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ORIGINAL ARTICLE

Lactobacillus reuteri accelerates gastric emptying and improves regurgitation in infants

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ABSTRACT

Background Young infants are frequently affected by uncomplicated regurgitation that may persist despite dietetic and conservative interventions. On this basis, we studied the putative effects of probiotics on the frequency of regurgitation and gastric emptying time in infants with functional gastroesophageal reflux (GER).

Patients and methods Forty-two infants with regurgitation were randomized to assume *Lactobacillus reuteri* DSM 17938 at a dose of 1×10^8 CFU per day and placebo for 30 days. The episodes of regurgitation were recorded by the parents each day. Gastric emptying time was recorded using real-time ultrasound at baseline and at the end of the study. Twenty-one infants without regurgitation were enroled to compare anthropometric and physiological parameters before the intervention diet.

Results Thirty-four infants completed the study (19 infants receiving probiotics and 15 placebo).At baseline, the whole group of infants was similar to the control group as regards anthropometric and physiological data. The median fasting antral area was significantly reduced, ($P = 0{\cdot}01$) the delta in gastric emptying rate was significantly increased ($P = 0{\cdot}01$) and the median episodes per day of regurgitation was reduced (, $P < 0{\cdot}001$) in the probiotic group compared to the placebo group. In the whole group, the frequency of regurgitation and the basal antral area showed a positive correlation ($r = 0{\cdot}53$, $P = 0{\cdot}004$).

Conclusions In infants with functional GER, *L. reuteri* DSM 17938 reduce gastric distension and accelerate gastric emptying. In addition, this probiotic strain seems to diminish the frequency of regurgitation.

Keywords Functional regurgitation, gastric motility, probiotic.

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Introduction

Functional gastroesophageal reflux (GER) is very common during infancy and most often manifests itself as episodes of regurgitation or vomiting [1]. Regurgitation is defined as the passage of refluxed gastric content into the oral pharynx, whilst vomiting is defined as expulsion of the refluxed gastric content from the mouth. The frequency of regurgitation may vary considerably in relation to age, and younger infants up to the first month of age are more frequently affected by regurgitation [2]. Most infants with regurgitation are happy and healthy even if they frequently spit up or vomit, and babies usually outgrow GER by their first birthday. The European and American Societies for Pediatric Gastroenterology, Hepatology and Nutrition have recommended dietary management for infants with uncomplicated regurgitation, such as parental reassurance and thickening of the formula [3,4]. Generally, parental education, anticipatory guidance and modification of feeding frequency and volume are sufficient for the management of uncomplicated GER [5,6]. Although some authors consider conservative therapy to be an efficient first choice for improving regurgitation [7], the administration of thickened formulas are considered a reliable dietary management for decreasing recurrent regurgitation and/or vomiting in infants with GER. However, significant regurgitation may persist, despite these interventions increasing parental stress and causing an additional workload for health care professionals.

During the last few years, the role of the intestinal microflora in health and disease has gained increasing recognition, and there are strong indications that diet can influence the relative

amount of microbial species in the gastrointestinal flora [8]. Consequently, one approach to fortify the biological function of formula feeds has been to use probiotics as constituents [9]. *Bifidobacteria* and *Lactobacillus* spp. are the most popular microorganisms for probiotic applications, and those of human origin are most effective [10]. Probiotic supplementation of infant formulas has shown that some strains may persist in the infant gut [11,12] and lower stool pH [13].

We have recently shown that *Lactobacillus reuteri* [14] administered to formula-fed preterm infants results in a significantly increased gastric emptying rate and in a significant decrease in regurgitation and mean daily crying compared with those given placebo. We planned this study to evaluate whether the effect of *L. reuterii* on the motor gastric activity was still present beyond the early neonatal period and whether this may translate in a clinical application for infants with functional regurgitation.

Patients and methods

Subjects and protocol

A randomized pilot double-blind, placebo-controlled, parallel-group trial was conducted in 42 consecutive infants younger than 4 months referred for uncomplicated regurgitation to the Gastrointestinal Unit of the Department of Pediatrics at the University of Bari, Italy from July 2008 to January 2010 (Table 1).

Uncomplicated infant regurgitation was defined according to the ROME III Criteria in presence of all of the following characteristics in otherwise healthy infants of 3 weeks to 12 months of age: (i) regurgitation 2 or more times per day for 3 or more weeks and (ii) no retching, hematemesis, aspiration, apnoea, failure to thrive, feeding or swallowing difficulties, or abnormal posturing [15]. The diagnosis of uncomplicated infant regurgitation was based on a clinical interview performed by the same expert paediatric gastroenterologist (FI).

To be eligible, infants have to: (i) be fed with the same infant formula, (ii) have normal growth and development and (iii) have no underlying predisposing factors or conditions.

Infants were excluded in presence of (i) any chronic/allergic disease; (ii) previous treatment with antibiotics/probiotics/aspirin/proton pump inhibitors; (iii) growth failure; (iv) gastrointestinal obstructions/stricture; (v) symptoms related to GER disease such as arching, irritability and refusal to feed; and (vi) previous abdominal surgery.

As there are no published normal values for the gastric emptying (GE) parameters with ultrasound in infants, 21 infants matched for age and weight with no history of regurgitation and fulfilling the inclusion criteria were recruited on voluntary bases to assess the cut off value of normal range of GE and referred as control group.

Table 1 Demographic and clinical characteristics at baseline

	Formula + LR *N* = 19	Formula + pacebo *N* = 15	Normal control *N* = 21
Age (days)	39 ± 8·2	40 ± 5·3	47 ± 1·3
Weight (g)	4990 ± 631	5100 ± 739	5230 ± 139
Length (cm)	51·3 ± 1·8	52·4 ± 1·5	53·1 ± 1·1
Head circumference	39·8 ± 1·3	38·1 ± 1·1	39·1 ± 0·3
Number regurgitation per day	5·0 [4·3–7·0]	6·0 [5·0–7·0]	1·1 ± 1·1

Values indicated as mean and SD apart from the number of regurgitation expressed as medians and 5 and 95 percentiles. *P* = NS.

Study design

The 4-week treatment period was preceded by a 2-week run-in phase. To undergo randomization (end of run-in period), patients had to have at least 2 or more regurgitation per day in absence of alarm symptoms for during the run-in phase. Infants were assigned consecutive numbers, starting with the lowest number available, and were randomly assigned, with the use of a computer generated randomization list to receive orally either probiotic or placebo. The active study product consisted of freeze-dried *L. reuteri* DSM 17938 suspended in a mixture of pharma grade sunflower and medium-chain triglyceride oils supplied in a dark bottle fitted with a dropper cap. Five drops of the formulation, delivering a dose of 1×10^8 colony-forming units (CFU) of *L. reuteri*, were administered to the children. The placebo consisted of an identical formulation in all respects except that the live bacteria were excluded. There were no differences in smell or taste between the two formulations. Both the *L. reuteri* and placebo study products were manufactured and donated by BioGaia AB (Stockholm, Sweden), which ensured that the study was blinded for investigators and patients. Infants were not allowed to consume any probiotic other than those provided or prebiotics, and they were instructed to continue their initial infant formula. Concomitant use of medications affecting gastrointestinal motility was not allowed, providing parents registered the intake. The institutional review board approved the study. Written informed consent was obtained from parents of children who were fully informed of the details of the study.

Symptom evaluation

Before entering the trial, parents were given written information about the study and instructed how to compile a structured

diary to record symptoms. Throughout the study, patients recorded on daily bases the number of episodes per day of regurgitation (defined as the passage of refluxed gastric contents into the oral pharynx) or any other symptom they may relate to the intervention. To ensure compliance, one investigator (AF) contacted the families every week to monitor the process of the study. Adherence was assessed by measuring the volume of oil returned; children who missed more than 20% of the medications were considered noncompliant.

Assessment of gastric emptying

GE was recorded at time 0 and at the end of the intervention period. The ultrasound gastric emptying examinations were always performed by the same investigator (FI) using a real-time apparatus (Image Point HX; Hewlett Packard Company, Palo Alto, CA, USA) equipped with a 3·5 MHz linear probe. The probe was positioned at the level of the trans-pyloric plane for simultaneous visualization of the antrum, superior mesenteric vein and the aorta. The antral measurements were always taken from the outer profile of the wall. As the cross-section of the gastric antrum, corresponding to the sagittal plane passing through the superior mesenteric vein, is elliptical in shape, its area can be calculated by measuring the longitudinal (L) and anteroposterior (AP) diameters and applying the formula for calculating the ellipse p-Greek L × AP/4[15]. Antral measurements were made before and immediately after the end of the test meal (time 0) and at regular 30-min intervals up to 120 min after the meal. In each patient, the gastric emptying rate was expressed as per cent reduction in antral cross-sectional area at time 0 and 120 min after meal ingestion [16]. The change (delta) in gastric emptying rate (the difference in the % GE rate values before and after intervention) was then calculated.

Data analysis

In an intervention diet like this, the evaluation of the physiological effects of probiotics can be clearly evaluated considering only the patients who strictly complied with the trial's protocol (per protocol analysis). Compliance covers exposure to treatment, availability of measurements and absence of major protocol violations. Besides, with the assumption that an increased gastric emptying would be expected in 75% of infants receiving the probiotic and in 15% of those receiving placebo, we calculated that a minimum of 11 infants per group would be required for the study to have an alpha error = 0·05 and a beta error of 0·8. The data were analysed first using simple descriptive statistics of centrality and dispersion. Anthropometric data were expressed as mean ± SD, whilst number of regurgitation and physiological data were expressed as medians and 5 and 95 percentiles. For clinical parameter (number of regurgitation), the values per day recorded over the last 7 days of the treatment period were calculated. The differences in anthropometric, clinical and physiological parameters were determined by the Mann–Whitney rank sum test, and differences were considered significant at the 5% level ($P < 0·05$). The correlation between GE parameters and frequency of regurgitation was calculated by means of Spearman correlation test. The software package used for the statistical analysis was STATA (STATA ver 4.0 Statistical Software; Stata Corporation, College Station, TX, USA).

Results

A total of 42 infants with functional and uncomplicated GER underwent randomization and took the product (placebo or probiotics). At the end of the study, 34 infants completed the trial. Eight infants (three from the probiotic group, five from the placebo group) were not included in the final analysis for noncompliance with the protocol or parents' decision not to continue the study, in absence of any side effects (see Consort form). The responses of the 34 infants were retained for the per protocol analysis: 19 in the probiotic group and 15 in the placebo group. Anthropometric and clinical parameters at the beginning of the intervention period are reported in Table 1. The gastric emptying parameters at baseline are reported in Table 2.

At the end of the intervention period, the fasting antral area was significantly reduced and the delta in gastric emptying rate was significantly increased in infants receiving probiotics compared to placebo (3·0 [2·0–4·2] cm^2 vs. 4·0 [2·4 – 5·9] cm^2, $P = 0·01$; and +11·7 [−3·9 to +24·0] % vs. +8·4 [−27·0 to +23·5] %, $P = 0·01$, respectively) (Figs 1 and 2). Besides, the formula-fed infants receiving the probiotic had a significant decrease in the frequency of regurgitation per day compared to placebo (1·0 [1·0–2·0] vs. 4·0 [3·0–5·0] median episodes per day calculated over the last 7 day of treatment, $P < 0·001$).

At the end of the intervention period, we observed that in the whole group the frequency of regurgitation positively correlated with the basal antral area ($r = 0·53$, $P = 0·004$) (Fig. 3).

No difference was seen in body weight and other growth parameters during and at the end of the trial; no infants had any reported adverse events related to the trial.

Table 2 Gastric emptying parameters for study group and control

	Study group ***N* = 34**	**Control group** ***N* = 21**
Fasting antral area (cm^2)	2·7 [2·0–3·1]	2·7 [1·4–3·1]
Gastric Emptying rate (%)	−54·9 [−75·6 to −44·2]	−55·3 [−85·2 to −44·3]

Values indicated as medians and 5 and 95 percentiles; *P* = NS.

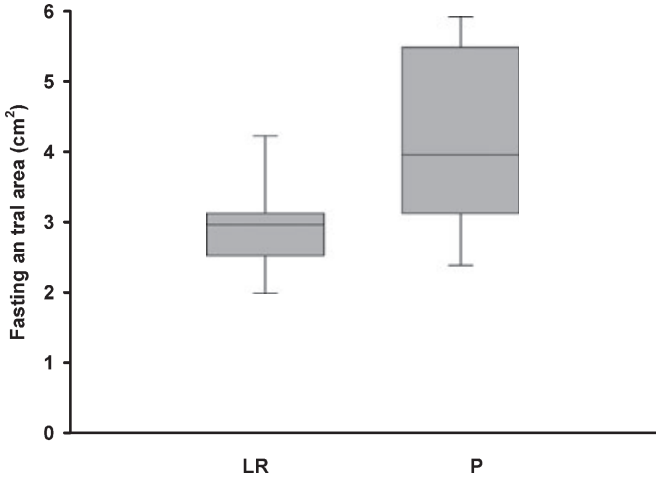

Figure 1 Fasting antral area recorded in infants at the end of the intervention diet in *Lactobacillus reuteri* (LR) or placebo (P)-supplemented formula-fed infants. Statistical analysis using the Mann–Whitney rank sum test showed significant difference between the groups (P = 0·01).

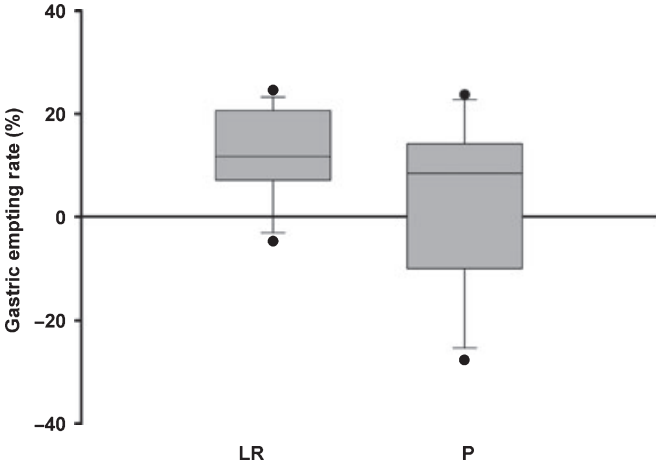

Figure 2 Gastric emptying recorded in infants at the end of the intervention diet in *Lactobacillus reuteri* (LR) or placebo (P)-supplemented formula-fed infants. In each infant, the gastric emptying rate was expressed as per cent reduction in antral cross-sectional area from time 0 to 120 min after meal ingestion. The change (delta) in gastric emptying (GE) rate was calculated as the difference in the % GE rate values before and after the intervention. Statistical analysis using the Mann–Whitney rank sum test showed significant difference between the groups (P = 0·01).

Discussion

Our study shows that the administration of *L. reuteri* DSM 17938 significantly accelerates gastric emptying and ameliorated regurgitation in infants affected by noncomplicated GER when used daily for 28 days with a dose of 1×10^8 (CFU). Reduced episodes of regurgitation, linked with an increased gastric emptying rate and reduced fasting antral area, were seen in healthy formula-fed preterm infants supplemented with *L. reuteri* ATCC 55730 [14]. The new strain was used because the parental strain is not more commercially available.

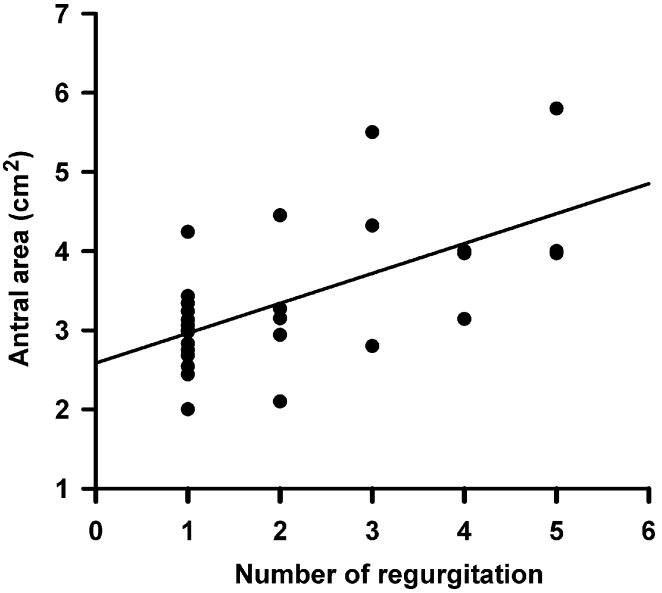

Figure 3 The correlation between basal antral area and frequency of regurgitation recorded from the whole group of infants is shown. Number of regurgitation corresponds to the episodes counted the day of gastric emptying examination. The Spearman correlation test showed a significant positive correlation (r = 0·53, P = 0·004).

To the best of our knowledge, the effect of probiotic is not been investigated on functional regurgitation neither on gastric motility in this kind of infant population.

Our infants had regurgitation but they were otherwise healthy with satisfactory growth parameters and neurodevelopment. In such infants, also called 'happy spitters', pharmacological treatment is typically not necessary and a conservative treatment is suggested as reported in the ESPGHAN–NASPGHAN guidelines [1]. In some cases, significant regurgitation may persist, despite these interventions increasing parental stress and causing an additional workload for health care professionals.

The pathophysiology of regurgitation is multifactorial, involving oesophageal, gastric and enteric nervous system abnormalities. Gastric distension and impaired fundic relaxation as a result of disturbed gastric motility might play a role in acid reflux to the oesophagus. In fact, transient lower

oesophageal sphincter relaxations, which are one of the main pathophysiological mechanism of GER, seem to be triggered by gastric distension via activation of stretch receptors in the stomach [15–18]. The enlarged fasting antral area and delayed gastric emptying time could be related with gastric distension and consequently provoke the regurgitation. This work on infants confirmed that regurgitation could be related to gastric emptying and antral dysfunction [19–22].

We also collected the gastric emptying parameter in normal infant matched for age and sex with the study group. The comparison with the normal value of gastric emptying in this age range allows us to define specifically the effect of probiotic on gastric motility. Actually, these children treated with *L. reuteri* had an acceleration of gastric emptying time. An intrigue experimental work on colonic motility in rat showed that *L. reuteri* ameliorate the rhythmic contraction of the colon [23]. The molecular and physiological pathways via which the commensal bacteria exert their effect on intestinal motility are far from being elucidated. Nevertheless, the mechanism of neuroimmune interaction may play a crucial role also in this age range infant [24]. It is reasonable to suppose that the structure responsible of the intestinal motility as enteric neurons, interstitial cells of Cajal and smooth muscle cells, could relay some of the actions that probiotic exerts, beyond the gut, on central and autonomic nervous system [25].

We are aware of the limitations of our study. Given strict enrolment criteria, a population study is extremely selected, and it is difficult to extend the conclusion to all infants affected by noncomplicated regurgitation, but this may be the starting point for a larger studies. Such studies are greatly warranted as probiotic are targeted to a specific gastrointestinal disorders, and the nutritional therapeutic approach remains the favourable intervention in small infants.

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Conflict of interest

All the authors disclose any others conflict of interest.

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References

1 Rudolph CD, Mazur LJ, Liptak GS, Baker RD, Boyle JT, Colletti RB *et al*. Guidelines for evaluation and treatment of gastroesophageal reflux in infants and children: recommendations of the North American Society for Pediatric Gastroenterology and Nutrition. *J Pediatr Gastroenterol Nutr* 2001;**32**:S1–31.
2 Martin AJ, Pratt N, Kennedy JD, Ryan P, Ruffin RE, Miles H *et al.* Natural history and familial relationships of infants splilling up to 9 years of age. *Pediatrics* 2002;**109**:1061–7.
3 Vandenplas Y, Ashkenazi A, Belli D, Boige N, Bouquet J, Cadranel S *et al.* A proposition for the diagnosis and treatment of gastro-oesophageal reflux disease in children: a report from a working group on gastro-oesophageal reflux disease. Working Group of the European Society of Paediatric Gastroenterology and Nutrition (ESPGAN). *Eur J Pediatr* 1993;**152**:704–11.
4 Vandenplas Y, Belli D, Benhamou PH, Cadranel S, Cezard JP, Cucchiara S *et al*. A critical appraisal of current management practices for infant regurgitation – recommendations of a working party. *Eur J Pediatr* 1997;**156**:343–57.
5 Orenstein S, McGowan J. Efficacy of conservative therapy as taught in the primary care setting for symptoms suggesting infant gastro-esophageal reflux. *J Pediatr* 2008;**152**:310–4.
6 Shalaby TM, Orenstein SR. Efficacy of telephone teaching of conservative therapy for infants with symptomatic gastroesophageal reflux referred by pediatricians to pediatric gastroenterologists. *J Pediatr* 2003;**142**:57–61.
7 Hegar B, Rantos R, Firmansyah A, De Schepper J, Vandenplas Y. Natural evolution of infantile regurgitation versus the efficacy of thickened formula. *J Pediatr Gastroenterol Nutr* 2008;**47**: 26–30.
8 Holzapfel WH, Haberer P, Snel J, Schillinger U, Huis in't Veld JH. Overview of gut flora and probiotics. *Int J Food Microbiol* 1998;**41**: 85–101.
9 Gibson GR, McCartney AL. Modification of the gut flora by dietary means. *Biochem Soc Trans* 1998;**26**:222–8.
10 Dunne C, O'Mahony L, Murphy L, Thornton G, Morrissey D, O'Halloran S *et al.* In vitro selection criteria for probioticbacteria of human origin: correlation with in vivo findings. *Am J Clin Nutr* 2001;**73**:386S–92S.
11 Bennet R, Nord CE, Zetterstrom R. Transient colonisation of the gut of newborn infants by orally administered bifidobacteria and lactobacilli. *Acta Paediatr* 1992;**81**:784–7.
12 Indrio F, Ladisa G, Mautone A, Montagna O. Effect of a fermented formula on thymus size and stool pH in healthy term infants. *Pediatr Res* 2007;**62**:98–100.
13 Langhendries JP, Detry J, Van Hees J, Lamboray JM, Darimont J, Mozin MJ *et al.* Effect of a fermented infant formula containing viable bifidobacteria on the fecal flora composition and pH of healthy full-term infants. *J Pediatr Gastroenterol Nutr* 1995;**2**:177–81.

14 Indrio F, Riezzo G, Raimondi F, Bisceglia M, Cavallo L, Francavilla R. The effects of probiotics on feeding tolerance, bowel habits and gastrointestinal motility in preterm newborns. *J Pediatr* 2008;**152**:801–6.
15 Hyman PE. Childhood functional gastrointestinal disorders: neonate/toddler. *Gastroenterology* 2006;**130**:1519–26.
16 Darwiche G, Bjorgell O, Thorsson O, Almer LO. Correlation between simultaneous scintigraphy and ultrasonographic measurement of gastric emptying in patients with type 1 diabetes mellitus. *J Ultrasound Med* 2003;**22**:359–66.
17 Massey BT, Simuncak C, LeCapitaine-Dana NJ, Pudur S. Transient lower esophageal sphincter relaxations do not result from passive opening of the cardia by gastric distention. *Gastroenterology* 2006;**130**:89–95.
18 Penagini R, Carmagnola S, Cantu P, Allocca M, Bianchi PA. Mechanoreceptors of the proximal stomach: role in triggering transient lower esophageal sphincter relaxation. *Gastroenterology* 2004;**126**:49–56.
19 Salvia G, De Vizia B, Manguso F, Iula VD, Terrin G, Spadaro R *et al*. Effect of intragastric volume and osmolality on mechanisms of gastroesophageal reflux in children with gastroesophageal reflux disease. *Am J Gastroenterol* 2001;**96**:1725–32.
20 Wildi SM, Tutuian R, Castell DO. The influence of rapid food intake on postprandial reflux: studies in healthy volunteers. *Am J Gastroenterol* 2004;**99**:1645–51.
21 Battaglia E, Grassini M, Navino M, Niola P, Verna C, Mazzocchi A *et al*. Water load test before and after PPI therapy in patients with gastro-esophageal reflux disease. *Dig Liver Dis* 2007;**39**:1052–6.
22 Barbieri CL, Troncon LE, Herculano JR Jr, Aprile LR, Moraes ER, Secaf M *et al*. Postprandial gastric antral contractions in patients with gastro-oesophageal reflux disease: a scintigraphic study. *Neurogastroenterol Motil* 2008;**20**:471–8.
23 Kunze WA, Mao YK, Wang B, Ma X, Forsythe P, Bienenstock J *et al*. Lactobacillus reuteri enhances excitability of colonic AH neurons by inhibiting calcium dependent potassium channel opening. *J Cell Mol Med*, 2009;**13**:2261–70.
24 Faussone-Pellegrini M, Vannucchi MG, Alaggio R, Strojna A, Midrio P. Morphology of interstitial cells of Cajal of the human ileum from foetal to neonatal life. *J Cell Mol Med* 2007;**11**:482–94.
25 Collins SM, Bersick P. The relationship between intestinal microbiota and the central nervous system in normal gastrointestinal function and disease. *Gastroenterology* 2009;**136**:2003–14.

