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Full Length Research Paper

The effect of fermented milk with *Bifidobacterium infantis* on intestinal disorders in the case of antibiotherapy with amoxicillin and contamination with enteropathogenic *Escherichia coli* (EPEC)

Hamma Samia[1]*, Nicoletti Cendrine[2] and Sadoun Djamila[3]

[1]Laboratory of Applied Microbiology, Department of Microbiology, Faculty of Natural and Life Sciences, University A.MIRA of Béjaia, 06000, Algeria.
[2]Faculté des Sciences et Techniques de St Jérôme, avenue Escadrille Normandie Niémen, 13397 Marseille cedex 20, Algeria.
[3]Laboratory of Applied Microbiology, Department of Microbiology, Faculty of Natural and Life Sciences, University A.MIRA of Béjaia, 06000, Algeria.

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This study deals with the ingestion of fermented milk with *Bifidobacterium infantis*, and its effect on intestinal disorders and on the intestinal lining during antibiotherapy with amoxicillin and contamination with enteropathogenic *Escherichia coli* EPEC O111.B4, the latter being responsible for 45% of infant diarrhoea in Algeria. Our results showed that the growth of *B. infantis* was not affected either by the presence of EPEC or by the administration of Amoxicillin. Inversely, an antagonistic effect of *B. infantis* on EPEC was observed with inhibition rates reaching 100% whether in presence of Amoxicillin or not, with survival rates of 100% versus zero in batches where *B. infantis* was not ingested. An inhibiting effect on Enterobacteria was observed. After dissection of all rabbits, macroscopic and microscopic observations of histological sections of the digestive tract (small intestine and colon), showed that rabbits that received amoxicillin associated or not with contamination with EPEC suffered from severe intestinal atrophy with degradation of intestinal tissues (lining and mucous membranes). However, a less significant impact was observed among rabbits that underwent antibiotherapy associated with contamination with EPEC but ingested fermented milk with *B. infantis.* Total regeneration of tissues was observed 15 days after the first dissection. On the other hand, no pathological anomaly was observed among rabbits that ingested fermented milk with *B. infantis* associated with contamination with EPEC or amoxicillin. These results showed that the number and the length of survival of *B. infantis* cells in the rabbit digestive tract during the ingestion of fermented milk with *B. infantis* and after ingestion ended were sufficient to enable it to exert probiotic effects.

Key words: *Bifidobacterium infantis*, EPEC O111.B4, amoxicillin, diarrhoea, intestinal atrophy.

INTRODUCTION

Intestinal flora comprise the first line of defence of the intestine against pathogens, the intestinal epithelium and its mucus form the second line, and the intestinal immune system the third. Intestinal flora is affected by different factors including the individual's state of health (Guggenbuhl, 2004). The nature of the microbial flora in the digestive tract can considerably influence the health of the infant and an imbalance in the normal flora can lead to colonization of the digestive tract by undesirable bacteria, which cause diarrhoeal infections. Diarrhoeal diseases are among the major causes of infant mortality in developing countries. According to W.H.O. estimates, diarrhoea is responsible for the death of 3.2 million child-

*Corresponding author. E-mail: hamma_samia@yahoo.fr. Tel: 213 034 21 47 62. Fax: 213 034 21 47 62.

ren under five every year all over the world (Simeoni, 2000). Diarrhoea is also the most frequent side effect of antibiotherapy. It has been demonstrated that the administration of certain bacterial strains of *Bifidobacteria* prevents the harmful effects of broad spectrum antibiotics (Rigaud, 2003). In a study carried out in mice, Moreau et al. (1994) showed that the Bifidobacteria in mother's milk considerably reduced the risk of diarrhoeas in infants. Many studies have shown that probiotics reduced the incidence and the severity of diarrhoea among children hospitalized for gastro-enteritis (Philipe, 2002). Similarly, probiotics appear to reduce the incidence of diarrhoea associated with antibiotics. In recent years, Bifidobacteria have attracted considerable attention due to their overall beneficial effects on health (Peter et al., 2001); they play a significant role in maintaining the balance of intestinal microflora by correcting intestinal disorders and by fighting against diarrhoea and gastro-enteritis. They exert antagonistic activity against entero-pathogenic *E. coli* (EPEC), the micro-organism generally associated with acute infantile diarrhoea (Fooks and Gibson, 2002).

In this study, we conducted a survey among paediatricians to determine the frequency of antibiotic treatments to enable us to classify antibiotics in order of their use. This investigation was followed by microbiological analysis of the stools of 120 infants who were the most affected by diarrhoea in order to classify the incidence of the diarrhoea as a function of the type of the germ responsible. Finally, with the aim of developing a pharmafood containing *Bifidobacterium infantis* associated with *per os* antibiotherapy, an *in vivo* study was carried out on rabbits that received amoxicillin (the most widely-used antibiotic among infants in Algeria) and that were contaminated with EPEC resistant to this antibiotic. This study enabled us to observe the effect of the association of *B. infantis* with *per os* antibiotherapy on the intestinal lining and in the treatment of diarrhoea.

MATERIAL AND METHODS

Bacterial strains

B. infantis is a type ATCC 15697 strain was acquired from the (LMA) laboratory collection. We chose this species because of its antagonistic effect on EPEC (Gibson and Wang 1994), its resistance to gastric juice (Biavati et al., 1992) and its capacity to survive at high rates (10^8 ¢g^{-1}) in the intestine (Marteau et al., 1992).

The strain of *E. coli* (EPEC O111 B4) used was isolated starting from the infant diarrhoeal stools. We chose this species after analysis of 120 coprocultures of diarrhoeal infant stools.

Culture conditions and growth

MRS Agar base for microbiological analysis (Merck) added to 0.5 gl^{-1} of L cysteine (Merck) and to 0.2 gl^{-1} of nalidixic acid (IPA) were used for enumeration of *B. infantis* in the inoculum and in rabbit faeces. EMB base medium (Eosine and Methylene blue, IPA) was used for enumeration of EPEC in the inoculum and in rabbit faeces. The growth of the two strains was monitored in a Petri box, in order to count the number of living bacteria in faeces over time (in days), and to monitor the colonies' development in the culture medium (for EPEC, the colonies were violet in colour with a metallic lustre on EMB medium).

Trial to optimize the resistance of *B. infantis* to amoxicillin

The trial to obtain resistance of *B. infantis* to this antibiotic was performed using the well method. Once the CMI of the selected antibiotic was determined, the strain was subjected to increasing doses of antibiotics to obtain resistance to even higher doses than the therapeutic doses generally given to new born babies.

Rabbits

The experiments were carried out on males and females of the same species (*Orycctolagis arniculus*) and of the same age (30 days), weighing between 450 and 500 g at the beginning of the experiment. The rabbits were kept separately in metal cages 50 cm in dimension in a well-aired animal house at a constant temperature of 21 ± 1°C, and 12 h of light from 8 a.m. to 8 p.m. The rabbits were fed with carrots and lettuce. Water was distributed in suitable drinking-bottles. The cages were cleaned every morning.

Preparation of stage 1 infant milk

The milk used in this study was Gigoz stage 1 infant milk (Nestle, Switzerland). The milk was prepared as follows: 114 g of milk powder were dissolved in 900 ml of distilled sterile water according to the manufacturer's instructions. Homogenisation was performed under a UV laminar flow hood.

Preparation of fermented milk

Milk fermented with *B. infantis* with added EPEC inoculum was prepared every day throughout the period of treatment: *B. infantis* ferment*:* 2 colonies were inoculated in 9 ml of the prepared infant milk and incubated for 18 h at 37°C. EPEC inoculum: 2 colonies were inoculated in 9 ml of prepared infant milk and incubated for 18 h at 44°C.

Preparation of the antibiotic

The antibiotic used was amoxicillin (125 mg), one bottle contained 30 g of powder (corresponding to 60 ml after addition of sterile distilled water (manufacturer Bristol-board-Myers Squibb s.r.i. 04010 Sermoneta, Italy). The usual daily dose for an infant is a syrup spoon containing 125 mg administered twice a day, i.e. 0.5 ml of syrup equivalent to 2.5 mg of antibiotic powder, administered to the rabbit twice a day to (amount adapted to the weight of the rabbit).

Analysis of rabbit intestinal flora

Twenty rabbits were separated into 5 batches of 4 rabbits each; each batch received a specific treatment (standard inoculated germ, duration of treatment). Before starting the study, a search was made for EPEC and *B. infantis* in the faecal flora of rabbits and the number of Enterobacteria was counted three days after the animals had been installed in their cages for the period of experimentation.

Collection of faeces

Faeces were recovered 4 h after treatment every day during the period of ingestion of the fermented milk by the young rabbits, and, a short time later, 1 g of faeces was diluted in 9 ml of physiological water (9 g NaCl/ml), and 1 ml of the suspension was removed for analysis.

Procedure for inoculation of rabbits with the germs to be tested

Batch 1: The rabbits received a therapeutic amount of amoxicillin (2.5 mg, twice/day) for one week.
Batch 2: The rabbits received a therapeutic amount of amoxicillin (2.5 mg, twice/day) for one week along with 1 ml (10^7 CFUml^{-1}) of EPEC inoculum.
Batch 3: The rabbits received a therapeutic amount of amoxicillin (2.5 mg, twice a day for one week with, successively, 1 ml of fermented milk with *B.infantis* (10^8 CFU ml^{-1})* and 1 ml (10^7 CFU ml^{-1})* of EPEC inoculum.
Batch 4: The rabbits received successively, 1 ml of fermented milk with *B. infantis* (10^8 CFU/ml) and 1 ml (10^7 CFU ml^{-1}) of EPEC inoculum for one week.
Batch 5: The rabbits received a therapeutic amount of amoxicillin (2.5 mg, twice a day) with 1 ml of fermented milk with *B. infantis* (10^8 CFU ml^{-1}) for one week.

*the numbers 10^8 of *B. infantis* and 10^7 *E. coli* were not chosen arbitrarily, but represent the numbers generally found in healthy infants fed with mother's milk (10^8 ¢ of *B. infantis)* and in diarrhoeal infants (10^7 ¢ of EPEC), respectively.

Microbiological analysis

B. infantis and EPEC were counted daily in fermented milk and in previous rabbit faeces during and after the different treatments for one week. The purpose of these analyses was to determine the number of living *B. infantis* and EPEC, and how long they survived in the intestine. Successive decimal dilutions were performed and a 1 ml dilution was added in the MRSc medium for the *B. infantis strain* and on the surface of EMB plates for the EPEC strain. The *B. infantis* was then incubated for 48 h at 37°C in an anaerobiosis jar and the EPEC at 44°C. In the curves, the values representing the number of colonies formed are log 10.

Statistical analysis

Results were analysed (analysis of variance and probability analyses) using ANOVA. Analysis of variance was carried out to determine the significance of the results.

Histological test – selection of samples

To study the impact of the different treatments (antibiotic, *B. infantis* and EPEC) on the intestinal mucus membrane, we dissected rabbits after each death or at the end of the study. The small and large intestines were stored in 10% formol-saline.

Preparation of histological blades

Preparation was carried out according to the following stages recommended by Hould (1998): macroscopic study, sample selection of cuts and deposits in the histocassettes, fixing with formol, dehydration with ethanol then with acetone, enlightenment with xylene, 1st paraffin bath, inclusion (2 h) and coating with paraffin, refrigeration and freezing, roughing-out at 20 µm (elimination of excess paraffin), sections made with a microtome (to obtain very thin 4 µm strips which were then spread on supports, staining with hematoxylin-eosin: H.E), and assembly between blade and slides (resin) and finally, microscopic observation.

RESULTS AND DISCUSSION

Selection of antibiotic

An epidemiologic survey revealed that amoxicillin is the most widely prescribed *per os* antibiotic in Bejaia (Algeria) and is prescribed with a frequency of 49.2%. The use of amoxicillin is also highly correlated with the occurance of diarrhoea. The cefixin and the association of amoxicillin with acid clavuranic, erythromycin and its associations may lead to 30% of diarrhoea when used (Gottrand, 2006). In fact, gastro-intestinal signs appear during the important use of antibiotic at a broad spectrum (Merad and Merad, 2001).

Selection of *B. infantis*

The use of *B. infantis* is justified by its predominance in the intestinal flora of infants fed with mother's milk. This species has been shown to play several different roles including antagonism against pathogenic agents, thanks to various substances such as bacteriocins. Maizke-Johnson (1997) and Lievin et al. (2000) identified a proteinic substance produced by *B. infantis* that appeared to have a bactericidal effect on EPEC. Lievin et al. (2000) observed a reduction of 5 logarithmic units in the initial number of EPEC after 3 h of contact with *B. infantis* supernatant at 10^8 CUF ml^{-1}. The strain of *B. infantis* in which resistance was optimized (Figure 1) could be used for the prevention or treatment of diarrhoea accompanying antibiotherapy. Tissier advised the administration of bifidobacteria to children suffering from diarrhoea, stating that the latter replace the bacteria that cause the disease (Schrezenmeir and Vrese 2001). Optimizing the resistance of *B. infantis* to amoxicillin is very important because all the antibiotic treatments, except colimazon, destroy the bifidobacteria populations. Amoxicillin, an antibiotic frequently prescribed for children, is active *in vitro* in most of strains of bifidobacteria (Pochart, 2007). In fact, amoxicillin prevents for a long time, the increase of a new bacterial family (Rousseau, 2002)

Selection of EPEC

The presence of pathogenic germs was observed in all the 120 coprocultures carried out on infants suffering from diarrhoea after having being treated with penicillin, particularly amoxicillin. The presence of pathogenic germs

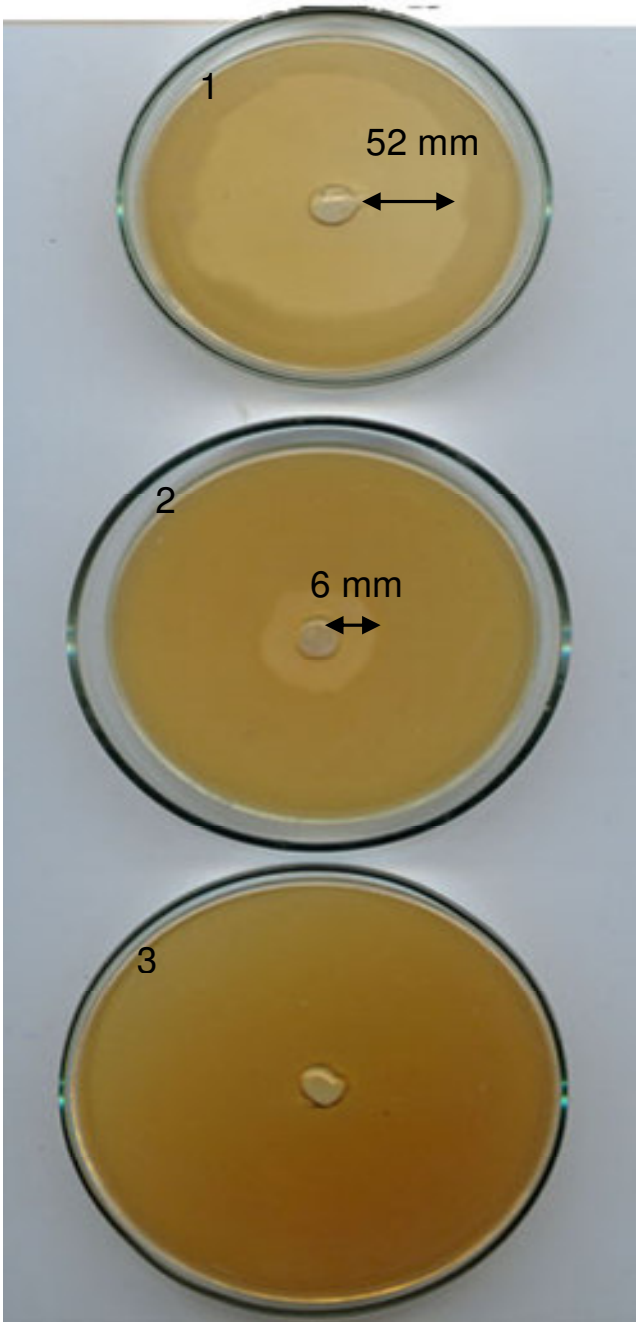

Figure 1. Observation of the inhibition zone by amoxicillin. 1 represents the CMB, 2 represents the CMI, and 3 show the resistance acquired by *B. infantis*.

made it possible to analyze the distribution of the diarrhoeal cases as a function of the type of germ: 45% *E. coli*, 24% Salmonellas, 10.66% Shigelles, 9% *Campylobacter*, 7.4% *Klebsiella* and 4% of parasitic origin (Figure 2). *E. coli* with 45% of the cases remains a frequent cause of diarrhoea in developing countries and is responsible for epidemics of gastro-enteritis in hospitals (Knut, 2001). The strain of *E. coli* involved was identified as EPEC O111. B4. Enteropathogenic *E. coli* is

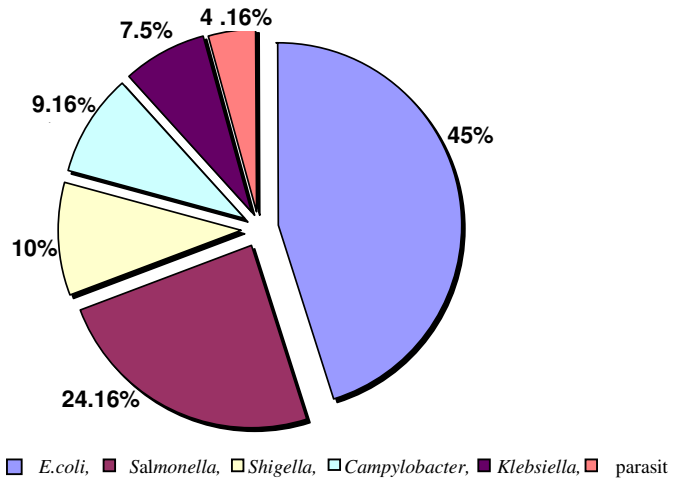

Figure 2. Identified germs-distribution on positive coprocultures during the experiment.

one of the principal causes of infant diarrhea (Crivelli et al., 2000). Diverse infections, agents are responsible for the intestinal infections, particularly those passed on children and of which *EPEC* remains the most responsible (Vallance and Finky, 2000; Clarke et al., 2003).

Enumeration of *B. infantis* and EPEC in the pre-fermentation stage

After 18 h of culture, the average number of cells of *B. infantis* and EPEC were 2 x 10^8 and 4 x 10^7 CFU ml^{-1}, respectively. These rates were reduced to 10^8 ¢ml^{-1} for *B. infantis,* and to 10^7 ¢ml^{-1} for EPEC in sterile milk in the pre-fermentation stage. This rate of *B. infantis* is essential because probiotic action is obtained only if the strain is established in the digestive tract in numbers equal to or higher than 10^7 germs g^{-1} of faeces (Dilmi and Sadoun 2002). 10^7 *E. coli* were not chosen arbitrarily, but represent the numbers generally found in diarrhoeal infants (Harris, 2006).

Development of *B. infantis* and EPEC in rabbit faeces

Bacteriological analysis of rabbit faeces in the different batches did not reveal the presence of any species involved in our study. *B. infantis* is considered to be a species of human origin. Among some *species E. coli* was only observed after the 45th day.

Results of the faecal counts of *B. infantis* and EPEC in all 5 batches are given in Figures 3 and 4. Concerning batches 1 and 2 (Figure 5), an increase in Enterobacteria was observed during the week of antibiotherapy alone or with EPEC, and contamination exceeded 10^{12} UFC/ml accompanied by diarrhoea after the 3rd day. After the 10th day, the death rate was 100%. However, a reduction in

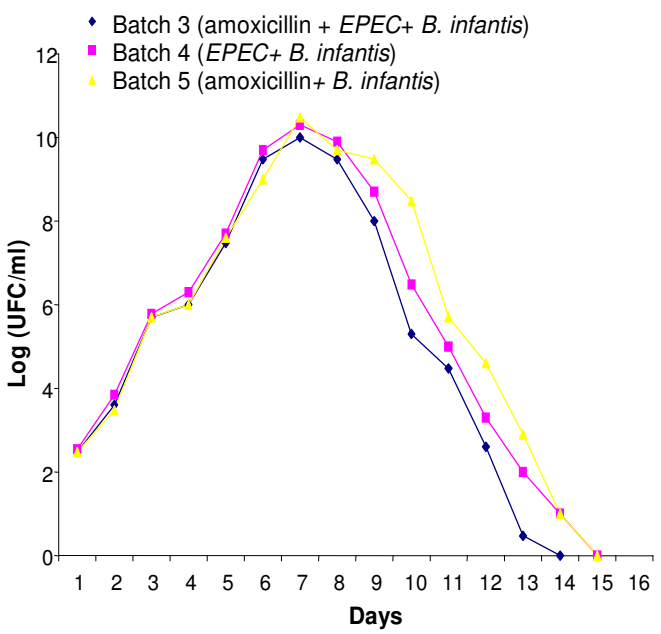

Figure 3. Changes in the number of *B. infantis* in batches 3, 4 and 5.

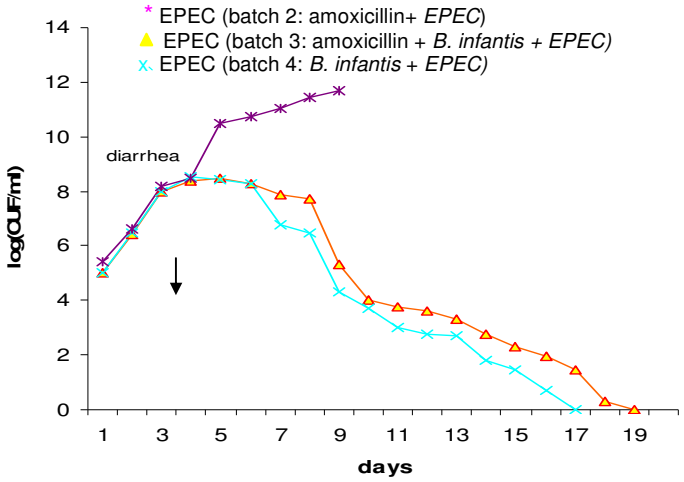

Figure 4. EPEC numbers evolution in rabbit's faeces.

the number of Enterobacteria in the faeces was observed after the 6th day in rabbits in batches 3 and 4 (Figure 5) that received *B. infantis*, and, four days later, the number reached the original number, that is, 10^5 CFU ml^{-1} observed in control rabbits. In batch 5, in which *B. infantis* was introduced at the same time as antibiotherapy but without contamination with EPEC, the number of Enterobacteria remained stable at approximately the original number throughout the period of ingestion of *B. infantis* and even after ingestion ended. A highly significant difference was observed between the number of Enterobacteria in faeces in rabbits in batches 1 and 2 and those in batches 3 and 5 in which *B. infantis* was ingested. Indeed, a viability rate of 100% was obtained for the latter.

The antagonistic effect of *B. infantis* against EPEC in batches 3, 4, and 5 appeared at the end of the 4th day when the number of *B. infantis* reached a rate of about 10^7 CFU/ml. This number continued to increase throughout treatment and finally reached a threshold of 10^{10} CFU ml^{-1}. However, the number dropped significantly ($p<0.05$) to reach 10^5 cells g^{-1} of stools 72 h after ending consummation of fermented milk with *B. infantis* and tended to disappear six days later. The works of Marteau et al. (1992) have demonstrated that after stopping the consummation, the concentrations in exogenous bifidobacteria diminished progressively and in parallel with the markers of transit to completely disappear in a period of 8 days. The strain of bifidobacteria analysed by Kuller et al. (1997) was eliminated after the consummation had been stopped. Nevertheless, non survival does not necessarily result in the absence of beneficial effects (Mgrilli et al., 2003).

We observed a non significant difference between changes in the *B. infantis* rates in the three batches (in presence or not of EPEC or and antibiotic). An increase in EPEC $ml^{-1}$was observed during the first five days of the study reaching respectively: 3 x 10^{10} ¢ml^{-1} for batch 5, 2 x 10^8 ¢ml^{-1} for batch 4, and 3 x 10^8 ¢ml^{-1} for batch 3, with diarrhoea beginning around the 3rd day. In batch 2, the number of EPEC ml^{-1} continued to increase even after contamination and antibiotic treatment was ended, reaching 3 x 10^{12} ¢ml^{-1} on the 9th day and resulting in a death rate of 100% by the 10th day concomitant with acute diarrhoea. However, among rabbits in batches 2, 3 and 4 that received fermented milk with *B. infantis*, the number of EPEC started to drop after the 5th day of administration, and continued to decrease after administration was stopped and throughout follow-up, and tended to disappear at the end of the 15th day of the study, by which time the rabbit faeces were again normal. Many studies have shown the efficiency of taking into account bifidobacteria in the treatment of infectious diarrhoea, notably pseudo membranous colitis (Neish, 2002)

Indeed, a highly significant difference between the number of ¢$ml^{-1}$of EPEC was observed between batches 3, 4 and 5 in which *B. infantis* was ingested, and in batch 2 in which *B. infantis* was not ingested. These results showed that administration of resistant *B. infantis* at the same time as antibiotherapy and during EPEC contamination ensured protection against the harmful effect of the latter (acute diarrhoea). Wolin and Coll (1998) noted that the administration of large numbers of Bifidobacteria reduced the risk of infantile diarrhoea. In practice, intestinal flora disorders among prematures due to antibiotherapy can be treated by administrating cultures of Bifidobacteria. Administration of a combination of *B. longum* and *L. acidophilus* (these strains being resistant to antibiotic treatments) to infants aged 13 days to 3 months, prevents digestive disturbances.

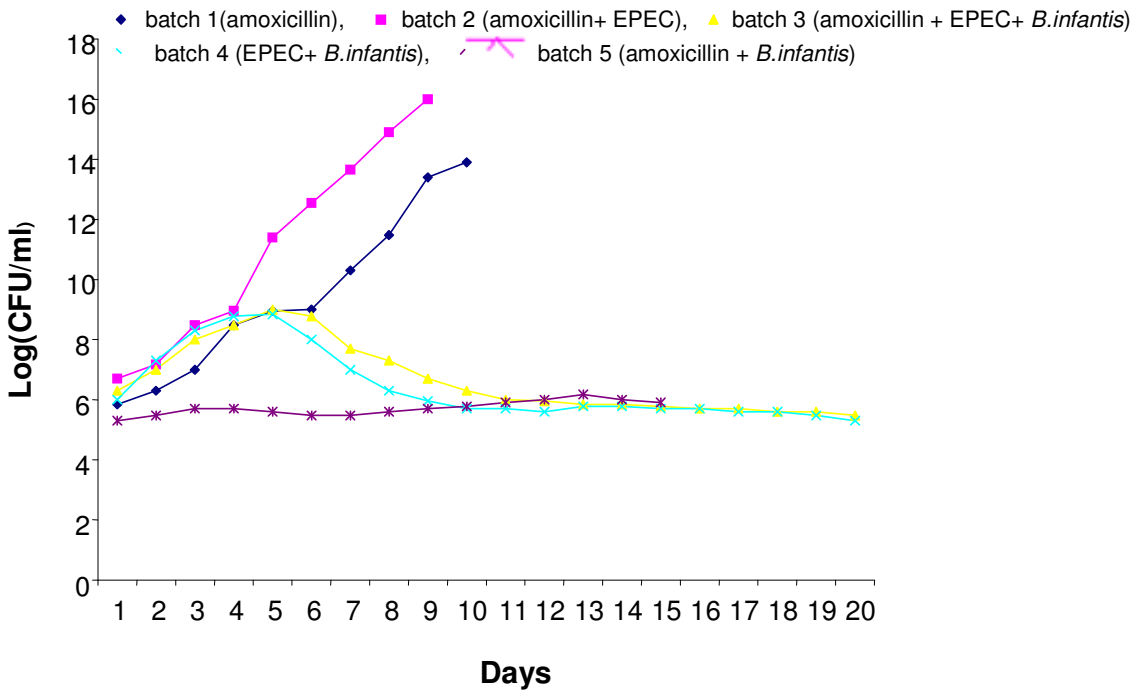

Figure 5. Changes in the number of Enterobacteria in rabbit faeces in the different batches.

Our results are noteworthy because *B. infantis* was able survive in the intestine at satisfactory rates and to exert a probiotic effect throughout the period of ingestion and even for a few days after ingestion ended, in spite of the presence of antibiotics.

Impact of the association of *B. infantis,* amoxicilline, and EPEC on the intestinal lining

Macroscopic study

Observations with the naked eye of the different intestinal segments (small intestine (ig) and the colon (Gi) after dissection of the rabbits were as follows:

Control rabbits (no treatment): the colic samples were of uniform size (0.5 cm). During dissection we observed that the lining was fibrous and whitish in colour. When the colon was sectioned, we observed it had two thin linings of firm consistency. The samples removed from the small intestine were of uniform size (0.3 cm) and thread-like in appearance.

Batch 1 (antibiotherapy only): the colic segments were of uniform size (1.2 cm in diameter) with a flattened mucous membrane, and were reddish brown in colour. This change in colour compared to controls (whitish), probably masked very serious intestinal infection. Sections from the small intestine revealed a very thin translucent lining of uniform size (0.1 cm in diameter). Contraction of the intestinal light was noted compared to controls (0.3 cm). Indeed, intestinal atrophy is a frequent consequence of antibiotherapy. A coloscopy made on patients suffering from post-antibiotic bleeding colitis by a derivative of penicillin has show variable lesions under the form of fragility of the mucous membrane and purpura (Marteau, 2005).

Batch 2 (antibiotic + EPEC): the colic samples displayed a flattened mucous membrane and were dark red in colour. When sectioned, the diameter of the colic segment was much smaller (0.1 cm) than in control rabbits (0.5 cm). We observed a very thin lining with a smooth surface and white pearly appearance with a reduced intestinal light. The intestinal samples (ig) had a very thin flat lining with a diameter of 0.4 cm; the slight increase in the intestinal light compared to the controls was probably due to the flatness of the intestine.

Batch 3 (EPEC + antibiotic + *B. infantis*): Contraction of the intestinal light was observed, the mucous membrane was flat and reddish brown in colour with a diameter of 1.3 cm. The small intestine was of uniform size with a slightly narrower intestinal light, 0.2 cm in diameter. The appearance (shape and colour) and contraction were less significant than those of the intestinal segments of the young rabbits in the preceding batches (batch 1 and 5).

To determine whether degradation of the rabbits' intestines resembled that of rabbits in batches 1 and 5, after dissection the same macroscopic study was carried

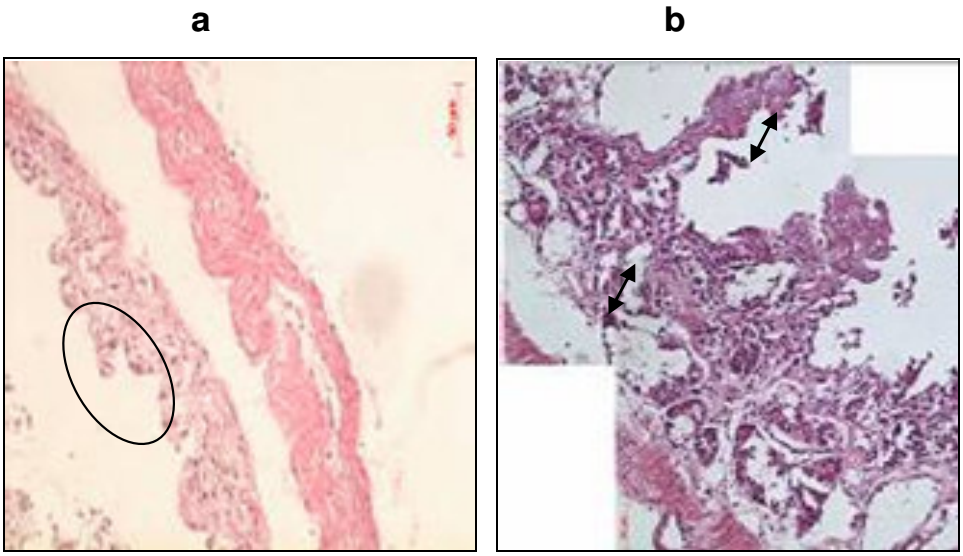

Figure 6. Microscopic observations of histological sections of the colon (a) and of the small intestine (b) of rabbits in batch 1 which displayed intestinal atrophy and degeneration of the mucous membrane.

out on young rabbits in the same batch two weeks later. The segments (ig and Gi) removed showed the same characteristics as those obtained in batches 2 and 4. These observations revealed that not only had the infection failed to develop but also that the intestines had recovered their normal appearance.

Batch 4 (EPEC + *B. infantis*): macroscopic observations of intestinal and colic segments did not reveal any change in colour, appearance or diameter compared to controls.

Batch 5 (antibiotic + *B. infantis*): the appearance of the samples was the same as the controls: a thin, fibrous lining 0.5 cm in diameter and whitish in colour. The intestinal samples (ig) showed the same characteristics as those observed in controls: uniform size, thread-like appearance and a diameter of 0.3 cm.

The results of the macroscopic study showed that the rabbits in batches 1 and 3 that did not receive *B. infantis* displayed symptoms of serious intestinal infection accompanied by marked contraction of the intestinal light (intestinal atrophy) probably due to the antibiotherapy or to contamination by EPEC. These after-effects disappeared (batch 3) or did not even appear (batches 2 and 4) in rabbits that received *B. infantis.* The clinical study of Mastrandrea et al. (2004), accomplished on human subjects (aged from 6 to 48 years) who show clinical symptoms of the irritated intestine syndrome has demonstrated the positive effect of probiotics in their treatment.

Lee et al. (2004) have show *in vitro* the inhibitor effect of *L. casei* and *B. longum* on the proliferation of tumour cells.

The *in vitro* and *in vivo* studies demonstrate that taking probiotics (*Streptococcus*, *Lactobacillus* and *Bifidobacterium*) reduces the colonization of the digestive tract by pathogenic bacteria and stimulates the specific immunity response of defence of the host by activating the lymphocytes, stimulating the anti-tumour activity and reducing infections like the vaginal colonization of coliforms and yeasts (Amrouche, 2005).

Microscopic study

The photographs of the histological sections of the digestive tract (small intestine and colon) taken after dissection of the young rabbits were taken with a microscope (Zeiss Axiovert 200 M-objective 20) and a Zeiss colour camera. These observations showed that the rabbits in batches 1 and 2 had the most diseased small intestine and colon.

The small intestine (Figure 6a) and the colon (Figure 6b) of rabbits in batch 1 (antibiotherapy only) were very seriously affected. The entire mucous membrane disappeared and the muscular membrane was very thin. Only phantoms of lamina propria remained in the mucous membrane. In the colon- where the mucous cells prevailed in controls (without any treatment) (Figure 7), the lamina propria were completely destroyed. These observations revealed the dilapidated state of the intestinal tissues of rabbits suffering from acute diarrhoea.

The same microscopic observations were made of rabbits in batch 2 (antibiotherapy plus contamination with EPEC). We observed degradation of the lining and of the intestinal mucous membrane of the small intestine (Figure 8b) and of the colon (Figure 8a) corresponding to

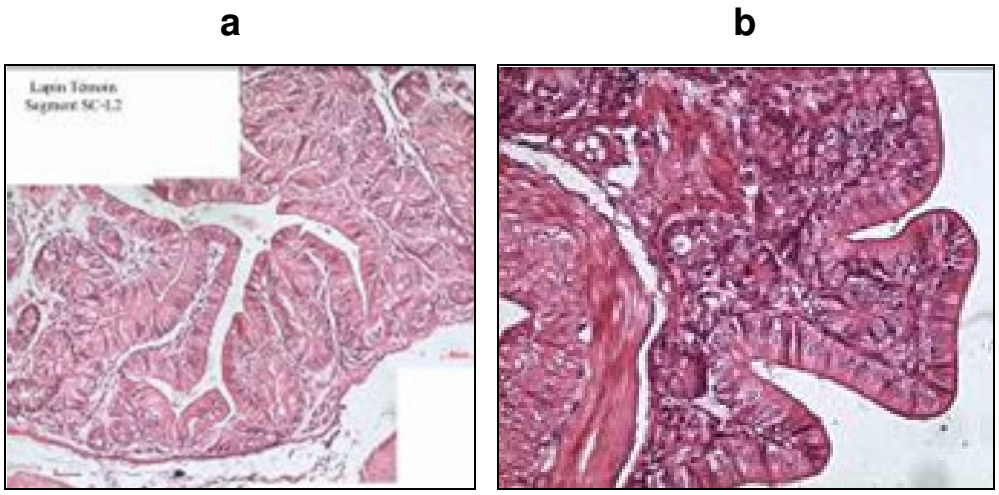

Figure 7. Microscopic observations of histological sections of the colon (a) and of the small intestine (b) of control rabbits which displayed no pathological anomaly.

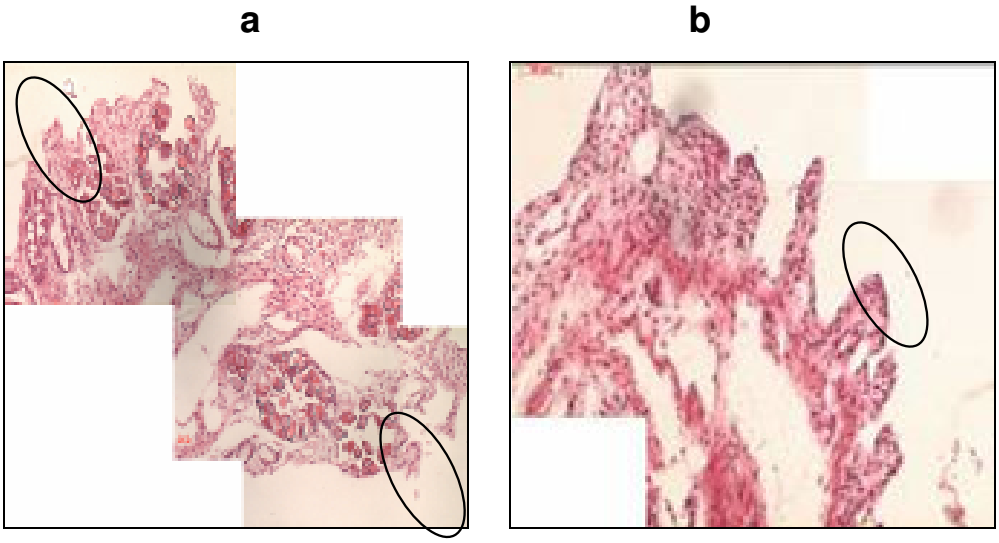

Figure 8. Microscopic observations of histological sections of the colon (a) and of the small intestine (b) of rabbits in batch 2 which displayed intestinal atrophy and degeneration of the mucous membrane.se

macroscopic observations in which we noted a contraction of the intestinal light and a change in appearance (shape, colour and consistency). The post-antibiotic diarrhoea is a condition that indicates that the intestine is irritated (Boclé, 2005). The pathogenic of diarrhoea associated with antibiotic treatments is not really known. In certain cases we invoke the toxicity of products on the intestinal mucous membrane (amoxicillin/ clavuranic acid, clindamicin). For rodents, disturbing the flora induced by the administration of antibiotics is susceptible of altering the maturation of the intestinal mucous (Scheman et al, 2005; Pochart, 2007).

In rabbits in batch 3 (EPEC + amoxicillin + *B. infantis*), the after-effects were less serious but nevertheless significant, and the small intestine was always seriously affected (Figure 9c), however in the colon (Figure 9a) the mucous membrane appeared to be less affected. The colic mucous membrane remained almost intact although the plate displayed a tendency to scale. In some observations only the crypt in the colon remained (Figure 9b). This colonization is attributed to the phenomenon of competitiveness for sites of adherence (Kelly et al., 2006). Hermendez-Manjarrez et al. (2000) showed that EPEC could induce lesions A/E (attaching/effacing) in the

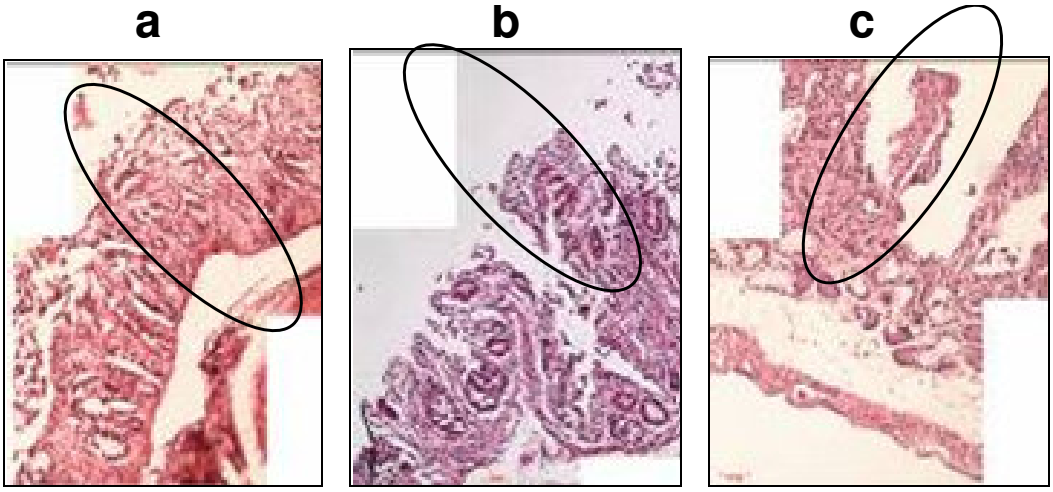

Figure 9. Microscopic observations of histological sections of the colon (a, b) and of the small intestine (c) of rabbits in batch 3 after the first dissection (at the end of the treatment): less severe atrophy of the intestinal mucous membrane.

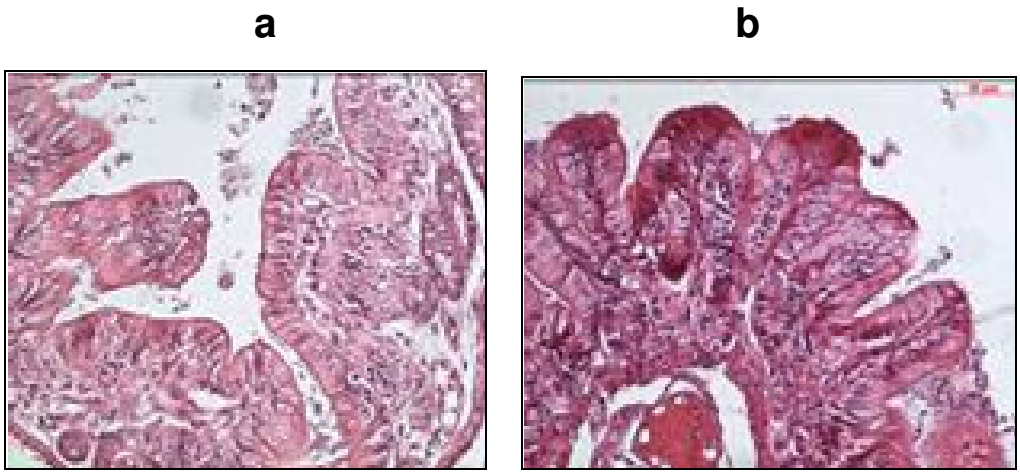

Figure 10. Microscopic observations of histological sections of the colon (a) and of the small intestine (b) of rabbits in batch 3, after the second dissection (15 days later): total regeneration of the intestinal mucous membrane.

intestinal epithelium. However, three weeks after stopping the treatment - 15 days after the first dissection - we ob-served complete recovery of the lining, with a return to its normal appearance (macroscopic observations), recovery of the mucous membrane of the small intestine (Figure 10b) and of the colon (Figure 10a), in rabbits in batch 3 which that ingested *B. infantis*. This leads us to the conclusion that the presence of *B. infantis* in the intestines for a given period facilitates the regeneration of intestinal tissues.

Concerning rabbits that ingested fermented milk with *B. infantis* associated with contamination by EPEC (batch 4), the administration of amoxicillin (batch 5), microscopic observations of the small intestine (Figures 11b and 12b) and of the colon (Figures 11a and 12a) did not reveal any significant modification of the intestinal lining or of the mucous membrane compared to controls (Figure 7). These results provide evidence for a probiotic and barrier effect, and/or protection exerted by *B. infantis* against the action of EPEC and amoxicillin.

Overall, microscopic observations confirmed the results of macroscopic observations and showed that ingestion of fermented milk with *B. infantis* at the same time as antibiotherapy, and in the case of contamination with EPEC, can reduce and even eliminate the harmful effects of EPEC (intestinal atrophy, destruction of the tissue) responsible for acute diarrhoea. The randomised studies on gnotobiotic mice have shown that the maintenance of normal intestinal flora may make the inflammatory signs completely disappear in mice (Neish,

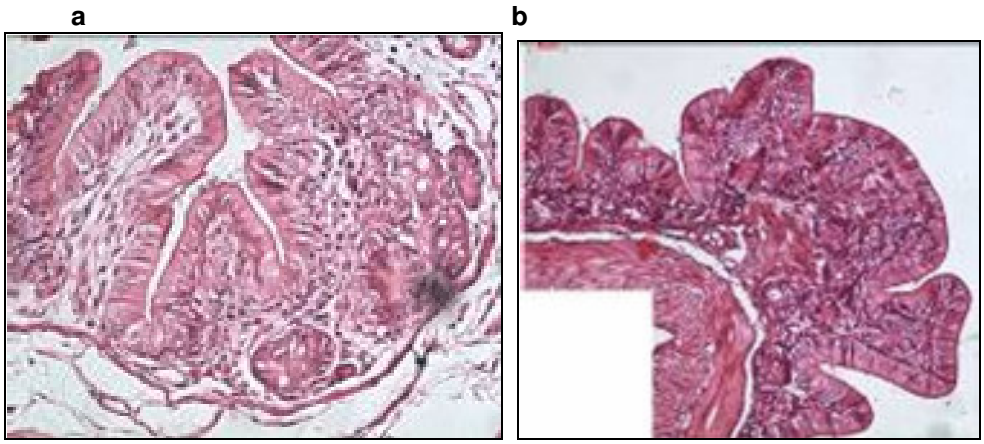

Figure 11. Microscopic observations of histological sections of the colon (a) and of the small intestine (b) of rabbits in batch 4, which displayed no pathological anomaly.

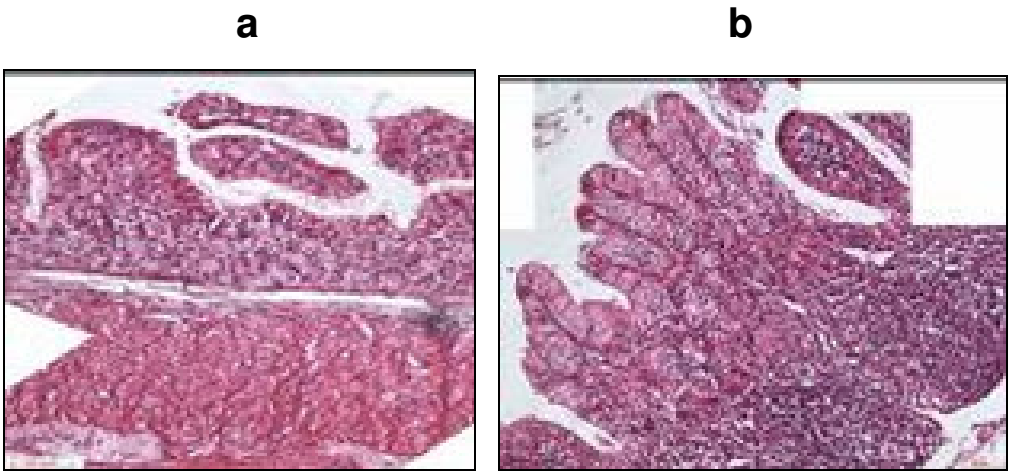

Figure 12. Microscopic observations of histological sections of the colon (a) and of the small intestine (b) of rabbits in batch 5 which displayed no pathological anomaly.

2002).

Indeed, bifidobacteria are widely used in yoghurt and other fermented milk products (Nebra and Blanch 1999). Probiotics with living bifidobacteria improve the microbial balance of the human gastro-intestinal tracts and can be used for treatment of infectious diarrhoea, chronic inflammatory intestinal diseases, and in experimental prevention of colon cancer (Macfarlane and Cummings, 2000).

Conclusion

The results of this study showed that ingestion of fermented milk with *B. infantis* antibioresistant at a rate of 10^8 ¢ml^{-1} was sufficient to enable the latter to withstand the acidity of gastric juice and survive in appreciable numbers during the entire period of ingestion of fermented milk (7 days). The number of cells remained significant as long as the rabbits consumed fermented milk with *B. infantis* and continued 62 h after consumption of the milk by the rabbits ended. *B. infantis* exerted an antagonistic effect on EPEC and on Enterobacteria. There was a highly significant difference between the numbers of EPEC and Enterobacteria in the faeces of rabbits treated with *B. infantis* and faeces of untreated rabbits, with a death rate of 100% among the latter suffering from acute diarrhoea. No significant difference was observed between changes in the rates of *B. infantis* in batches where the latter was ingested by rabbits (in presence or not of the antibiotic or of EPEC). Indeed, the results of macroscopic and microscopic observations of histological sections from the digestive tract (small intestine and colon) after dissection of all the rabbits showed that the rabbits that received antibiotics associated or not with contamination with EPEC showed signs of acute

intestinal atrophy accompanied by almost complete destruction of intestinal tissues (lining, mucous membrane). However a less significant impact was observed in young rabbits that received antibiotherapy combined with contamination with EPEC that ingested fermented milk with *B. infantis*. Full recovery of the intestinal lining was observed 15 days after the first dissection. Rabbits that ingested fermented milk with *B. infantis* combined with contamination with EPEC or with administration of an antibiotic showed no pathological anomaly.

Overall, our results showed that the number of cells and the length of the survival period of *B. infantis* in the digestive tract during ingestion and until the 5th day after ingestion ended were sufficient to enable *B. infantis* to exert its probiotic effect (antagonistic effect on EPEC and protection against the harmful effects of amoxicillin on the intestinal lining).

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