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Efficacy of the probiotic strain *Enterococcus faecalis* CECT7121 in diarrhoea prevention in newborn foals

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Abstract

Rivulgo, V.M.; Ceci, M.; Haeublin, G.; Sparo, M.; Sanchez Bruni, S.: ***Efficacy of the probiotic strain Enterococcus faecalis CECT7121 in diarrhea prevention in newborn foals.*** *Rev. vet. 27: 1, 3-6, 2016.* The aim of this work was to assess the effectiveness of the probiotic strain *Enterococcus faecalis* CECT7121 for the prevention of neonatal diarrhea in one to three day-old foals. Fecal samples from twenty foals divided randomly in experimental and control groups were studied. The experimental group (n=10) received *E. faecalis* CECT7121 (1 x 10^{10} CFU/ml) orally in the first day of life and for a 6 days period. The control group (n=10) received distilled water for the same period. In 4/10 of the foals from the control group diarrhea was observed, conversely diarrhea cases were not observed in any of the foals belonging to the experimental group. The differences between groups were statistically significant ($p<0.001$). *E. faecalis* CECT7121 was isolated in faeces from the second day of treatment until six days after the end of the probiotic administration. Therefore, *E. faecalis* CECT 7121 may be considered as a biological tool for the prevention of neonatal diarrhea in foals. Any adverse effects, at least under the conditions of this assay, but more efficacy studies should be performed to evaluate this organism in the prevention or treatment of diseases in foals.

Key words: foal, neonatal diarrhoea, *Enterococcus faecalis* CECT7121, probiotic, efficacy.

Resumen

Rivulgo, V.M.; Ceci, M.; Haeublin, G.; Sparo, M.; Sanchez Bruni, S.: ***Eficacia del probiótico Enterococcus faecalis CECT7121 en la prevención de diarreas neonatales en potrillos recién nacidos.*** *Rev. vet. 27: 1, 3-6, 2016.* El objetivo de este trabajo fue evaluar la eficacia del probiótico *Enterococcus faecalis* CECT7121 en la prevención de la diarrea neonatal en potrillos de uno a tres días de edad. Se estudiaron muestras de materia fecal de veinte potrillos divididas al azar en grupos experimental y control. Los animales del grupo experimental (n=10) recibieron *E. faecalis* CECT7121 (1 x 10^{10} UFC/ml) por vía oral en el primer día de vida y por un período de 6 días. El grupo control (n=10) recibió agua destilada por el mismo período. Se observó que el 40% de los potrillos del grupo control presentaron diarrea, mientras que no se observaron casos de diarrea en ninguno de los potrillos pertenecientes al grupo experimental. Las diferencias entre los grupos fueron estadísticamente significativas ($p<0,001$). *E. faecalis* CECT7121 se aisló en heces desde el segundo día de tratamiento hasta seis días después del final de la administración del probiótico. Por lo tanto este hallazgo indicaría que *E. faecalis* CECT7121 podría ser considerado como una herramienta biológica para la prevención de la diarrea neonatal en potrillos sin ningún efecto adverso, al menos bajo las condiciones del presente ensayo, aunque más estudios de eficacia se deberían realizar para evaluar este organismo en la prevención o el tratamiento de enfermedades de los potrillos en los haras.

Palabras clave: potrillo, diarrea neonatal, *Enterococcus faecalis* CECT7121, probiótico, eficacia.

INTRODUCTION

The occurrence of certain infectious diseases in newborn foals such as neonatal diarrhoea can lead to great economical losses because of the high cost of treatment or the death of foals with systemic sepsis [5, 9, 10-12]. However, the current antimicrobial treatment for diarrhoea control presents high failure rates due to the use of broad-range antimicrobials. Hence, the following selection of multiresistant bacteria besides the bacterial bidirectional transfer between human and animal is causing serious therapeutic concerns in clinical prac-

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tice [2] . In order to prevent the above-mentioned situation new therapeutic strategies have been investigated like the biological control through the use of probiotic bacterial strains [13] .

Probiotics are traditionally defined as viable but non-pathogenic microorganisms that when ingested, have beneficial effects in the prevention and treatment of several enteric diseases [9] . *Enterococcus faecalis* CECT7121, recovered from natural corn silage, is considered a probiotic strain because of its viability in the gastrointestinal tract, gastric pH tolerance, bile salts tolerance, intestinal epithelium adhesion capability, harmless behaviour, antimicrobial compounds production (peptide AP-CECT7121) as well as the specific and non-specific immune response triggering.

Due to these properties *E. faecalis* CECT7121 could be an alternative and a new strategy for the prevention of diarrhoea in newborn foals. The aim of this study was to assess the efficacy of the probiotic strain *E. faecalis* CECT7121 for the prevention of neonatal diarrhoea in newborn foals.

MATERIAL AND METHODS

Experimental design. Twenty newborns foals with 25-50 kg weight were selected to this study from a thoroughbred horse stud farm set up in the Province of La Pampa (Argentina), in which previous cases of neonatal diarrhoea were documented. The foals were kept with their dams in individual stalls during the experiment with *ad libitum* access to grass hay and water. The foals were randomly divided in, the experimental group (n= 10) that received *E. faecalis* CECT7121 (1 x 10^{10} CFU/ml) orally in the first day of life and every 24 h along a 6 days period, and the control group (n= 10) that received distilled water (placebo) under the same conditions and time period than the experimental group. Diarrhoea was defined as the presence of faeces with abnormally liquid consistency on more than one occasion in 24 h. Foals were checked by the veterinarian daily for signs of diarrhoea, the presence of watery faeces, smearing of faeces and loss of the hair in the perianal area. The faecal samples were collected from the rectum of the foals with sterile gloves from the birth and every 24 h until the sixth day of life and then, every 48-72 h until the twelfth day of life. The diarrhoea was characterized by the veterinarian of the stud farm with a score of 1 = mild, 2 = moderate, 3 = liquid diarrhoea, the veterinarian don't know which foals treated with probiotics and which with placebo (blinded study).

E. faecalis CECT7121 preparation for experimental group treatment. An aliquot (200 ul) of defrosted *E. faecalis* was inoculated in 3 ml of brain-heart infusion (BHI) broth and incubated at 35°C for 24 h. A new inoculation was performed and incubated at 35°C for 24 h.

Visual, physico-chemical and microbiological analysis of fecal samples. A) Visual analysis of the fresh sample: direct observation was performed to recognize any of parasitic forms following the method described by [3] . B) Physico-chemical analysis: pH, presence of blood and glucose were evaluated. Measurements were made using semi-quantitative test strips (Siemens Medical Solution Diagnostics). C) Bacteriological analysis of intestinal indigenous microbiota and the presence of *E. faecalis*: for arerobic bacterial cultures, suspensions of faecal sample in sterile distilled water with an equivalent density to the 0.5 point of McFarland scale were prepared. Each one of these suspensions was inoculated on enrichment media using blood agar for 24 h at 35°C, and then inoculated in different selective media, using conventional biochemical methods according to standard microbiological techniques [8,11] . D) Determination of resistance to antimicrobial agents was determined using disk diffusion method. The following antimicrobial agents were used: gentamicin, fluorquinolones, fosfomycin, streptomycin and beta-lactamases according to CLSI [1] .

Statistical Analysis. Statistical analysis (Fischer exact test) was performed using the program SPSS, version 6.0 for comparing the clinical cure of the foals involved in this trial.

RESULTS

Clinical results. After administration of the probiotic no signs and symptoms that reflect changes in the health status of the foals were observed. In the control group (placebo) 4/10 of the foals showed mild to moderate diarrhoea (score between 1-2 grade). There was no record of neonatal septicaemia or death in these foals before conventional antimicrobial treatment established (streptomycin and activated charcoal). In contrast, in the experimental group diarrhoea was not observed in any of foals. The Fisher exact test results showed that the differences among the groups were significant ($p<0,001$).

Physical-chemical and microbiological analyses of faecal samples. Inoculum control count in the order of the administered bacterial concentration (1 x10^{10} UFC / ml) was obtained. In the physico-chemical analysis there were no glucose and blood detected, the change in pH was 5 to 6.3. The experimental and control group were negative for parasitic and the bacterial species isolated in both groups were: *Escherichia coli, Streptococcus* spp *and Staphylococcus* spp. In 100% of the experimental group, *E. faecalis* CECT7121 was isolated from the second day of treatment until six days after the end of the probiotic administration (Figures 1 and 2). In fours foals with diarrhoea (control group) the only bacteria isolated was *Klebsiella pneumoniae*. The susceptibility test to the antimicrobial agents used, detected multi- resistance of the strain isolated. The screening test confirmed the presence of the ESBLs-producer.

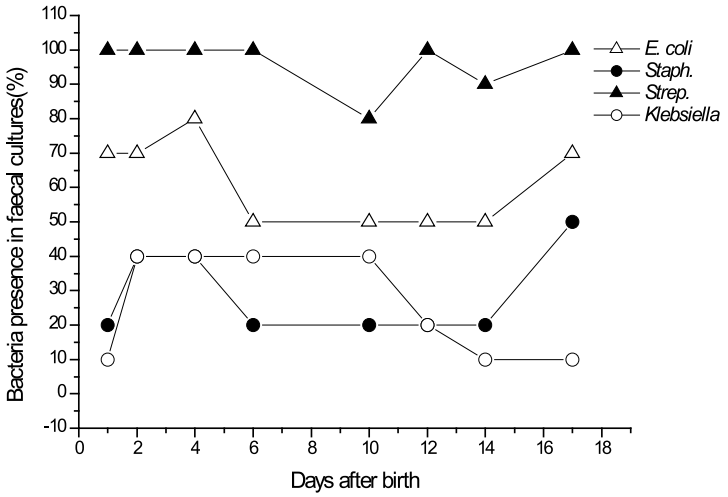

Figure 1. Percentage of aerobical bacteria existing in faecal cultures throughout the experimental period belonging to control group (n=10). It is observed the isolates of *E. coli; Staphylococcus* spp*; Streptococcus* spp. in non-diarrheic foals and *Klebsiella pneumoniae* only isolated in diarrheic foals.

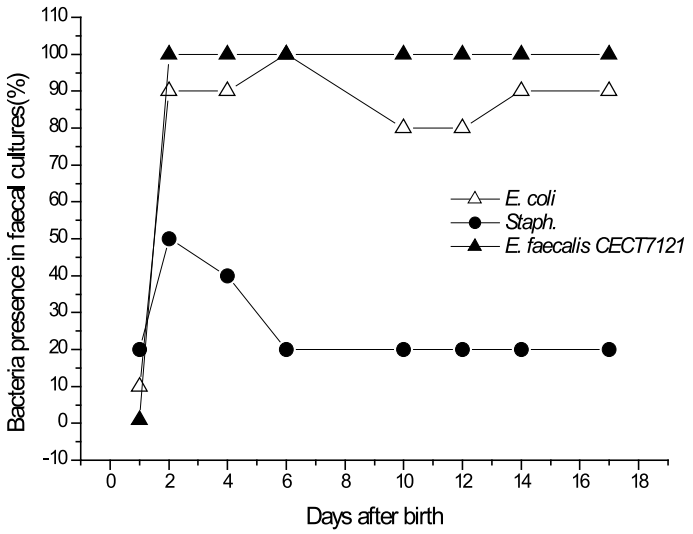

Figure 2. Percentage of bacteria existing throughout the experimental period on the experimental group (n=10) with *E. coli; Staphylococcus* spp. and *E. faecalis* CECT7121 isolations in faecal cultures.

DISCUSSION

Despite of the large use of probiotic strains in human and veterinary medicine there are few published works about the application and efficacy of these products in foals. In our study the administration of the probiotic strain *E. faecalis* CECT7121 for a 6-day period was effective for mild to moderate neonatal diarrhoea prevention. One of the best-studied probiotics in human medicine is *Lactobacillus rhamnosus* strain GG, which has been able to survive the gastric acid and bile digestion in order to colonize the gastrointestinal tracts of humans [4].

However in one study employing *Lactobacillus pentosus* WE7 as a probiotic for neonatal diarrhoea prevention reported an increase in the number of diarrhoea cases together with the appearance of clinical abnormalities [14]. However, in this study after given *E. faecalis* CECT7121 by 6 consecutive days no adverse effects were observed in any of the treated foals and our results indicated that *E. faecalis* CECT7121 was isolated from the second day of treatment until six days after the end of the probiotic administration. And the latter will represent a passive bacterial movement through the intestinal tract, although not its colonization.

The results of our isolates of intestinal indigenous microbiota of the foals without diarrhoea were consistent with other reports [5, 6, 7, 10]. The difference in bacterial isolates between the different studies could be because of geographic differences, temporal differences or also due to a compromised gastrointestinal tract leading to overgrowth of certain bacteria with subsequent translocation of these bacteria, also the management and stressful environmental conditions [15].

Interestingly, the high resistance to gentamicin and the sensitivity to streptomycin based on the antibiogram of the *Klebsiella pneumoniae* ESBL-producer make us suspect that this strain would come from a human source. This could explain the fact that some antimicrobial agents (such as streptomycin) is not used in human medicine while it is widely used in veterinary medicine combined with penicillin for treating horses and because for the fact that the development of resistance to streptomycin is documented in foals. Monitoring the development of antimicrobial resistance is pivotal for the rational selection of appropriate antimicrobial drugs to initiate treatment of foals [3].

In conclusion this work shows that the probiotic strain *E. faecalis* CECT 7121 can be considered as a biologic tool for the prevention of neonatal diarrhoea in foals without any adverse effects, but more efficacy studies should be performed to evaluate this organism in the prevention or treatment of diseases in foals in other farms.

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