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Establishment of streptococci in the upper respiratory tract: longitudinal changes in the mouth and nasopharynx up to 2 years of age

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As part of a series of longitudinal studies on the development of the indigenous microflora of the upper respiratory tract, the establishment of streptococci in the oral cavity and nasopharynx and IgA1 protease production by the early streptococcal flora was examined in 50 healthy Caucasian infants at the ages of 2, 6, 12, 18 and 24 months. In the oral cavity, streptococci were found in all infants on every sampling occasion, *Streptococcus mitis* biovar 1 being the main finding in each age group. *S. salivarius* and *S. mitis* biovar 2 reached their highest prevalence during the first year of life, whereas the prevalence of *S. oralis* and *S. sanguis* showed no significant increase before 12 months of age. Salivary streptococci mainly consisted of the above-mentioned species during the follow-up period. In contrast to the oral cavity, no stable colonisation pattern was observed for viridans streptococci in the nasopharynx. *S. mitis* biovar 1 and *S. pneumoniae*, a traditional respiratory pathogen, were the principal streptococcal species among nasopharyngeal isolates. IgA1 protease production by early streptococci was common in infancy. Among the oral streptococcal microflora, *S. mitis* biovar 1 (especially during the first year of life) and *S. oralis* and *S. sanguis* constituted the main species responsible for this enzyme activity. In the nasopharynx, IgA1 protease was produced by *S. mitis* biovar 1, *S. oralis* and *S. pneumoniae*. In conclusion, streptococcal colonisation differs in these two close habitats in the upper respiratory tract.

Introduction

The genus *Streptococcus* may be divided into six major clusters based on 16S rRNA gene sequencing: anginosus group, bovis group, mitis group, mutans group, pyogenic group and salivarius group. Each of these six groups contains several phylogenetically close species [1]. Viridans streptococci, including the anginosus, mitis, mutans and salivarius groups, are considered to be principal members of the indigenous microflora in the upper respiratory tract.

Viridans streptococci are the primary early colonisers in the oral cavity and are present during the first days of life [2, 3]. Different reports on predentate infants have suggested that *Streptococcus salivarius*, *S. mitis* biovar 1, *S. mitis* biovar 2 or *S. oralis* are the predominant streptococcal species in infancy [2–5]. Soon after teeth erupt, *S. sanguis* [4, 6, 7] and, later, mutans streptococci [8], become established in the mouth. Recent studies on the development of the early microflora [9, 10] indicate that the acquisition and succession of different bacterial species in the oral cavity is a selective process in terms of the age at which infants are susceptible to colonisation. In this respect, only fragmentary longitudinal data on oral streptococci [2–4, 6, 7] and no data on the streptococcal colonisation pattern in the nasopharynx appear to exist in the literature.

The nasopharyngeal microflora has mainly been examined as the reservoir of potential respiratory pathogens. Indeed, except for *S. pneumoniae* (pneumococcus) limited data exist on the colonisation of other streptococcal species in the nasopharynx, although α-haemolytic streptococci are frequently mentioned as primary inhabitants of the pharyngeal microflora and

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play an important role in interfering with the establishment of respiratory pathogens [11, 12]. The current information on nasopharyngeal colonisation is not clear, because of unspecified sampling site (nasopharynx or oropharynx) and lack of detailed identification of the streptococci isolated.

Bacterial adherence to mucosal surfaces is the first step in the colonisation of the upper respiratory tract. Therefore, inhibition of adherence is the major mechanism in the host immune regulation of colonisation. The main protective antibody on mucosal surfaces of the upper respiratory tract is secretory immunoglobulin A (S-IgA). The subclass S-IgA1 dominates in oral and nasopharyngeal secretions as well as in maternal milk [13, 14]. Interestingly, some viridans streptococci belonging to the mitis group are capable of producing IgA1 protease that specifically cleaves antibody molecules of the IgA1 subclass [15]. All strains of *S. pneumoniae*, *S. oralis* and *S. sanguis* produce this enzyme, whereas activity varies among populations of *S. mitis* biovar 1 [16, 17]. Protease activity may aid the colonisation of streptococcal strains on mucosal surfaces despite the presence of IgA1. Interestingly, high numbers of streptococci that are capable of producing IgA1 protease have been reported to be present in infants' mouths [18, 19].

The aim of the present study was to examine the establishment of streptococci during the first 2 years of life in two habitats of the upper respiratory tract, i.e., the oral cavity and the nasopharynx, and the distribution of IgA1 protease-producing streptococci in relation to age.

Material and methods

Subjects

The study population consisted of 50 consecutive infants who were 2 months old at baseline (23 boys, 27 girls) and originated from a cohort of 329 healthy, full-term, Caucasian infants participating in the Finnish Otitis Media (FinOM) Cohort Study. The selection of the study infants has been presented in detail previously [9]. Before enrolment of the infants in the study, written informed consent was obtained from their parent(s). The study protocols were approved by the Ethical Issues' Committees of the National Public Health Institute, Department of Social and Health Care of Tampere City, and Tampere University Hospital.

Bacterial sampling, culture and presumptive identification

Unstimulated saliva was collected from the buccal sulcus area as described previously [9]. Nasopharyngeal samples were taken by inserting a flexible metal-shaft swab with a calcium-alginate tip through the nasal cavity into the nasopharynx (the posterior part of the nasal cavity) and left in place for 5 s. After withdrawal the swab shaft was cut and the tip was placed in VMGA III transport medium [20]. Specimens were sent *via* express mail to the Anaerobe Reference Laboratory of the National Public Health Institute, Helsinki, Finland, where they were processed within 24 h of collection. Samples were available from all 50 infants at 2 months (±2 weeks), 48 infants at 6 months (±2 weeks), 44 and 45 infants (salivary and nasopharyngeal samples, respectively) at 12 months (±4 weeks), 45 infants at 18 months (±4 weeks) and 42 infants at 24 months (±4 weeks) of age. Serially diluted samples were cultured on blood agar containing sheep blood 5% and on chocolate agar for the growth of aerobic and facultative bacteria, on mitis salivarius agar containing bacitracin [21] for the selective isolation of mutans streptococci, and on crystal violet-nalidixic acid-gentamicin agar [22] for the selective isolation of *S. pneumoniae*. The plates were incubated in an aerobic atmosphere with CO_2 5% at 37°C for 2 days. A representative of each distinct colony type detected with a dissecting microscope was isolated and subcultured for presumptive identification, which included Gram's staining, determination of cell morphology, catalase reaction and haemolytic activity. Optochin disk test and commercial API 20 Strep test kits (bioMérieux, Marcy l'Etoile, France) were performed for α-haemolytic and non-haemolytic, catalase-negative, gram-positive cocci. Lancefield serological groups were determined for β-haemolytic streptococci by the commercial Streptex rapid latex test (Murex Biotech, Dartford). All streptococcal isolates were frozen at -70°C for further examination.

Identification of viridans streptococcal isolates

Further species identification of viridans streptococcal isolates was based on the scheme by Kilian *et al.* [15] combined with the scheme of Whiley and Beighton [23]. Subcultures of frozen stocks were made on blood agar for testing of hydrolysis of arginine and aesculin, production of acetoin (Voges-Proskauer test), alkaline phosphatase and β-N-acetylglucosaminidase with individual Rosco Diagnostic Tablets (Rosco, Taastrup, Denmark) and for testing of amygdalin, inulin, mannitol and sorbitol fermentation. Selected reactions were verified with broth media as described previously [15]. The type of haemolysis was confirmed on blood agar plates. To record the colonial morphology and the production of extracellular polysaccharides, subcultures were made on mitis salivarius agar incubated in an anaerobic atmosphere (H_2 5%, CO_2 5%, N_2 90%) at 37°C for 2 days. The production of extracellular polysaccharides, noted as hard coherent colonies on mitis salivarius agar, was used as the key reaction for separation of *S. oralis* from IgA1 protease-producing *S. mitis* biovar 1.

Testing of IgA1 protease production

The streptococcal isolates were subcultured on Todd-Hewitt agar (Difco Laboratories, Detroit, MI, USA) for testing IgA1 protease production as described previously [17]. Briefly, a loopful of bacteria from a 24-h subculture was suspended in 40 μl of IgA1 solution and incubated overnight at 37°C. IgA1 degradation was revealed by immuno-electrophoresis.

Results

A total of 1265 clinical isolates identified as viridans streptococci and 12 reference strains (*S. sanguis* SK-1, *S. oralis* SK-2 and SK-23, *S. gordonii* SK-3, *S. mutans* SK-28, *S. anginosus* SK-52, *S. constellatus* SK-53, *S. intermedius* SK-54, *S. salivarius* SK-56, *S. mitis* SK-142, *S. vestibularis* SK-227 and *S. crista* SK-231) [15] were examined.

The mitis group dominated among the early streptococcal populations of the upper respiratory tract in the infants examined (Table 1). The division of the salivary and nasopharyngeal isolates into different streptococcal species and their proportions at each age group revealed that *S. mitis* biovar 1 dominated among the salivary isolates on all five sampling occasions and among the nasopharyngeal isolates on the first two sampling occasions (Table 2). In addition to *S. mitis* biovar 1, *S. pneumoniae* and *S. oralis* also constituted a large proportion of the nasopharyngeal isolates on the last three and two sampling occasions, respectively. Of 1265 viridans streptococcal isolates tested, 53 (4.2%) isolates remained unidentified. Four separate groups were distinguished among 44 unidentified isolates according to their similar phenotypic profile (data not shown).

The prevalence rates of the five most frequently encountered streptococcal taxa in saliva by 2 years of

Table 1. Prevalence of streptococcal groups in the saliva and nasopharynx of 50 infants during the first 2 years of life

	Percentage of infants with group at									
	2 months (n = 50)*		6 months (n = 48)*		12 months (n = 44/45)*		18 months (n = 45)*		24 months (n = 42)*	
Streptococcal group	S	NP	S	NP	S	NP	S	NP	S	NP
Mitis	98	24	100	60	100	53	100	51	100	38
Salivarius	46	4	69	10	70	11	44	18	60	2
Anginosus	2	4	0	0	2	0	0	0	0	0
Mutans	0	0	0	0	2	0	7	0	5	0
Pyogenic†	0	2	0	4	0	4	0	2	0	0

S, saliva; NP, nasopharynx.
*Number of infants sampled.
†Includes Lancefield group B and G streptococci (group A not detected).

Table 2. Distribution of streptococcal species within the saliva and nasopharynx of 50 infants on five sampling occasions during the first 2 years of life

	Percentage of isolates at									
	2 months		6 months		12 months		18 months		24 months	
Streptococcal group/species	S (n = 171)	NP (n = 21)	S (n = 189)	NP (n = 63)	S (n = 202)	NP (n = 36)	S (n = 218)	NP (n = 61)	S (n = 343)	NP (n = 27)
Mitis group										
S. mitis 1	63.7	38.1	52.9	54.0	46.0	30.6	39.4	24.6	38.8	14.8
S. mitis 2	11.1	9.5	15.3	4.8	9.4	5.6	8.3	9.8	3.5	7.4
S. oralis	2.9	0	2.6	4.8	6.9	5.6	18.3	18.0	16.3	33.3
S. sanguis	0	0	1.1	0	7.9	2.8	10.1	1.6	17.2	0
S. crista	1.2	0	0	0	4.5	0	2.3	0	0.9	0
S. gordonii	0.6	0	0	0	2.0	0	2.3	0	0.3	0
S. parasanguis	1.8	0	1.1	0	0	2.8	0.9	0	0	0
S. pneumoniae	0.6	19.0	0.5	14.3	0.5	33.3	0.9	24.6	1.5	37.0
Salivarius group										
S. salivarius	13.5	9.5	18.0	4.8	15.3	11.1	11.9	14.8	13.7	3.7
S. vestibularis	2.3	0	5.8	1.6	3.5	2.8	1.4	0	1.5	0
Anginosus group	0.6	10.0	0	0	0.5	0	0	0	0	0
Mutans group	0	0	0	0	1.5	0	0.5	0	0.9	0
Pyogenic group	0	4.8	0	3.2	0	5.6	0	1.6	0	0
Unidentified isolates	1.8	9.5	2.6	12.7	2.0	0	3.7	4.9	5.5	3.7

S, saliva; NP, nasopharynx.

age, i.e., *S. mitis* biovar 1, *S. mitis* biovar 2, *S. salivarius*, *S. oralis* and *S. sanguis*, are shown in Fig. 1. The infants were predentate at 2 months, predentate or dentate at 6 months and dentate at 12 months onwards. *S. mitis* biovar 1 was by far the most frequent finding in each age group, being present in the saliva of nearly all infants at each of the five sampling times. *S. salivarius* and *S. mitis* biovar 2 reached their highest prevalence during the first year of life, whereas *S. oralis* and *S. sanguis* showed no significant increase before 12 months of age. Other streptococci had much lower prevalence rates: *S. vestibularis* was found in *c.* 10% of the infants from 2 months onwards and *S. crista* from 12 months onwards. *S. gordonii*, *S. parasanguis*, the anginosus group and mutans streptococci were rarely detected in saliva during the first 2 years of life. *S. pneumoniae* was occasionally isolated from saliva during the follow-up period (Table 2).

Viridans streptococci were isolated from the nasopharyngeal samples relatively frequently, the collective prevalence rates varying between 22% and 56% on different sampling occasions. The prevalence rates of the five streptococcal taxa most frequently isolated from the nasopharynx by 2 years of age, i.e., *S. mitis* biovar 1, *S. mitis* biovar 2, *S. salivarius*, *S. oralis* and *S. pneumoniae*, are shown in Fig. 2. Except for *S. mitis* biovar 1, the prevalence rates of other viridans streptococci remained <20% during the follow-up period. *S. pneumoniae* was the most prevalent streptococcal species after 6 months of age, the prevalence increasing steadily from 8% at 2 months to 33% at 18 months, then decreasing to 24% at 24 months (Fig. 2). One infant had a positive *S. pneumoniae* finding in the nasopharynx on every sampling occasion, but in most cases this species – like other streptococcal species – was found on one occasion only.

Streptococci positive for IgA1 protease were frequently isolated from the upper respiratory tract during early childhood (Table 3). At 2 years, all children harboured IgA1 protease-positive streptococci in their saliva. Due to increasing frequencies of *S. oralis* and *S. sanguis* in the oral cavity with advanced age, more than half of the salivary streptococcal microflora was composed of strains of IgA1 protease-positive streptococci during the second year of life, although *S. mitis* biovar 1 constituted the main streptococcal species responsible for this enzyme activity (Table 3). Within salivary *S. mitis* biovar 1 populations, the proportion of IgA1 protease-positive isolates increased with age, being 55% at 2 and 6 months, 58% at 12 months, 66% at 18 months and 69% at 24 months. In most infants, either IgA1 protease-positive or simultaneously both IgA1 protease-positive and IgA1 protease-non-producing *S. mitis* biovar 1 strains were isolated from saliva. In the nasopharynx, *S. pneumoniae* and *S. mitis* biovar 1 were the main IgA1 protease-positive streptococcal species during the first year of life and, in addition to them, *S. oralis* during the second year of life (Table 3). Strains of IgA1 protease-positive streptococci dominated in the nasopharynx from the age of 6 months onwards.

Discussion

In this longitudinal study, where both predentate and dentate infants were examined, saliva was chosen as an oral specimen, which reflects the streptococcal microflora shedding from different oral surfaces. Another

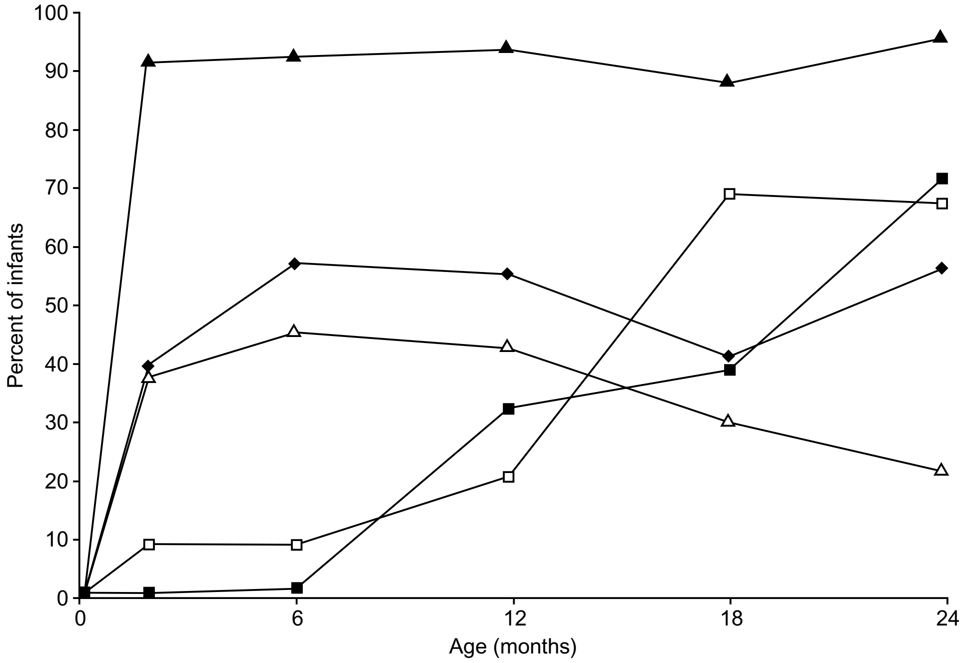

Fig. 1. Prevalence (%) of infants with *S. mitis* biovar 1 (▲), *S. mitis* biovar 2 (△), *S. oralis* (□), *S. salivarius* (◆) and *S. sanguis* (■) in saliva at 2, 6, 12, 18 and 24 months of age.

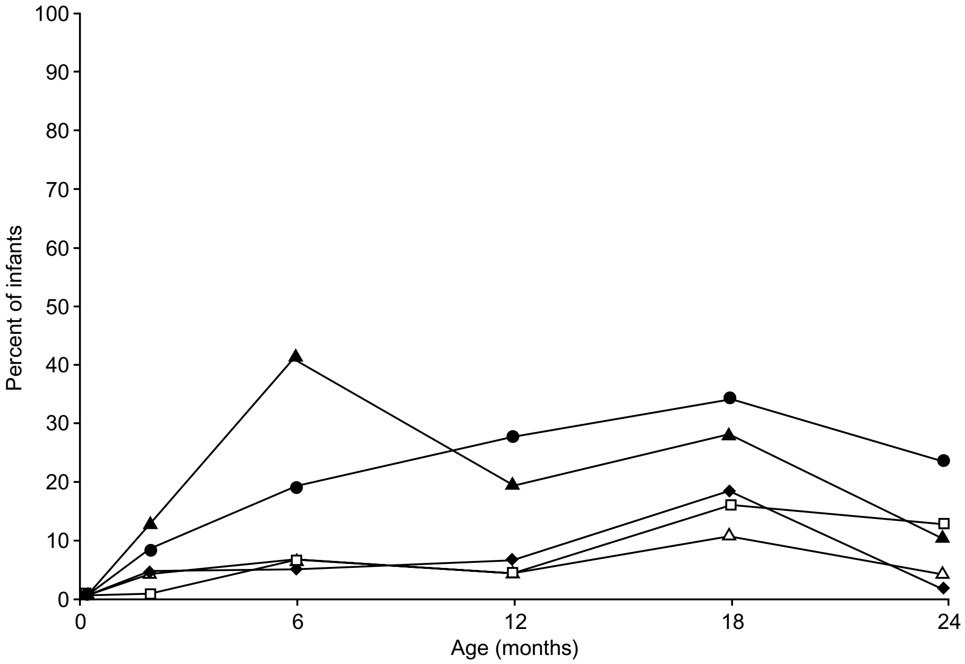

Fig. 2. Prevalence (%) of infants with *S. mitis* biovar 1 (▲), *S. mitis* biovar 2 (△), *S. oralis* (□), *S. salivarius* (◆) and *S. pneumoniae* (●) in the nasopharynx at 2, 6, 12, 18 and 24 months of age.

Table 3. IgA1 protease production by streptococci isolated from the saliva or nasopharynx of infants at five scheduled follow-ups

Age (months)	Number (%) of infants with IgA1pr$^+$ streptococci	Number (%) of IgA1pr$^+$ isolates	Proportion of indicated species among IgA1 pr$^+$ isolates				
			S. mitis 1	*S. oralis*	*S. sanguis*	*S. pneumoniae*	Unidentified species
Saliva							
2	38/50 (76)	71/171 (41.5)	87.3%	7.0%	0	1.4%	4.2%
6	38/48 (79)	67/189 (35.4)	82.1%	7.5%	3.0%	1.5%	6.0%
12	40/44 (91)	84/202 (41.6)	63.1%	16.7%	19.0%	1.2%	0
18	42/45 (93)	129/218 (59.2)	45.0%	31.0%	17.1%	1.6%	5.4%
24	42/42 (100)	221/343 (64.4)	41.6%	25.3%	26.7%	2.3%	4.1%
Nasopharynx							
2	8/50 (16)	8/21 (38.1)	25.0%	0	0	50.0%	25.0%
6	28/48 (58)	45/63 (71.4)	55.6%	6.7%	0	20.0%	17.8%
12	20/45 (44)	22/36 (61.1)	31.8%	9.1%	4.5%	54.5%	0
18	23/45 (51)	35/61 (57.4)	57.4%	31.4%	2.9%	42.9%	2.9%
24	18/42 (43)	24/27 (88.9)	16.7%	37.5%	0	41.7%	4.2%

IgA1pr$^+$, IgA1 protease-positive.

aspect of choosing saliva was its potential role as a transmission vehicle for bacteria between the two study habitats of the upper respiratory tract. Nasopharyngeal samples were collected through the nasal cavity, thus avoiding streptococcal contamination from the oral cavity. Streptococcal isolates were selected with a dissecting microscope on the basis of their colony morphology. This practice may lead to failure to isolate all streptococcal species present in samples. However, the results of the present study are comparable with previous studies [3–5] where a similar isolation technique was used for oral streptococci.

The precise species identification of viridans streptococci is tedious; the main difficulties encountered in the present study were with the mitis group. As in previous studies of oral streptococci in infancy [3, 4], attempts to identify the streptococcal isolates left a small proportion (4.2%) unidentified. Most unidentified isolates formed various groups with similar profiles, indicating that considerable heterogeneity exists among streptococcal populations of the upper respiratory tract in infancy. This observation may be due to the presence of unrecognised species or may reflect a high degree of recombination between native clones and species among streptococcal populations in the immature mouth. Indeed, Kawamura *et al.* [24] described two further species in Japanese infants to be included in the mitis group, namely *S. infantis* and *S. peroris*. These two species can be separated from *S. mitis* biovar 1 only by sequence analysis of 16S rRNA genes. Some heterogeneity also exists among arginine-hydrolysing *S. mitis* biovar 2 populations [15, 25]. Recently, a novel arginine-positive oral streptococcus isolated from

Australian infants, *S. australis*, has been added to the mitis group [26]. An arginine-positive *S. mitis*-like group was observed in the present study; however, it remained unidentified because of the production of IgA1 protease. At present it is not known whether *S. australis* produces this enzyme.

Viridans streptococci become established in the mouth soon after birth; Carlsson *et al.* [2] detected *S. salivarius* from the oral cavity in 75% of infants studied from the second day of life onwards. According to Pearce *et al.* [3], the streptococcal isolates from oral mucosal surfaces in infants during the first 3 days comprised mainly *S. oralis*, but 2 and 4 weeks later *S. salivarius, S. mitis* biovar 1 and *S. oralis* accounted equally for *c*. 90% of the total streptococcal microflora. Previous reports on high isolation rates of *S. oralis* from saliva and the oral mucosa in predentate infants [3–5] differ from the present study, in which *S. oralis* showed a somewhat similar colonisation pattern to *S. sanguis*. The nearly ubiquitous presence of *S. mitis* biovar 1 in the infants studied here corroborates well with similar findings by Smith *et al.* [4]; however, the prevalence of *S. mitis* biovar 2 in predentate infants was higher in the present study. In contrast, the cross-sectional data produced by Tappuni and Challacombe [5] on predentate and dentate infants reported *S. mitis* biovar 2 as the main streptococcal finding in saliva. As identification schemes vary regarding biochemical reactions and nomenclature, discrepancies between studies are expected. Lucas *et al.* [27] found that the oral streptococcal microflora was fairly stable at species level and *S. salivarius*, *S. oralis* and *S. mitis* were the predominant species in repeated oral rinse samples from school-age children. The results of the present study demonstrate the predominance of these species at a young age. The relatively high prevalence of *S. parasanguis* reported by Lucas *et al.* [27] and the low prevalence in the present study suggest that this species colonises the oral cavity in later childhood rather than infancy.

Early studies by Carlsson *et al.* [2, 6] pointed out the significance of an amenable ecological environment for oral streptococcal colonisation. They used repeated sampling to demonstrate how the eruption of teeth subsequently resulted in the establishment of *S. sanguis* in infants' mouths [6]. Recently, Caufield *et al.* confirmed this connection and suggested a discrete 'window of infectivity' at a median age of 9 months for *S. sanguis* [7], as previously shown for *S. mutans* at a median age of 26 months [8]. The results of the present study are in agreement with these findings based on culture. By using a checkerboard DNA probe assay for identification of target bacteria in samples from teeth and tongue, Tanner *et al.* [28] frequently detected *S. mutans* in children under 18 months of age and suggested that the tongue serves as a reservoir for many tooth-associated species. The high prevalence rate of *S. mutans* was probably due to a specific population investigated in terms of high caries incidence [28]. Frandsen *et al.* [29] examined the ecology of viridans streptococci in detail and demonstrated that each streptococcal species associated with specific oral/oropharyngeal surfaces. *S. mitis* biovar 1 was isolated from most mucosal surfaces sampled, *S. mitis* biovar 2 and *S. salivarius* from the tongue surface, *S. sanguis* from buccal mucosae and initial dental plaque, and *S. oralis* from initial dental plaque, whereas the anginosus group dominated in subgingival plaque [29]. These findings may explain the failure of the present study to detect the latter group in infants.

The source and acquisition of the indigenous nasopharyngeal microflora and the succession of colonising species with age are poorly understood. The composition of the indigenous microflora in a certain niche may influence the outcome of colonisation of pathogens. The nasopharynx is considered to be the main reservoir for frequently encountered respiratory pathogens, particularly *S. pneumoniae* [30]. Aniansson *et al.* [31] reported various bacterial recoveries from nasopharyngeal cultures in an unselected sample of Swedish infants that were similar to the preliminary results of studies in Finnish infants [32]. The prevalence rates of *S. pneumoniae* in Swedish infants were similar to the present results but much lower for α-haemolytic streptococci, i.e., 13% at 1–3 months *versus* 22% at 2 months, 16% at 4–7 months *versus* 56% at 6 months, 20% at 8–12 months *versus* 36% at 12 months, and 20% *versus* 42% at 18 months, respectively. Except for *S. pneumoniae*, the mitis group streptococci have been considered as members of the non-pathogenic flora of the mouth and nasopharynx. Indeed, α-haemolytic streptococci isolated from human pharynx have been the target of several studies in terms of their interference with respiratory pathogens [11, 12, 33–35]. On the other hand, potentially virulent *S. mitis* isolates have been detected in association with respiratory diseases [36]. In the present study, both viridans streptococcal species and *S. pneumoniae* were detected on only one or two sampling occasions during the follow-up. This observation indicates that the streptococcal colonisation pattern of infants' nasopharynges differs from that of infants' mouths.

It has often remained unclear in the literature as to whether investigators were studying the oropharynx or nasopharynx. Obviously, the oropharynx constitutes a mixed environment where both oral and nasopharyngeal species can be present. Rasmussen *et al.* [37] investigated the aerobic microflora of the nasal cavity in 10 healthy adults and stated that no single streptococcal species was a frequent inhabitant at this body site and demonstrated a clear difference between the bacterial composition of the nasal cavity and that of oropharynx. In the present study, nasopharyngeal samples were collected through the nasal cavity, thus contamination by streptococci within the mouth was excluded. Despite the previous observation of the absence of *S. oralis* in the posterior wall of the oropharynx [29], this species was a relatively frequent finding in nasopharyngeal samples collected from infants during the second year of life, when the prevalence of *S. oralis* increased significantly in the oral cavity.

The antigenic variation and capability of producing IgA1 protease may favour the successful early establishment of *S. mitis* biovar 1, which was by far the most prominent species capable of cleaving IgA1 during the first 2 years of life, and other IgA1 protease-positive streptococcal species on mucosal surfaces in the upper respiratory tract. IgA1 antibodies are the main mediators of specific immunity relevant to mucosal surfaces of the upper respiratory tract, although salivary concentrations of S-IgA are low in infancy [13]. Both factors conceivably facilitate the early colonisation of mucosal surfaces by this particular streptococcal species. This hypothesis is supported by the observation that initial dental plaque is dominated by *S. mitis* biovar 1, *S. oralis* and *S. sanguis*, all of which are capable of IgA1 protease production [29, 38]. Similarly, the most prevalent streptococcal species detected in the nasopharynx, *S. pneumoniae*, *S. mitis* biovar 1 (the main proportion of isolates were IgA1 protease-positive) and *S. oralis*, are IgA1 protease producers. The presence of strains capable of IgA1 protease production may even facilitate the colonisation of IgA1 protease-non-producing strains in the same micro-environment. In the present study strains of IgA1 protease-positive and IgA1 non-protease-producing *S. mitis* biovar 1 were isolated simultaneously from saliva of infants, thus confirming the observed heterogeneity within *S. mitis* biovar 1 populations colonising an immature mouth [39, 40]. It has been suggested that increased proportions of IgA1 protease-positive streptococci on the oropharyngeal mucosa may jeopardise the local immune barrier and allow potential allergens to penetrate mucosal surfaces in the presence of this enzyme [41]. On the other hand, young infants can mount a specific secretory immune response against antigenic stimulation by IgA1 protease-positive streptococci [19].

The results of the present study indicate that despite the close proximity of the oral cavity and the nasopharynx, separate streptococcal colonisation patterns develop in the different parts of the upper respiratory tract during infancy. Early indigenous species, such as streptococci, may facilitate or inhibit the later establishment of pathogens at each body site. In this respect, the potential interplay of certain viridans streptococci with the host and other colonising bacteria of the upper respiratory tract is of specific interest to future research activity.

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