Background: This study was designed to assess gut microflora changes in children in Finnish day-care centers (DCCs).

Methods: Ninety-four children in four DCCs were randomised to receive a combination of Lactobacillus acidophilus La 5 and Bifidobacterium lactis Bb 12 or placebo for six months. Faecal samples were collected monthly and during bouts of diarrhoea. The parents kept a daily record. These groups were similar to infections and antibiotic treatments during the last month before the study. Altogether 14/76 (18%) children developed diarrhoea, and 13 healthy children who did not were studied as controls from the same DCCs at the same time. The gut microflora of altogether 26 children was examined by fluorescent in situ hybridisation at the start of the study, and before and after diarrhoea. Results: Twelve of 26 subjects (46%) had initially an aberrant microflora as determined by high levels of clostridia, the remaining 14 (54%) had balanced microflora. In the group with aberrant microflora, 9/12 (75%) manifested diarrhoea during follow-up, whereas in the group with balanced microflora, 4/14 (29%) fell ill with diarrhoea (p = 0.04). Diarrhoea reduced the number of all bacteria for at least a month. Antibiotic therapies increased the numbers of bacteria, mostly the number of clostridia. The initial total number of bacteria in the probiotic group decreased significantly in the late follow-up samples; p = 0.0075, this being due to the stabilising effect of probiotics. During treatment with probiotics aberrant microflora tended to approach the pattern in balanced microflora. Conclusions: A smaller and more stable amount of bacteria in the gut microflora was associated with healthy outcome of children during the study. Not only infections and antibiotics caused disruption of the gut microflora; aberrance of the gut microflora itself seems to predispose a child to diarrhoea episodes and other infections. Probiotics reduced the aberrance.

Key words: gut microflora; gastrointestinal and respiratory infections; probiotics; children in day care centers

ABBREVIATIONS
BAC, amount of bacteroides in faeces
BIF, amount of bifidobacteria in faeces
CI, confidence interval
CLOS, amount of clostridia (bacteria belonging to Clostridium histolyticum group) in faeces
DCC, day-care center
FISH, fluorescent in situ hybridisation
IQR, interquartile range
LAB, amount of lactobacilli and enterococci in faeces
TOT, total amount of microbes in faeces

INTRODUCTION
Babies are born with a sterile gastrointestinal tract. Bacterial colonisation of the intestine takes place soon after birth and is influenced by contamination from the mother’s birth canal and the surrounding environment (6). The gut is initially colonised by enterobacteria, Escherichia coli and enterococci, followed by anaerobic organisms, particularly Bacteroides, Bifidobacterium and Clostridium. Certain Bifidobacterium species seem to dominate in breastfed babies due to the presence of specific growth factors in human milk (3, 15, 24, 35). After the introduction of solid foods and discontinuation of breastfeeding, obligate anaerobes increase in numbers and diversity (31). The colonisation is a gradual process, in which during the first years of life the bacterial population in the gastrointestinal tract becomes stable in size, and specific for the host. Infants evince larger day-to-day and diet-induced variations and fluctuations in faecal microbial populations than adults (6). At two to four years of age the child has developed an adult-like flora, which may consist of more than 500 different culturable bacterial species (8).

Children in day-care centers (DCCs) are at an increased risk of infections. In one Finnish study (41) the mean number of illness periods was reported as 4.9 per
child per follow-up year. In Finland 140580 children attended DCCs in 1999 (44). Diarrhoea occurs at a rate of 0.7 to 2.6 episodes per child and 0.8 to 3.0 outbreaks per center each year in DCCs in the USA (2). The infections, especially diarrhoea, and the hygienic status of DCCs, including the microbial load of the immediate environment, can affect the intestinal microflora. Faecal contamination may take place from hand to hand due to inadequate hand-washing (25). It may be assumed that the gut microflora is at least partly similar among children in the same DCC.

The objective in the present study was to determine how consistent the gut microflora was in children attending DCCs. We specifically evaluated the effects of diarrhoea and vomiting, fever and antimicrobial treatments on the gut microecology of the children. In addition a combination of two probiotics: Lactobacillus acidophilus La 5 and Bifidobacterium lactis Bb 12 was administered to children in DCCs for half a year to study the effects of a probiotic combination on the gut microflora and infections. The composition of the faecal microflora was monitored by fluorescent in situ hybridisation (FISH), which is now widely adopted for the detection of bacterial groups in the gut microflora (12, 26). Measurement of the amounts of bifidobacteria (BIF), bacteroides (BAC), lactobacilli (LAB), clostridia of the Clostridium histolyticum group (CLOS) and the number of total microbes (TOT) in faeces gives us a good point of view to the changes in the gut microflora (17).

MATERIALS AND METHODS

Subjects. Ninety-four children from four DCCs in Pori, Finland, were admitted to the study with parents’ permission. The study was carried out between December to the end of May, the months which are the most intensive time for infections in DCCs. The subjects and the outcome of the study are presented in Fig. 1. Sixteen of the participating children discontinued for various reasons. In one case this took place after 4 months upon the onset of diabetes mellitus juvenilis: a 6.5 year-old-boy (in the probiotic group); his younger brother (placebo) also discontinued at this point.

Study design. The study was randomised, double-blind and placebo-controlled. The enrolled 94 subjects were randomised to receive placebo (microcrystalline cellulose) or a combination of two probiotic bacteria: Bifidobacterium lactis Bb 12 and Lactobacillus acidophilus La 5 (both from Chr. Hansen, Hørsholm, Denmark) in two capsules each containing: $2.9 \times 10^{10}$ colony-forming units (CFU) Bb 12 and $1.3 \times 10^{10}$ CFU La 5. The number of subjects eventually receiving placebo was 36, and the number of subjects receiving the probiotic preparation was 42. 78 children thus completed the study. The capsules were given in the DCCs once a day, and during weekends and days of absence at home. For infants who were unable to swallow, the capsules were opened and the contents suspended in a small amount of milk. Randomisation and packaging of the capsules was by Chr. Hansen & Chr. Hansen BioSystems A/S, Denmark, and the manufacturer kept the numbered codes until the results were analysed. The capsules were stored at $+4^\circ$C.

The parents kept a daily record in which stool frequency and consistency, diarrhoea, vomiting, fevers, other health complaints and antimicrobial treatments were recorded. Diarrhoea was defined as at least three loose stools per day for at least two consecutive days, and a vomiting period as at least two vomitings per
day. The parents were requested to contact the researcher in the case of diarrhoea during the follow-up, for additional faecal samples and for characterisation of the clinical situation, dehydration and aetiology of the disease. Visits during episodes of diarrhoea were arranged at the Paediatric Department of Satakunta Central Hospital, Pori, Finland.

The faecal samples from healthy children in the DCCs were collected at the beginning of this study and once a month for six months, a mean of 6.5 (range two to seven) samples per child. All samples were stored at -20°C until analysis. Additional samples were collected, when possible, during bouts of diarrhoea, in six of them faecal culture for bacteria (Salmonella, Shigella, Yersinia and Campylobacter) and latex for rotavirus and adenovirus were done at Satakunta Central Hospital; additional viral determinations by PCR techniques were performed on eight samples at the Virology Department of Tampere University, Tampere, Finland.

The faecal microflora was determined three times: at the start of the study, before diarrhoea, and at convalescence in 13 diarrhoeal patients and in 13 non-diarrhoeal age-matched controls from the same DCCs at the same time points, applying the FISH method. These groups were matched for age, gender and antibiotic treatments one month before the intervention.

**Analysis of the bacteriology of faecal samples using genetic probes.** FISH was performed as described by Langendijk and colleagues 1995 (26). Briefly, faecal samples were weighed and phosphate-buffered saline (PBS; 8 g NaCl, 0.2 g KCl, 1.44 g Na2HPO4 and 0.24 g KH2PO4/l) added to make up a 1/10 suspension (w/v). The sample was homogenised and a 5 ml portion removed, vortexed with glass beads for 30 s, and subsequently centrifuged at 250 x g for 2 min. The bacterial cells were fixed in 4% (v/v) paraformaldehyde solution overnight at 4°C. The cells were then washed twice in PBS and resuspended in 1 ml of PBS:ethanol (1:1, v/v). A portion of the cell suspension was then hybridised overnight in hybridisation buffer (HB; 20 mM Tris-HCl, 0.9 M NaCl) with a Cy3 indocarbocyanin-labeled probe. Bifidobacteria were counted with probe BIF164 (5'-CATCCGGCATATCCAACC) (26), bacteroides with BAC303 (5'-CCAATGTGGGG-GACCTT) (32), lactobacilli/enterococci with LAB158 (5'-GGTATTACGA(T/C)CTGTTTCCA) (16), and clostridia (Clostridium histolyticum group) with HIS150 (5'-TTATGCCGTATTAATCT(C/T)CTTTT) (17). Total cell numbers were counted using 4',6-diamidino-2-phenylindole (DAPI) as nucleic acid stain. Cells were washed with the hybridisation buffer, applied to a 0.2 μm polycarbonate filter (Millipore Corporation, Bedford, USA) and mounted on a glass slide with Slow Fade (Molecular Probes Inc., Eugene, USA). Fluorescent cells were counted visually using a Leica Laborlux D epifluorescence microscope. Fifteen microscopic fields were counted per assay. The bacteria determined were bifidobacteria, bacteroides, lactobacilli and enterococci, clostridia (belonging to Clostridium histolyticum group) and the total.

**The stability and aberrance of the gut microflora.** The number of BIF was used in the evaluation of the stability of the microflora, while an increased level of CLOS was considered to represent the unbalanced part of the gut microflora (4, 11, 20, 23). Those who had initially high amounts of CLOS (> 5.5 x 10^8) being above the geometric mean of the measured amounts were arbitrarily classified as cases with aberrant microflora and those below the geometric mean were classified as having balanced gut microflora.

**Statistics.** Using the 76 children with available diaries the study groups were compared with respect to the clinical results during the follow-up i.e. fever, vomiting, diarrhoea and antibiotic treatments. The occurrence of fever, vomiting and diarrhoea were analysed using χ²-square test and the number of antibiotic treatments were analysed using Mann-Whitney U test. The number of days with fever were analysed using the t-test for independent samples. The connection between age and infectious conditions was analysed by using the t-test for independent groups. In addition, the test for linear trend was conducted to study the occurrence of infections in age groups.

Nested case-control design with 26 subjects was applied to study the gut microflora and the connections between gut microflora, probiotic treatment and diarrhoea. Before any microflora determination 13 randomised subjects having diarrhoea during the study and 13 individually matched healthy controls were selected. The connection between the characteristics of the initial microflora, probiotic treatment and diarrhoea was analysed by χ²-square test. The results were given as odds ratios with 95% confidence intervals (CI). Paired t-test (Wilcoxon signed rank test) was used to test the within-subjects changes in the number of bacteria to study the impact of diarrhoea, antibiotics and probiotics. T-test for independent samples (Mann-Whitney U test) was used to compare the study groups with respect to the changes in the number of bacteria. Geometric means with 95% CI were used for clinical results, and medians with interquartile ranges (IQR) for changes in the amounts of bacterial strains.
Ethical considerations. The parents gave their written consent, and the study was approved by the ethics committees of Turku University Hospital, Satakunta Central Hospital and the city health center of Pori.

RESULTS

Clinical Results

The study groups were comparable in age and gender. These groups were similar to infections and antibiotic treatments during last month before the study, some cases of otitis media occurred. The most common infectious condition experienced by the study subjects, 56/78 (72%), was fever, altogether 113 fever periods (60 probiotic/53 placebo) and 3.3 fever days (range 0–17) per child due mainly to respiratory tract infections. Five subjects had chickenpox during the study. Altogether 36 children (46%) were prescribed 67 courses of antibiotic therapy. Antibiotics were used mainly for otitis media and upper respiratory tract infections, in two cases for skin infections and in two for tonsillitis caused by Streptococcus pyogenes. Two children were on continuous antibiotic therapy for recurrent otitis media. Amoxicillin, sulfa-trimethoprim and penicillin were the most commonly used antibiotics. One subject, aged 1.2 years, was prescribed six courses of antibiotic therapy and had 19 days of illness.

Altogether 14/76 children (18%) manifested diarrhoea, one of them during the first two days of the intervention (excluded from microflora analysis), and 16/76 (21%) vomiting. The mean duration of diarrhoea being 2.6 days (range 2–5) and that of vomiting: 1.4 days (range 1–3), thus vomiting and diarrhoea episodes were infrequent and with short duration. The children with diarrhoeal episodes were significantly younger, mean age of 3.3 (95% CI 2.6–4.1) years, as compared to those with no diarrhoea episodes, 4.6 (95% CI 4.3–5.0) years (p = 0.002). In like manner, children with periods of vomiting were younger, 3.6 (95% CI 2.8–4.4) years, as compared to those with no vomiting 4.6 (95% CI 4.2–4.9) years (p = 0.018); likewise children with fever: 4.1 (95% CI 3.8–4.4) years, and with no fever 5.2 (95% CI 4.6–5.8) years (p = 0.001). Thus the occurrence of fever, diarrhoea and vomiting was significantly affected by age; older children had fewer infections than younger (test for linear trend; p = 0.001 for fever; p = 0.007 for diarrhoea; p = 0.018 for vomiting).

Eleven children with diarrhoea manifesting mild dehydration visited the paediatric outpatient clinic and seven of them received oral rehydration therapy. A viral aetiology of the diarrhoea was confirmed in six cases: astrovirus in three, Norwalk-like viruses in two and Sapporovirus in one; bacterial aetiology in none. Seven children with diarrhoea episodes had repeated infections being in vicious circle of infections during the study period and needed 20 antibiotic courses; in addition two of them were on continuing antibiotic therapy.

The Stability of the Gut Microflora

In general the faecal microflora in children in Finnish DCCs remained stable during the study period. We used the number of BIF in evaluating of the stability of the microflora; the initial number of BIF, in the placebo group, median IQR 8.6 x 10^8 g (6.1 x 10^8–1.2 x 10^9) was comparable to that in the followup sample 3 months later 1.2 x 10^9 g (2.6 x 10^8–1.6 x 10^9); p = 0.406; correspondingly in the probiotic group initially median IQR 5.7 x 10^8 g (3.3 x 10^8–1.1 x 10^9) and later 5.4 x 10^8 g (2.4 x 10^8–6.7 x 10^8); p = 0.401. None of the genera investigated decreased below the detection level. The initial mean total number of bacteria was on the level of 6 x 10^9 g and that of clostridia 5 x 10^8 g.

Aberrant and Balanced Microflora

In the study subjects whose microflora was evaluated (n = 26; 13 diarrhoea cases and 13 age-matched non-diarrhoea children from the same DCCs) there was initially a subgroup of 12 subjects (46%), who evinced an aberrant microflora as defined by high amounts of initial CLOS, and nine of them also had levels of total bacteria above the geometric mean. Altogether 14 subjects (54%) were shown to have an initial balanced microflora defined as lower amounts of the clostridia and total bacteria (Fig. 2). In the group with aberrant microflora, 9/12 (75%) manifested diarrhoea during follow-up, whereas in the group with balanced microflora, 4/14 (29%) fell ill with diarrhoea (p = 0.04). The balanced microflora was also shown to be more stable during follow-up. The subjects with balanced status had a lower risk of infections (diarrhoea, p = 0.016; vomiting, p = 0.014; all illness days including fever days, p = 0.009) and hence of fewer antibiotic treatments (p = 0.048) compared to subjects with aberrant microflora.

The Impact of Probiotics, Antibiotics, and Diarrhoea

Probiotics. The number of infections during the follow-up was not associated with the individual DCC; neither with the intervention with placebo or probiotics with respect to fever, diarrhoea and vomiting, Table 1.

At the beginning of the study, the faecal microflora of children receiving placebo and probiotics was com-
GUT MICROFLORA CHANGES AND PROBIOTICS IN CHILDREN IN DAY-CARE CENTERS

Fig. 2. Differences in placebo and probiotic groups with balanced and aberrant gut microflora. Mean number of bacteria (per gram of faeces) in gut microflora determined by FISH. placebo group, n = 9; probiotic group, n = 17. 1, at the start of the study. 2, before diarrhoea (later follow-up). 3, one month after diarrhoea (latest follow-up).

Table 1. Infectious conditions in children in DCCs during the 6 months study.

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Probiotics</th>
<th>Total</th>
<th>Probiotics vs. placebo p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>Incidence*</td>
<td>27/35 77%</td>
<td>29/41 71%</td>
<td>56/76 72%</td>
</tr>
<tr>
<td></td>
<td>Mean number of days (range)</td>
<td>3.1 (0-17)</td>
<td>3.5 (0-11)</td>
<td>3.3 (0-17)</td>
</tr>
<tr>
<td></td>
<td>Mean number of periods (range)</td>
<td>1.4 (0-5)</td>
<td>1.4 (0-6)</td>
<td>1.4 (0-6)</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>Incidence</td>
<td>5/35 14%</td>
<td>9/41 22%</td>
<td>14/76 18%</td>
</tr>
<tr>
<td></td>
<td>No. of days (range)</td>
<td>0.3 (0-3)</td>
<td>0.7 (0-5)</td>
<td>0.5 (0-5)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>Incidence</td>
<td>7/35 20%</td>
<td>9/41 22%</td>
<td>16/76 21%</td>
</tr>
<tr>
<td></td>
<td>No. of days (range)</td>
<td>0.4 (0-5)</td>
<td>0.3 (0-2)</td>
<td>0.3 (0-5)</td>
</tr>
</tbody>
</table>

*Condition at least once during the study.

parable. The initial median total number of bacteria, in the probiotic group: $6.5 \times 10^9$ g (IQR $3.2 \times 10^9-9.7 \times 10^9$) decreased to $2.8 \times 10^9$ g (IQR $1.9 \times 10^9-4.5 \times 10^9$), in the late follow-up samples; $p = 0.008$, which was attributable to the stabilising effect of probiotics. In the placebo group no significant decrease was noted: from $6.7 \times 10^9$ g (IQR $4.7 \times 10^9-8.9 \times 10^9$) to $6.2 \times 10^9$ g (IQR $4.7 \times 10^9-8.9 \times 10^9$); $p = 0.595$. The total numbers of bacteria apart from clostridia in the subjects with aberrant microflora tended to approach those of the pattern in balanced microflora, as shown in Fig. 2, when given probiotics. The group with aberrant microflora receiving probiotics ($n = 7$) had initial median total numbers of bacteria $9.7 \times 10^9$ g (IQR $8.1 \times 10^9-1.2 \times 10^{10}$), which decreased to $4.1 \times 10^9$ g (IQR $2.4 \times 10^9-4.6 \times 10^9$); $p = 0.006$; and CLOS decreased from $1.7 \times 10^9$ g (IQR $9.7 \times 10^8-2.8 \times 10^9$) to $8.2 \times 10^8$ g (IQR $2.8 \times 10^8-1.3 \times 10^9$); $p = 0.031$, during the treatment. The group with an aberrant microflora receiving placebo ($n = 5$) had an initial median number of total bacteria $7.9 \times 10^9$ g (IQR $5.6 \times 10^9-1.5 \times 10^{10}$), and in the late follow-up samples: $6.2 \times 10^9$ g (IQR $5.4 \times 10^9-7.4 \times 10^9$); $p = 0.411$.

**Antibiotics.** There was no difference in the number of antibiotic treatments between the probiotic and placebo group ($p = 0.790$), Table 2.

Altogether 10/26 children received no antibiotic treatments during the study. Figure 3 shows the impact of antibiotics on the gut microflora, which was greatest with respect to the amount of clostridia, initially $p = 0.0355$; before diarrhoea $p = 0.0622$ and after $p = 0.0595$; partly significant.

**Diarrhoea.** Diarrhoea was associated with a decrease in the numbers of all bacteria compared to cases with
no episodes of diarrhoea. This effect was still evident a month after cessation of diarrhoea. When comparing the microflora as assessed before the episodes of diarrhoea and one month after convalescence, a significantly lower number of bacteria was detected; LAB, median from $9.0 \times 10^9$/g (IQR $5.5 \times 10^8$–$1.7 \times 10^9$) to $5.1 \times 10^8$/g (IQR $4.1 \times 10^8$–$8.6 \times 10^8$) ($p = 0.031$); CLOS, median from $8.1 \times 10^8$/g (IQR $3.0 \times 10^8$–$1.9 \times 10^9$) to $4.1 \times 10^8$/g (IQR $2.3 \times 10^8$–$1.0 \times 10^9$); $p = 0.026$, and TOT, median from $6.3 \times 10^9$/g (IQR $5.3 \times 10^9$–$1.0 \times 10^{10}$) to $4.8 \times 10^9$/g (IQR $2.7 \times 10^9$–$6.0 \times 10^9$); $p = 0.015$.

**DISCUSSION**

The stability of the gut microflora in children under different circumstances is not well known. The gut microflora has been shown to be of great importance in maintaining good general health, protecting the host from colonisation by pathogenic microorganisms. In addition there is interaction between the host’s immune system and luminal antigens which is influenced by the presence of the gut microflora (50). The acquisition of a normal microflora may be a never-ending process as new strains of endogenous or exogenous origin proliferate and adhere to the intestinal ecosystem under the influence of allogenic or autogenic factors (46). This randomised, double-blind, placebo-controlled study is the first to bring out microflora changes in infants in DCCs by evaluating the impact of diarrhoea, probiotics and antibiotics.

The beneficial bifidobacterial microflora in the children in the Finnish DCCs in our study was shown to be stable. In a study of Hopkins and associates (21) the mean number of total anaerobes in ten children aged 16 months to seven years (ages equal to the 26 children in the current study) was at a level of $5 \times 10^{10}$ g and correspondingly the mean number of clostridia $2 \times 10^7$/g, in our study the mean number of total anaerobes was thus lower ($6 \times 10^8$/g) and the number of clostridia ($5 \times 10^9$/g) higher. The smaller and more stable number of bacteria in the gut microflora was associated with healthy outcome of children during this study. Clinically, our study confirms the results given in previous reports (28, 42): the youngest are at the greatest risk of infections in DCCs. The youngest children who repeat-
long-term effects. In studies by groups under Vanderhoof flora vulnerable for several weeks and having clear microflora lasted for a long time rendering the micro-
that the reducing effect of diarrhoea on the faecal more harmful exogenous bacteria (5). Our study showed colonisation resistance, allowing the establishment of pathogens (22, 27, 30), and may lead to decreased protective effected barrier and result in overgrowth of infections like gastroenteritis can be detrimental to the enhanced the stability of the gut microflora.

In the present study a large number of clostridia was inhibition, and fluctuations in faecal microbial populations are greater in infants than in adults with larger day-to-day and diet-induced variations (6). Bifidobacteria play an important role in the breakdown of complex carbohydrates, which are resistant to hydrolysis by human digestive enzymes (29). B. infantis is usually found in infants and children (33). In our study we used bifidobacteria as an indicator of stability. While stability in species composition may be a feature of the normal microflora, stability at the level of bacterial strains may be less common. Fluctuations in the composition of the population of E. coli in human faecal samples, detected through the use of serotyping, have been reported (18). In a study by McCartney and co-workers (34) the gut microflora of two adults was followed for 12 months and considerable stability was shown, but, without visible cause, variation and fluctuations also took place. In the present study a large number of clostridia was shown to be a sign of aberrant microflora leading to dysfunction and exposing a child to infections, but oral administration of probiotics reduced the aberrance and enhanced the stability of the gut microflora.

Alterations in the gut microflora by antibiotics or infections like gastroenteritis can be detrimental to the protective effect barrier and result in overgrowth of pathogens (22, 27, 30), and may lead to decreased colonisation resistance, allowing the establishment of more harmful exogenous bacteria (5). Our study showed that the reducing effect of diarrhoea on the faecal microflora lasted for a long time rendering the microflora vulnerable for several weeks and having clear longterm effects. In studies by groups under Vanderhoof (49) and Arvola (1), Lactobacillus GG reduced the percentage of antibiotic-associated diarrhoea clinically. Our own results demonstrate some mechanisms by which antibiotics and probiotics act in the gut microflora: antibiotics act by increasing the overgrowth and aberrance and probiotics by reducing the overgrowth and stabilising.

Prevention of infections and control measures in DCCs are useful and cost-effective (7, 48). Appropriate steps include careful hand-washing of personnel and children, changing diapers in sanitary facilities, separation of these areas from food handling and eating areas, careful routine cleaning of environmental surfaces, and exclusion of any child or child care worker with diarrhoea (47). This study showed that the state of hygienic functions in Finnish DCCs is fairly good judging from the low level of infections, especially diarrhoea; the local rotavirus epidemia was, it is true, small this year. However, some infants in DCCs can develop a vicious circle of infections. Aberrance of the gut microflora is probably a part of this hazard and we can speculate that it may lead to the poorer appetite often found in these children. Probiotics in the prevention of infections in DCCs would offer quite a new means of addressing this problem (19), although this study was not able to show direct preventive effect on infections.

Probiotics have been used in preventing diarrhoea (43) and they may modulate the intestinal microflora (13). Our results showed that an aberrant microflora predisposes to diarrhoea and other infections, and probiotic therapy renders the aberrant microflora more balanced and prevents the overgrowth of bacterial strains. The welfare of children includes the prevention of microflora disruption, and it is essential to safeguard the complex equilibrium in the ecology of the gut microflora.

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REFERENCES

1. Arvola T, Laiho K, Torkkeli S, Mykkänen H, Salminen S,


(44) Stakes reports for OECD, Early Childhood Education and Care Policy in Finland. Available at: http://www.vn.fi/stm/suomi/julkaisu/julk011fr.htm