Research Journal of Dairy Sciences 2 (2): 35-44, 2008
ISSN: 1993-5277

The Effect of Fermented Milk with *Bifidobacterium infantis* on Intestinal Disorders in the Case of Antibiotherapy with Amoxicillin and Contamination with EPEC

[1]Hamma Samia, [2]Nicoletti Cendrine and [2]Sadoun DjamiLa
[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

Abstract: 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 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 are 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 children under 5 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 Bifido-bacteria prevents the harmful effects of broad spectrum antibiotics (Rigaud, 2003). In a study carried out in mice, Moureau 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 enteropathogenic flora within *E. coli* (EPEC), the micro-organism generally associated with acute infantile diarrhoea (Fooks and Gibson, 2002).

Corresponding Author: Hamma Samia, Laboratory of Applied Microbiology, Department of Microbiology, Faculty of Natural and Life Sciences, University A.MIRA of Béjaia, 06000, Algeria

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.

MATERIALS AND METHODS

Bacterial strains: *B. infantis* is a type ATCC 15697 strain 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 (Agar, lactobacillus for microbiological analysis according to Man, Rogosa and Sharpe, ref. Merck) added to 0.5 g l-1 of L cysteine (C3H8ClNO2S H_2O, Merck) and to 0.2 g l-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^{-1}) 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 1mL (10^7 CFU mL^{-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 (10^7CFU mL^{-})[1] of EPEC inoculum.

Batch 4: The rabbits received successively, 1 mL of fermented milk with *B. infantis* (10^8 CFU mL^{-1}) and 1mL (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.

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 log10.

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).
- 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.

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.* (2002) identified a proteinic substance produced by *B. infantis* that appeared to have a bactericidal effect on EPEC. Lievin *et al.* (2002) 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 (Fig. 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).

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 a penicillin, particularly amoxicillin. The presence of pathogenic germs 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 (Fig. 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 *Escherichia coli* is one of the principal causes of infant diarrhea (Crivelli *et al.*, 2000).

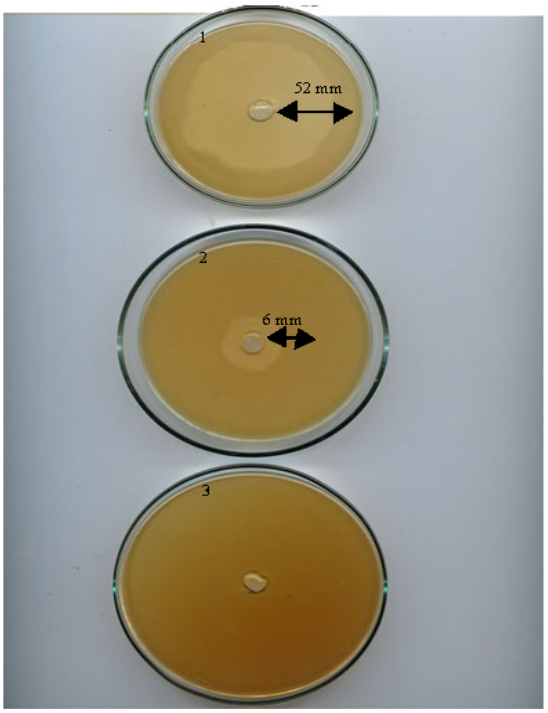

Fig. 1: Observation of the inhibition zone by *amoxicillin*, Box1 represent the CMB, Box 2 represent the CMI, Box 3 show the resistance acquired by *B. infantis*

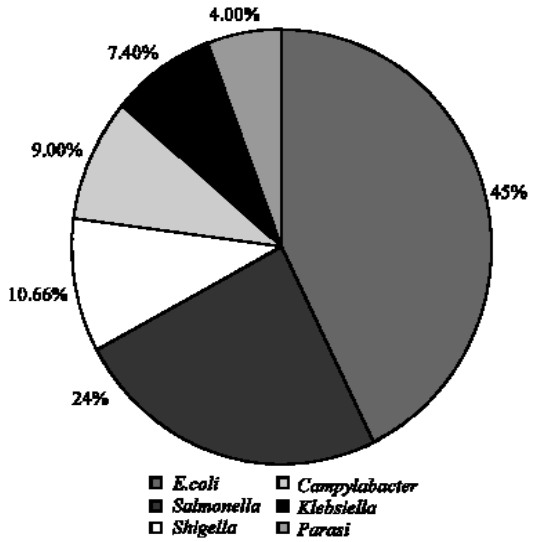

Fig. 2: Identified germs-distribution on positive coprocultures during the experiment

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, respectively 2.10^8 and 4.10^7CFU mL^{-1}. These rates were reduced to 10^8 ¢ mL^{-1} for *B. infantis* and to 10^7 ¢ mL^{-1} for EPEC in

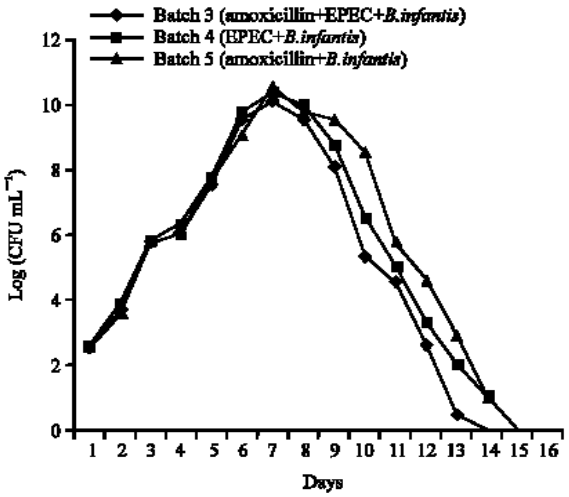

Fig. 3: Changes in the number of B. infantis in batches 3, 4 and 5

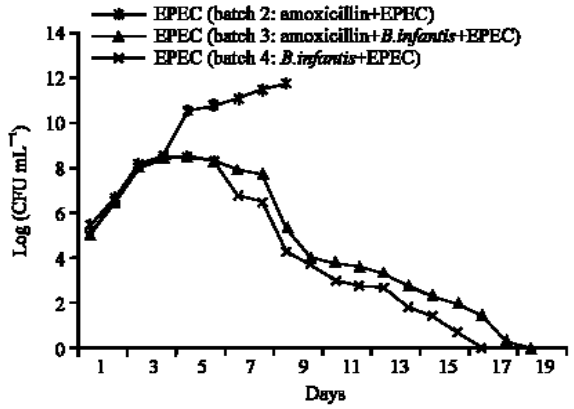

Fig. 4: EPEC numbers evolution in rabbit's faeces

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).

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 Fig. 3 and 4. Concerning batches 1 and 2 (Fig. 5), an increase in Enterobacteria was observed during the week of antibiotherapy alone or with EPEC and contamination exceeded 10^{12} UFC mL^{-1}

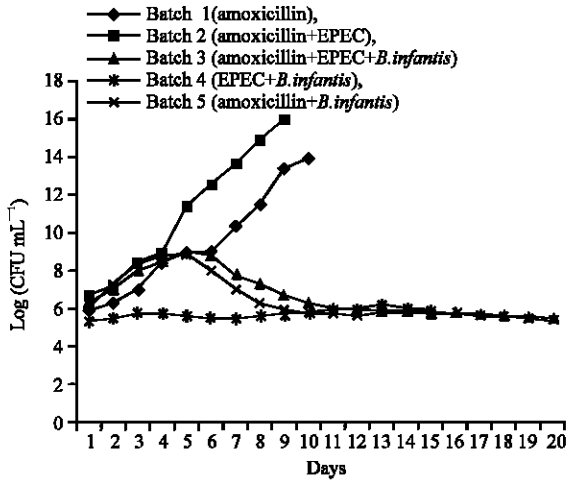

Fig. 5: Changes in the number of Enterobacteria in rabbit faeces in the different batches

accompanied by diarrhoea after the 3rd day. After the 10th day, the death rate was 100%. However, a reduction in the number of Enterobacteria in the faeces was observed after the 6th day in rabbits in batches 3 and 4 (Fig. 5) that received *B. infantis* and, four days later, the number reached the original number, i.e., 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^{-1}. 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 72h after ending consumption of fermented milk with *Bf infantis* and tended to disappear 6 days later.

We observed a significant difference ($p<0.05$) 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: 3.10^{10} ¢ mL^{-1} for batch 5, 2.10^8 ¢ mL^{-1} for batch 4 and 3.10^8 ¢ 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 3.10^{12} ¢ mL^{-1}on the 9th day and resulting in a death rate of 100 % by the 10^{th} 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.

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 inwhich *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 *et al.* (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 *Lb. acidophilus* (these strains being resistant to antibiotic treatments) to infants aged 13 days to 3 months, prevents digestive disturbances.

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 (0.3cm). Indeed, intestinal atrophy is a frequent consequence of antibiotherapy.

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 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 obser-vations 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*.

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 (Fig. 6a) and the colon (Fig. 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) (Fig. 7), the lamina propria were completely destroyed. These observations revealed the dilapidated state of the intestinal tissues of rabbits suffering from acute diarrhoea.

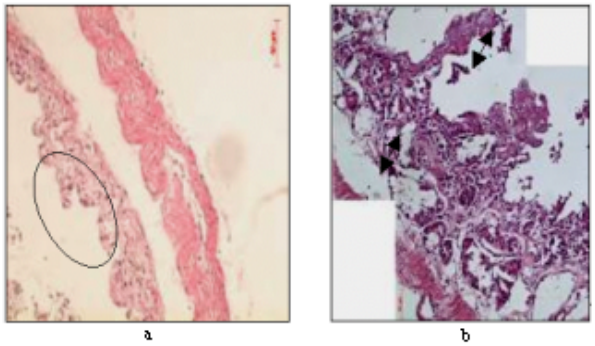

Fig. 6: Microscopic observations of histological sections of the colon (a) and of the small intestine (b) of rabbits in batch 1 which displayed intestinal atrophy and degeneration of the mucous membrane

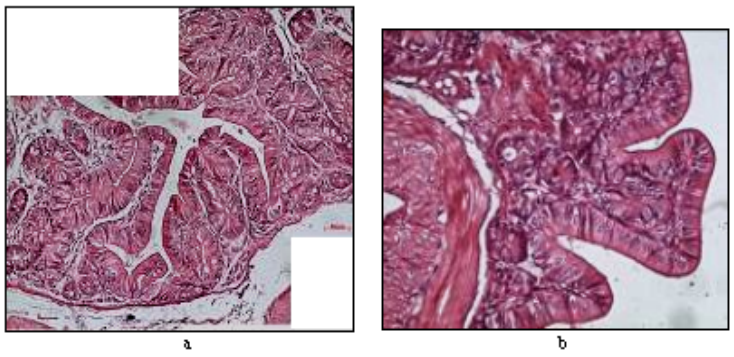

Fig. 7: Microscopic observations of histological sections of the colon (a) and of the small intestine (b) of control rabbits which displayed no pathological anomaly

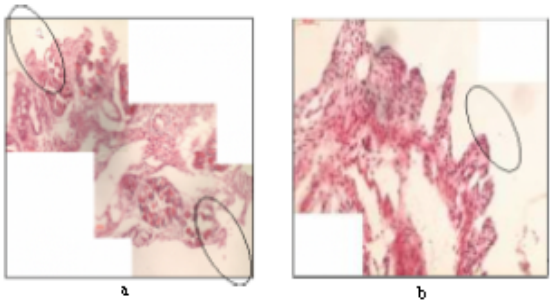

Fig. 8: Microscopic observations of histological sections of the colon (a) and of the small intestine (b) of rabbits in batch 2 which displayed intestinal atrophy and degeneration of the mucous membrane

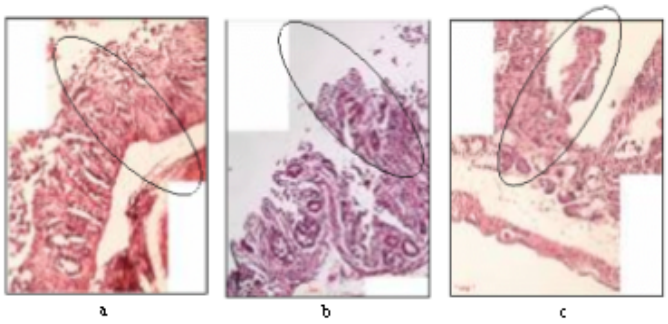

Fig. 9: Microscopic observations of histological sections of the colon (a, b) and of the small intestine (c) of rabbits in batch 3 after the first dissection (at the end of the treatment): less severe atrophy of the intestinal mucous membrane

The same microscopic observations were made of rabbits in batch 2 (antibiotherapy plus contamination with EPEC) (Fig. 8). We observed degradation of the lining and of the intestinal mucous membrane of the small intestine (Fig. 8b) and of the colon (Fig. 8a) corresponding to macroscopic observations in which we noted a contraction of the intestinal light and a change in appearance (shape, colour and consistency). In rabbits in batch 3 (EPEC+amoxicillin+*B. infantis)*, the after-effects were less serious but nevertheless significant (Fig. 9) and the small intestine was always seriously affected (Fig. 9c), however in the colon (Fig. 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 (Fig. 9b). Hernandez *et al.* (2000) showed that EPEC could induce lesions A/E (attaching/effacing) in the intestinal epithelium. However, 3 weeks after stopping the treatment-15 days after the first dissection - we observed complete recovery of the lining, with a return to its normal appearance (macroscopic observations) (Fig. 10), recovery of the mucous membrane

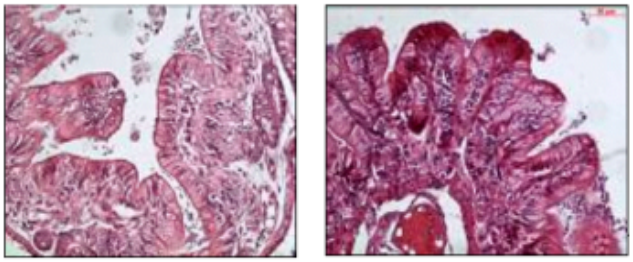

Fig. 10: Microscopic observations of histological sections of the colon (a) and of the small intestine (b) of rabbits in batch 3, after the second dissection (15 days later): total regeneration of the intestinal mucous membrane

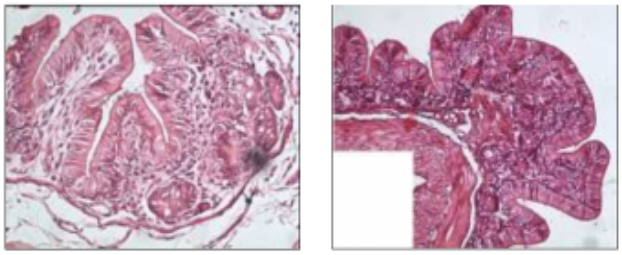

Fig. 11: Microscopic observations of histological sections of the colon (a) and of the small intestine (b) of rabbits in batch 4, which displayed no pathological anomaly

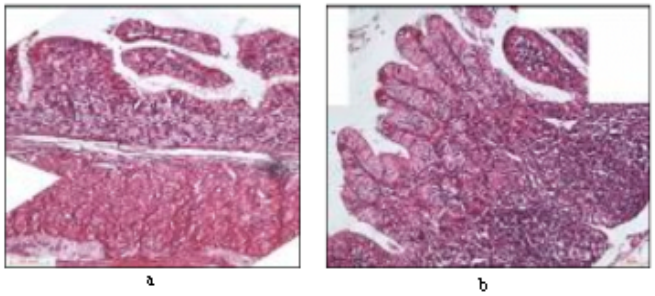

Fig. 12: Microscopic observations of histological sections of the colon (a) and of the small intestine (b) of rabbits in batch 5 which displayed no pathological anomaly

of the small intestine (Fig. 10b) and of the colon (Fig. 10a), in rabbits in batch 3which 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) (Fig. 11), the administration of amoxicillin (batch 5) (Fig. 12), microscopic observations of the small intestine (Fig. 11b and 12b) and of the colon (Fig. 11a and 12a) did not reveal any significant modification of the intestinal lining or of the mucous membrane compared to controls (Fig. 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. 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).

REFERENCES

Biavati, B., T. Sozzi, P. Mattarelli and L.D. Trovatelli, 1992. Survival of *Bifidobacterium* from human habit in acidified milk. Microbiological, 15: 197-200

Crivelli, A.P., J.M. Grivili-Parissi and J. Gibson, 2000. A recognition of enteropathogenic *Escherichia coli* virulence dominants by human. Colostrum and serum antibodies. J. Clin. Microbiol., 7: 2696-2700.

Dilmi-Bouras, A. and D.J. Sadoun, 2002. Effet du yaourt à *Streptoccoccus thermophilus* et *Lactobacillus delbruchii ssp bulgaricus* sur le cholestérol sanguin chez le lapin. Méd. Nutr., 38 (1): 24-32.

Fooks, L.J. and G.R. Gibson, 2002. *In vitro* investigation of the effect of probiotics and prebiotics on selected human intestinal pathogens FEMS. Microbiol. Ecol., 39: 67-75.

Guggenbuhl, N., 2004. Des probiotiques. Health and Food, 64: 1-3.

Gibson, G. and X. Wang, 1994. Regulatory effect of Bifidobacteria on the growth of their colonic bacteria. J. Appl. Bacteriol., pp: 412-420.

Hermendez-Manjarrez, H., S. Parra-Gavillanes, E. Berrocal-Chavez, A. Ocana-Navarro and A. Cravioto, 2000. Antigen-detection in enteropathogenic. *Echerichia coli* using secretory immunno globulin A antibodies isolated from human breast milk. Inf. Immune, 68 (9): 5030-5034.

Hould, R., 1998. Techniques d'histopathologie et de cytopathologie. Maloine-Editeur. Paris, pp: 400.

Knut, J.H., 2001. Probiotic bacteria in fermented foods: product characteristics and starter organism. Am. J. Clin. Nuts, 73 (2): 374-379.

Lievin, V., I. Perffer, S. Hudault, F. Rochrt, D. Brassart, J.R. Nesser and A.R. Servin, 2002. *Bifidobacterium* strains from resident infant human gastrointestinal microflora exert antibacterial activity. Gut, 47: 646-652.

Macfarlane, G.I. and J.H. Cummings, 2000. Probiotics and prebiotics can regulating the activity of intestinal bacteria benefit health? B.M.J., 318: 999-1003.

Maizke-Johnson, M.B., 1997. Inhibition of Gram-positive and Gram-negative pathogens by *Bifidobacterium* sp. M.Sc. Thesis submitted to the graduate school of Minnesota, USA, pp: 68.

Marteau, P., P. Pochart, Y. Bouhnik, I. Goderel, P. Bourlioux and J.C. Rambaud, 1992. Survival of Bifidobacteria ingested via fermented milk during their passage through the human small intestine: an *in vivo* using intestinal perfusion. Am. J. Clin. Nutr., 55: 78-80.

Nebra, Y. and A.R. Blanch, 1999. A new selective medium for *Bifidobacterium* sp. Appl. Environ. Microbiol., 65: 5173-5176.

OMS/UNICEF, 1990. Déclaration d'innocenté sur: l'allaitement maternel dans les années 90, une initiative mondiale. Cahier d'étude et de recherche, pp: 8.

Peter, L.M., J. Flemming, H. Olec, M. Soren and S. Peter, 2001. Intra-and extracellular B Galoctosidaser from *Bifidobacterium bifidum* and *Bifidobacterium infantis*: Molecular (cloning) Helerologous expression and comparative characterization. Applied Environ. Microbiol., pp: 67.

Philipe, P., 2002. Probiotique et antibiotique, une association gagnante. Yaourt et turista, pp: 1.

Rigaud, D., 2003. An intestine: the prodigal of adaptation and cooperation. Professor in CHU. The Bocage, Dijon. Objective Nutrition Number, 67: 1-6.

Schrezenmeir, J. and M. Vrese, 2001. Probiotics, prebiotics and synbiotics-approching. A definition. Am. J. Clin. Nutr., 73: 2 361-364.

Simeoni, U., 2000. Diarrhée aigue du nourrisson. Service de pédiatrie. Faculté de Medecine, université Louis Pasteur-Strasbourg, pp: 1-11.

Wolin, M.J., Y. Zhang, S. Bank, S. Yerry and T.L. Miller, 1998. NMR detection of (13) ch (3) from 3-(13) L.glucose: A signature for Bifidobacteria fermentation in the intestinal tract. J. Nutr., 128: 191-196.