keefe, 1997). Until three decades ago, GBS were a well-known cause of bovine mastitis, leading to economic losses in the dairy industry. Clinicians also focused attention on the organism, which emerged as a major pathogen in purulent meningitis (early onset and late onset) in newborns as well as adults (Schuctat & Wenger, 1994). In adults, the prevalence of GBS is higher in certain groups, such as pregnant and postpartum women. The prevalence of GBS colonization in different populations ranges from 5 %, to over 35 % in pregnant women (Koneman *et al.*, 1997).

Traditionally, the classification and identification of streptococci from clinical and veterinary sources have been based on the serogrouping and serotyping of the carbohydrate and protein antigens of the cell walls by the method of Lancefield (Devriese, 1991). Up to now, GBS have been classified according to nine known major polysaccharide antigens (Ia, Ib, II, III, IV, V, VI, VII and VIII) and three protein antigens (Ic, R and X) (Jelinkova, 1977; Spellerberg, 2000; Shet & Ferrieri, 2004).

Bacterial adherence to host cells appears to be a multifactorial phenomenon involving specific as well as non-specific interactions. Structures that are thought to be responsible for bacterial adhesion include fimbriae and non-fimbrial adhesins, such as lipoteichoic acid and proteins. The ability of bacteria to attach to and agglutinate erythrocytes may be used *in vitro* as a model to study host–bacterium interaction and the mechanism of attachment (Kurl *et al.*, 1989; Wibawan *et al.*, 1993).

Lectins are ubiquitous, found in animals, plants and micro-organisms. A lectin usually contains two or more binding sites for carbohydrate units; some lectins contain oligomeric structures with multiple binding sites (Ottensooser *et al.*, 1974). Because lectins are able to bind to antibodies and thereby inhibit competitively the reaction between cellular antigen and antibody, they have been used in some typing studies (Niewerth, 1987; Slifkin & Cumbie, 1987; Kellens *et al.*, 1993; Munoz *et al.*, 1994).

Abbreviation: GBS, group B streptococci.

This study was performed to demonstrate the epidemiological importance of serotype distribution and the haemagglutination and lectin-agglutination activities of bovine and human GBS strains isolated in and around Van, Turkey.

METHODS

GBS strains. A total of 148 GBS cultures were examined; 76 and 72 strains, respectively, were isolated from bovine milk samples, and from vaginal samples of pregnant and non-pregnant women from maternity clinics.

Reference strains. The reference strains *Streptococcus agalactiae* serotype Ia (090), Ib (H 36 B), Ic (A 909), II (18 RS 21), III (6313), IV (3139), V (SS 1169), VII (7271), VIII (JM9 130013), R (25/60 Compton), X (24/60 Compton) and *Staphylococcus aureus* Cowan I (NCTC 8530) were kindly provided by Professor Dr Christoph Lämmler, Institut für Pharmakologie und Toxikologie Fachbereich Veterinärmedizin, Justus Liebig Universität.

Serogrouping. The cultures were grown overnight on Blood Agar Base plates (Oxoid) with 5 % sheep blood. Serological grouping of isolates was performed with a commercial group B latex agglutination kit (Avipath-Strep, Omega Diagnostics) according to the manufacturer's instructions.

Serotyping. Each reference GBS serotype culture was grown overnight in 300 ml Todd–Hewitt broth (THB) and centrifuged (10 000 ***g***, 15 min); the bacterial pellet was suspended in one-tenth of the original volume of culture medium in 0·2 M PBS (pH 7·6). The suspensions were inactivated at 60 °C for 30–60 min in a water bath. After a sterility control, the inoculum suspensions were stored at 4 °C.

Type-specific antisera were prepared in rabbits by the intravenous injection of heat-killed suspensions of reference GBS serotype Ia, Ib, Ic, II, III, IV, V, VII, VIII, R and X antigens. After immunization, polyspecific antisera were tested for homologous and heterologous antibodies. Type-specific antisera were obtained by the adsorption of the polyspecific antisera with each cross-reactive reference GBS serotype culture (Mosabi *et al.*, 1997; Jelinkova, 1977; Ainsworth & Capley, 1986).

A staphylococcal co-agglutination test was used for serotyping the isolates. The test was performed, with minor modifications, according to the procedure described by Christensen *et al.* (1973).

Haemagglutination test. The human and bovine GBS isolates were cultivated in 10 ml THB for 18 h at 37 °C. The cultures were washed three times in 0·002 M PBS (pH 6·8) and suspended in 400 µl of the same buffer. The suspension was aliquoted into two portions. One portion was pretreated with trypsin (5 µg per 200 µl bacterial suspension) for 1 h at 37 °C, washed, and resuspended in the initial volume of PBS (Swenshon *et al.*, 1998); the other portion was not treated with trypsin.

Haemagglutination tests were carried out with 20 µl of rabbit erythrocytes (2 %) and 20 µl of culture (trypsinized or non-trypsinized) on microscope slides. The suspensions were mixed and the slides were rotated gently, and within 30 s the haemagglutination was recorded as strong agglutination (++), agglutination (+) or no agglutination (−) (Wibawan *et al.*, 1993; Korhonen & Finne, 1985).

Lectin-agglutination tests. The isolates were grown in 10 ml THB for 18 h at 37 °C. After centrifugation, the pellet was washed three times with 0·05 M PBS (pH 7·5) and resuspended in 400 µl PBS. The suspensions were divided into two portions; one was pretreated with a trypsin (5 µg per 200 µl bacterial suspension) for 1 h at 37 °C. The suspension was washed three times with PBS and resuspended in the initial volume of PBS (Schaefer *et al.*, 1979; Swenshon *et al.*, 1998).

The lectins from *Arachis hypogea* (Sigma L 0881), *Canavalia ensiformis* (Sigma L 7647), *Dolichos biflorus* (Sigma L 2785), *Helix pomatia* (Sigma L 3382) and *Triticum vulgaris* (Sigma L 9640) were prepared in 0·05 M PBS according to the manufacturer's instructions.

For lectin-agglutination tests, 20 µl of the lectin preparation was mixed with 20 µl of streptococcal suspension on microscope slides. The slides were rotated gently and examined for an agglutination reaction within 1 min; the reaction was recorded as strong agglutination (++), agglutination (+) or no agglutination (−) (Schaefer *et al.*, 1979; Motlova *et al.*, 1986; Swenshon *et al.*, 1998).

Statistical evaluation. The chi square test was employed to test for association.

RESULTS AND DISCUSSION

In this study, the prevalence of GBS serotypes from bovine milk and human vaginal specimens was determined, and the haemagglutination and lectin-agglutination properties of GBS were also determined and evaluated. This study is the first report on the distribution of bovine and human GBS serotypes from Turkey.

Serotyping results

Thirty-four (44·7 %) of the bovine and 49 (68 %) of the human GBS isolates could be serotyped by the co-agglutination test. The serotype distributions of both bovine and human GBS isolates are shown in Table 1. GBS serotype VII and III polysaccharide antigens could only be detected in bovine GBS isolates, whereas GBS serotype Ic, IV, V and R antigens were mostly found in human GBS isolates. GBS serotype VIII was detected in both bovine and human GBS isolates. Only one bovine GBS isolate had GBS serotype X antigens. The combined antigenic patterns displayed by bovine GBS isolates were II, IV; II, X; II, IV, X; and IV, R; whereas human GBS isolates showed the combined antigenic patterns III, V, VII; and VII, VIII (Table 1).

It has been reported that human GBS isolates are more readily typable (69–100 %) than those of bovine origin, which are less readily typable (47–85 %) (Finch & Martin, 1984; Pasaribu *et al.*, 1985; Mosabi *et al.*, 1997). In agreement with these reports, the results of the present study revealed that 68 % of the human isolates and 44·7 % of the bovine isolates were typable, mostly as single serotypes or as combinations of two or more serotypes by the co-agglutination test.

Previous reports have shown a relative heterogeneity in the distribution of different serotypes of bovine and human isolates in diverse geographic areas (Bopp & Lämmler, 1995; Mosabi *et al.*, 1997; Duarte *et al.*, 2004). It has been reported that the Ia, II, III, V and X antigens are commonly observed in GBS strains isolated from bovine milk (Finch & Martin, 1984; Pasaribu *et al.*, 1985; Kutschke, 1986; Mosabi *et al.*,

Table 1. Serotype distribution of 76 bovine and 72 human GBS isolates

Values show the number of typable strains; the percentage of typable strains is given in parentheses.

Serotype	Culture source	
	Bovine	Human
Ia	–	–
Ib	–	–
Ic	5 (6·5)	24 (33·3)
II	1 (1·3)	–
III	8 (10·5)	–
IV	1 (1·3)	6 (8·3)
V	2 (2·6)	4 (5·5)
VII	9 (11·8)	–
VIII	3 (3·9)	5 (6·9)
R	–	4 (5·5)
X	1 (1·3)	–
II, IV	1 (1·3)	–
II, IV, X	1 (1·3)	–
II, X	1 (1·3)	–
IV, R	1 (1·3)	–
III, V, VII	–	2 (2·7)
VII, VIII	–	4 (5·5)
Number of typable strains	34 (44·7)	49 (68)
Number of non-typable strains	42 (52·3)	23 (32)

1997; Duarte *et al.*, 2004), whereas Ia, Ib, Ic, II, III, IV and V antigens are found in GBS from human vaginal specimens (Jelinkova, 1977; Schuctat & Wenger 1994; Berg *et al.*, 2000; Spellerberg, 2000; Ko *et al.*, 2001). In the present study, GBS serotype III and VII were found to be the most frequent serotypes in bovine isolates, but not in human isolates. The importance of the invasive GBS serotype III strains among human isolates is well known (Berg *et al.*, 2000; Ko *et al.*, 2001). GBS serotype III has been incriminated as the major colonizing strain in pregnant women (Finch & Martin, 1984; Berg *et al.*, 2000; Moyo *et al.*, 2002).

These findings showed that bovine milk could be regarded as a risk factor, and that it could play an important role in transmission, especially of invasive GBS serotype III strains from bovine to human.

Harrison *et al.* (1995) have reported that the isolation rate of GBS serotype V has increased from 2·6 to 20 % in human samples. In the present study, serotype V was frequently found in the human isolates.

These results could be considered as the basis of an epidemiological marker for use in Turkey. The distribution of GBS serotypes can vary in different geographical regions, and this may be an important characteristic for the development of vaccine and treatment strategies.

Haemagglutination test results

Because seven (9·2 %) of 76 bovine and eight (11·1 %) of 72 human GBS strains showed auto-agglutination, these strains could not be used in both haemagglutination and lectin-agglutination tests. Of the 69 bovine GBS strains, eight (11·6 %) agglutinated rabbit erythrocytes, but 23 (33·3 %) gave a positive reaction after trypsin treatment. On the other hand, none of the human GBS strains agglutinated rabbit erythrocytes, although 15 (23·4 %) human GBS strains were positive after trypsin treatment (Table 2). The difference between bovine and human GBS strains with respect to haemagglutination properties without trypsin treatment was found to be statistically significant ($P<0·05$). In addition, comparable differences were observed between the typable and non-typable human GBS strains after treatment with trypsin.

It has been reported that the adherence to erythrocytes that leads to haemagglutination is a common property of streptococci of serological group G. Wibawan *et al.* (1993)

Table 2. Comparison of haemagglutination test results of examined GBS cultures

ST, serotypable strain; NT, non-serotypable strain.

Culture source	Typability	*n**	Haemagglutination activity (%)	
			Non-trypsinized culture	Trypsinized culture
Bovine	ST	29	6 (20·7)	9 (31)
	NT	40	2 (5)	14 (35)
	Total	69	8 (11·6)†	23 (33·3)
Human	ST	45	0 (0)	4 (8·8)‡
	NT	19	0 (0)	11 (58)‡
	Total	64	0 (0)†	15 (23·4)

*Seven bovine and eight human cultures showed autoagglutination and were not evaluated.

†Significant differences between hosts.

‡Significant differences between ST and NT.

Table 3. Lectin-agglutination test results of examined GBS cultures

Values show the number of isolates that showed lectin agglutination; the percentage of isolates is given in parentheses.

Culture source	Typability	*n**	Lectin				
			Arachis hypogea	*Canavalia ensiformis*	*Dolichos biflorus*	*Helix pomatia*	*Triticum vulgaris*
Bovine	ST	29	1 (3·4)	4 (13·8)	7 (24·1)	6 (20·7)	8 (27·6)
	NT	40	3 (7·5)	4 (10)	14 (35)	6 (15)	12 (30)
	Total	69	4 (5·8)†	8 (11·6)†	21 (30·4)†	12 (17·4)	19 (27·5)
Human	ST	45	8 (17·8)	20 (44·4)‡	2 (4·4)	4 (8·8)‡	8 (17·8)
	NT	19	4 (21·1)	4 (21·1)‡	4 (21·1)	9 (47·4)‡	1 (5·3)
	Total	64	12 (18·8)†	24 (37·5)†	6 (9·4)†	13 (20·3)	9 (14·1)

*Seven bovine and eight human cultures showed autoagglutination and were not evaluated.
†Significant differences between hosts.
‡Significant differences between ST and NT.

have indicated that 43·4 % of bovine GBS isolates, but no human isolates, have haemagglutination activity. The results of other studies have also revealed that bovine GBS isolates display this activity, although to different extents, and that human strains do not. In the present study, 11·6 % of bovine GBS, but no human GBS cultures, agglutinated rabbit erythrocytes. However, 33·3 % of bovine and 23·4 % of human GBS isolates showed a positive haemagglutination reaction after treatment of the cultures with trypsin. As indicated in Table 2, the haemagglutinating properties also appeared not to be directly related to the occurrence of the type antigens, because haemagglutination was observed among both typable and non-typable GBS strains.

In contrast to our results, Kurl *et al.* (1989) have reported that one of 130 human GBS isolates shows haemagglutination activity, and that haemagglutination properties do not increase, even if the cultures are treated with trypsin. Although no report is available about this mechanism, changes in the adhesion activities of GBS cultures treated with trypsin may occur much less under *in vitro* conditions than in the infectious process *in vivo* (Kurl *et al.*, 1989; Wibawan *et al.*, 1993).

Lectin-agglutination test results

Lectins are glycoproteins, which combine specifically with defined sugar structures on the cell wall. This has often been applied to the identification and characterization of microorganisms, exploiting the specificity of binding to cell surface sugars (Ottensooser *et al.*, 1974; Slifkin & Cumbie, 1987; Kellens *et al.*, 1993). In the present study, none of the GBS strains agglutinated with the lectins used. However, bovine and human GBS strains did agglutinate with lectins when the bacteria were treated with trypsin, as shown in Table 3. In agreement with our findings, Kellens *et al.* (1993) have reported that of 95 untreated β-haemolytic streptococci, none gave a visible reaction with any of the lectins tested, whereas 42 strains agglutinated with one or more lectin when the cells were treated with trypsin. The results suggest that certain adhesins are exposed after the bacteria have been treated with trypsin.

In contrast to earlier reports (Slifkin & Gil, 1983; Schaufuss *et al.*, 1986), 30·4 % of bovine GBS strains showed significant agglutination with *Dolichos biflorus* lectin, whereas 18·8 and 37·5 % of human GBS strains showed significant agglutination reactions with *Arachis hypogea* and *Canavalia ensiformis* lectins, respectively. On the other hand, the agglutination reaction with *Triticum vulgaris* lectin was observed in both bovine (27·5 %) and human (14·1 %) GBS strains, as reported elsewhere (Niewerth, 1987). Additionally, there were no significant differences between typable and non-typable bovine strains of GBS with respect to their lectin-agglutination reactions. However, typable strains of human GBS gave a significant reaction with *Canavalia ensiformis* lectin, whereas non-typable strains showed significant agglutination with *Helix pomatia* lectin.

As reported elsewhere (Slifkin & Gil, 1983; Niewerth, 1987), the *Dolichos biflorus* lectin-agglutination reaction is especially recommended for rapid identification of group C streptococci. In the present study, comparable results were also observed with bovine GBS strains. This relationship could be based on the cross-reactive components of C-polysaccharide antigens of both GBS and group C streptococci. Although the results of the present study indicated that the pattern of lectin-agglutination reactions might be characteristic of bovine and human GBS, it could not be safely used for rapid identification of GBS or differentiation of streptococci.

In this first report, the serotype distribution of GBS isolates in Turkey was found to be similar to those of GBS isolated in various regions of the world. The results of the study indicated also that the treatment of GBS with trypsin is important for the demonstration of the haemagglutination and lectin-agglutination properties of GBS. The lectin-agglutination tests used in the study could be useful for differentiating bovine and human GBS strains, but not for

identification of GBS strains. Further surveillance of GBS strains is important to provide data on serotype distribution, the formulation of a possible GBS vaccine, antibiotic prophylaxis and treatment recommendations for Turkey.

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REFERENCES

Ainsworth, A. J. & Capley, G. (1986). Monoclonal antibodies produced to *Streptococcus agalactiae*. *Am J Vet Res* **47**, 1211–1213.

Berg, S., Trollfors, B., Lagergard, T., Zackrisson, G. & Claesson, A. (2000). Serotypes and clinical manifestations of group B streptococcal infections in Western Sweden. *Clin Microbiol Infect* **6**, 9–13.

Bopp, V. & Lämmler, C. (1995). Comparative studies on group-B streptococci isolated from bovine milk samples in Thuringia and Hesse. *Zentralbl Veterinarmed B* **42**, 427–433.

Christensen, P., Kahlmeter, G., Jonsson, S. & Kronvall, G. (1973). New method for the serological grouping of streptococci with specific antibodies adsorbed to protein A-containing staphylococci. *Infect Immun* **7**, 881–885.

Devriese, L. A. (1991). Streptococcal ecovars associated with different animal species: epidemiological significance of serogroups and biotypes. *J Appl Bacteriol* **71**, 478–483.

Duarte, R. S., Miranda, O. P., Bellei, B. C., Brito, M. A. V. P. & Teixeira, L. M. (2004). Phenotypic and molecular characteristics of *Streptococcus agalactiae* isolates recovered from milk of dairy cows in Brazil. *J Clin Microbiol* **42**, 4214–4222.

Finch, L. A. & Martin, D. R. (1984). Human and bovine group B streptococci: two distinct populations. *J Appl Bacteriol* **57**, 273–278.

Harrison, L. H., Dwyer, D. M. & Johnson, J. A. (1995). Emergence of serotype V group B streptococcal infection among infants and adults. *J Infect Dis* **171**, 513.

Jelinkova, J. (1977). Group B streptococci in the human population. *Curr Top Microbiol Immunol* **76**, 127–165.

Keefe, G. P. (1997). *Streptococcus agalactiae* mastitis: a review. *Can Vet J* **38**, 429–437.

Kellens, J. T., Jacobs, J. A., Peumans, W. J. & Stobberingh, E. E. (1993). The agglutination of beta-haemolytic streptococci by lectins. *J Med Microbiol* **39**, 440–445.

Ko, W. C., Lee, H. C., Wang, L. R., Lee, C. T., Liu, A. J. & Wu, J. J. (2001). Serotyping and antimicrobial susceptibility of group B streptococcus over an eight-year period in southern Taiwan. *Eur J Clin Microbiol Infect Dis* **20**, 334–339.

Koneman, E. W., Allen, S. D., Dowell, V. R., Janda, W. M., Schreckenberger, P. C. & Winn, W. C. (1997). The Gram-positive cocci, part II: streptococci, enterococci and streptococcus-like bacteria. In *Color Atlas and Text Book of Diagnostic Microbiology*, 5th edn, pp. 577–629. Philadelphia, New York: Lippincott.

Korhonen, T. K. & Finne, J. (1985). Agglutination assays for detecting bacterial binding specificities. In *Enterobacterial Surface Antigens: Methods for Molecular Characterization*, pp. 301–313. Edited by T. K. Korhonen, E. A. Dawes & P. H. Mäkelä. Amsterdam: Elsevier.

Kurl, D. N., Haataja, S. & Finne, J. (1989). Hemagglutination activities of group B, C, D and G streptococci: demonstration of novel sugar-specific cell-binding activities in *Streptococcus suis*. *Infect Immun* **57**, 384–389.

Kutschke, V. C. (1986). Zur serologischen Typendifferenzierung von Streptokokken der Gruppe B. *Monatsh Vetmed* **41**, 227–228.

Mosabi, J. M., Arimi, S. M. & Kangethe, E. K. (1997). Isolation and characterization of group B streptococci from human and bovine sources within and around Nairobi. *Epidemiol Infect* **118**, 215–220.

Motlova, J., Wagner, M. & Jelinkova, J. (1986). A search for new group B streptococcal serotypes. *J Med Microbiol* **22**, 101–105.

Moyo, S. R., Maeland, J. A. & Bergh, K. (2002). Typing of human isolates of *Streptococcus agalactiae* (group B streptococcus, GBS) strains from Zimbabwe. *J Med Microbiol* **51**, 595–600.

Munoz, A., Lopez, A. & Llovo, J. (1994). Lectin typing of beta-haemolytic streptococci of groups A and B. *J Med Microbiol* **41**, 324–328.

Niewerth, B. (1987). *Wechselwirkungen zwischen Gram-positiven Bakterien, Lektinen und Körperzellen*. Dissertation, Fachbereich Veterinärmedizin der Justus Liebig Universität, Gießen.

Ottensooser, F., Nakamizo, Y., Sato, M., Miyamoto, Y. & Takizawa, K. (1974). Lectin detecting group C streptococci. *Infect Immun* **9**, 971–973.

Pasaribu, F. H., Lämmler, C. & Blobel, H. (1985). Serotyping of bovine and human group B streptococci by coagglutination. *IRCS (Int Res Commun Syst) Med Sci* **13**, 24–25.

Schaefer, R. L., Keller, K. F. & Doyle, R. J. (1979). Lectins in diagnostic microbiology: use of wheat germ agglutinin for laboratory identification of *Neisseria gonorrhoeae*. *J Clin Microbiol* **10**, 669–672.

Schaufuss, P., Lämmler, C. & Blobel, H. (1986). Rapid differentiation of streptococci isolated from cows with mastitis. *J Clin Microbiol* **24**, 1098–1099.

Schuctat, A. & Wenger, J. D. (1994). Epidemiology of group B streptococcal disease. *Epidemiol Rev* **16**, 374–402.

Shet, A. & Ferrieri, P. (2004). Neonatal & maternal group B streptococcal infections: a comprehensive review. *Indian J Med Res* **120**, 141–150.

Slifkin, M. & Cumbie, R. (1987). Identification of Group B streptococcal antigen with lectin-bound polystyrene particles. *J Clin Microbiol* **25**, 1172–1175.

Slifkin, M. & Gil, G. M. (1983). Rapid biochemical tests for identification of group A, B, C, F and G streptococci from throat cultures. *J Clin Microbiol* **18**, 29–32.

Spellerberg, B. (2000). Pathogenesis of neonatal *Streptococcus agalactiae* infections. *Microbes Infect* **2**, 1733–1742.

Swenshon, M., Lämmler, C. & Siebert, U. (1998). Identification and molecular characterization of beta-hemolytic streptococci isolated from harbour porpoises (*Phocoena phocoena*) of the North and Baltic Seas. *J Clin Microbiol* **36**, 1902–1906.

Wibawan, I. W. T., Lämmler, C., Seleim, R. S. & Pasaribu, F. H. (1993). A haemagglutinating adhesin of group B streptococci isolated from cases of bovine mastitis mediates adherence to HeLa cells. *J Gen Microbiol* **139**, 2173–2178.