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EXPERIMENTAL *E. COLI* INFECTION IN RABBITS – CLINICAL AND MORPHOLOGICAL STUDIES AND ATTEMPTS FOR CONTROL WITH AN ACIDIFYER

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Summary

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An enteropathy was experimentally reproduced in rabbits 2 weeks after weaning via a reference *E. coli* U83/39 (O15:H-) strain (EPEC). Attempts for control of the artificial infection by an acidifyer were performed.

Two groups of weaned White New Zealand rabbits, fed on pelleted forage with and without supplementation with the acidifyer Biotronic P® were used. The time for appearance of diarrhoea (incubation period), the clinical manifestation, the course and the outcome of the experimental disease were followed out. The epidemiological parameters morbidity rate, death rate and lethality in both groups were compared.

The results showed that Biotronic P® did not completely prevent the clinical manifestation of the enteropathy. The effect of its application was manifested as significant decrease in lethality and the cumulative death rate.

Key words: acidifyer, diarrhoea in rabbits**,** EPEC

INTRODUCTION

Alimentary pathology is among the commonest problems faced by intensive rabbit breeding. The causes resulting in milder or stronger expression of the enteropathy syndrome in rabbits, especially in the post weaning period, are multiple. In most cases, they could hardly be distinguished as primary or secondary.

The leading cause according to some authors is the unbalanced diet for the respective age and the subsequent impaired antibacterial activity of volatile fatty acids (VFAs), resulting in extensive colonization of the intestinal tract by various microbial agents, some of them pathogenic (Prohaszka,1980; Morisse *et al.*,1985; Peeters *et al.*,1993).

Some authors put emphasis on the microbial aetiology of enterites – different bacteria, viruses, protozoa (*Eimeria*, *Cryptosporidium*) (Whitney, 1976; Prescot, 1978; Löliger, 1980). The commonest bacterial agents are various *Esherichia coli* serotypes, belonging to the group of enteropathogenic *E. coli* (EPEC) (Cantey and Blake, 1977; Varga and Pesti,1982; Sansonetti, 1985; Camguilhem and Milon, 1989; Peeters, 1994; Finazzi *et al.*, 2000).

With this respect, the information about the mechanism of EPEC effect and

EPEC-induced diarrhoea – appearance, development, outcome, options for prevention and control is of considerable significance to rabbit breeders and veterinary specialists.

The aim of the present study was to evaluate the effect of an acidifying combination, containing organic and inorganic acids and plant extracts, used as a forage supplement, on diarrhoea syndrome in newly weaned rabbits, experimentally induced by the enteropathogenic *E. coli* U83/39 (O15:H-) strain with respect to the prophylaxis and control of this infection.

MATERIALS AND METHODS

Experimental animals

White New Zealand rabbits at the age of 45 days with average body weight 1.48 kg were used in the experiment. The animals were weaned at the age of 30 days and vaccinated against haemorrhagic disease with heat inactivated vaccine Hebovac–88T® (Vetbiopharm, Bulgaria). All animals were free from *Eimeria* oocysts. The rabbits were bred in two-floor metal cages (modules) with a slat floor, 5 animals per cage at room temperature (20–22 ^{o}C). They were fed with pelleted forage ad libitum and had a permanent access to tap water.

E. coli strain

The experimental diarrhoea was induced by a non-invasive and non-producing thermostable or thermolabile toxins enteropathogenic E. coli U83/39 (O15: H-) strain, kindly supplied by Dr. J. E. Peeters, National Institute of Veterinary Research, Brussels, Belgium. The strain was used to prepare a bacterial suspension with a density of 2.7 $\times 10^7$ cfu/mL.

Acidifyer

Biotronic P® (BIOMIN, Austria) contains formic acid, propionic acid and plant extracts. It is intended for stimulation of alimentary functions (salivation, degradation of pepsinogen to pepsin, regulation of stomach emptying, stimulation of pancreatic secretion and creation of favourable conditions for development of the useful intestinal microflora). The effect of the preparation is based upon decreasing stomach pH, activation of pepsin with subsequent optimal utilization of proteins, accompanied by a better weight gain.

Experimental design

After being weaned at the age of 30 days, the rabbits were divided into two groups with 20 animals each.

The rabbits from group I were fed a pelleted forage and were infected orally at the age of 45 days with 2 mL bacterial suspension of E. coli U83/39 (O 15:H-) with a density of 2.7 $\times 10^7$ cfu/mL, by means of a sterile non-pyrogenic feeding tube (2.0–3.0 mm/25 cm).

The animals from group II were identically infected, but their diet was supplemented with Biotronic P® at 2.5 kg/tonne at the age between 30 and 66 days.

Both groups were observed up to age of 66 days. Within the 3-week period following the infection, the appearance of diarrhoea, the development and severity of clinical symptoms and the outcome were followed out.

Pathoanatomical study

A complete necropsy was performed of each dead animal immediately after occurence of death. Specimens for histopathological study were obtained from various parts of the alimentary tract (ileum, caecum, colon). The material was fixed in 10% neutral formaline for 48 h, processed

in an ascending ethanol series, cleared in xylene and embedded in paraffine. The cross sections (5 μm) were stained with haematoxylin/eosin (H/E).

Bacteriological study

Samples obtained during the necropsy from small and large intestine and caecum were inoculated on McConkey agar (Difco). Materials from liver, spleen, kidneys, lungs and cardiac blood were cultivated on blood agar containing 5% sheep erythrocytes. The cultures were incubated aerobically at 37 ^{o}C for 24 h.

Other studies

Coprological samples and intestinal content was studied for presence of *Eimeria* oocysts by the method of Fuleborn.

The parameters morbidity rate, death rate and lethality in both experimental groups (with and without Biotronic P® supplementation) were compared.

Statistical analysis

The data for rabbits with diarrhoea syndrome and the number of dead rabbits were statistically processed by the non-parametric method for determination of χ^2 (Chi-square).The epidemiological parameters were compared by non-parametric comparison of percentages using the t-criterion of Student (Statsoft 1994–2000 Inc.)

RESULTS

The manifestation and the degree of the diarrhoea syndrome as well as epidemiological data are presented in Table 1.

The first signs of diarrhoea in rabbits from group I (without Biotronic P® supplementation) were observed 5 days after the infection. Within the 3–week post infection period, the diarrhoea syndrome appeared in 16 animals (morbidity rate 80%) with different severity (Table 1). The consistency of faeces varied from pulpous, semi-liquid to aqueous, mixed with mucus in some individuals. Moreover, anorexia, adynamia, dehydratation, progressive exhaustion, in some animals

Table 1. Clinical findings in rabbits, experimentally infected with *E. coli* U83/39 (O 15:H-) strain – untreated (control) and treated with Biotronic P®, supplemented to the diet at 2.5 kg/tonne

Parameters	Group I (untreated) (n=20)	Group II (treated) (n=20)
Diarrhoea syndrome	16	12**
Faeces – liquid with mucus	9	2
Faeces – semi-liquid	4	3
Faeces – pulpous	2	4
Faeces – soft	1	3
Dead	9	2*
Morbidity rate, %	80	60
Death rate, %	45	10**
Lethality, %	56.25	16.67**

*p< 0.05; ** p< 0.01 vs group I.

bruxism were noticed. The average duration of the incubatin period was 5.75 days and that of the course – 6.43 days. The outcome of the artificial infection was lethal for 9 rabbits with death occurring 10–14 days after the infection.

In rabbits, supplemented with Biotronic P®, the first signs of diarrhoea appeared by post infection day 7. The intestinal disorder was expressed in 12 animals. The clinical signs were similar to those described for group I, although with a various degree. The average duration of the incubation period was 8.25 days and that of the course – 5.87 days. Lethal outcome occurred only in two animals, by post infection days 12 and 13 respectively.

Statistically significant differences between groups were observed by respect to the number of diseased animals, the number of lethal outcomes, the death rate and the lethality.

The changes observed during the gross examination and the necropsy of dead rabbits were characteristic for dehydratation. The anal and the perianal areas were stained with aqueous, mucous faeces. The principal findings were observed in the alimentary tract. The stomach content was pulpous, covered with whitish, sticky, difficult to be disjoined mucus. The small intestine was semi-empty and filled with gas. The ileal wall was obviously thinned (Fig. 1). The caecum was filled with liquid, green-brownish content, mixed with gas bubbles (Fig. 2). At some places, the intestinal mucosa was ulcerated and haemorrhagic. The mesentherial lymph nodes were enlarged, succulent and with a moist cross-section (*Lymphadenitis simplex*). Often, petechial haemorrhages were present within. The kidneys were hyperaemic. No macroscopic changes were detected in the other abdominal or thoracic organs.

The histopathological examination revealed that the most obvious microscopical changes were those in the ileum, the caecum and the colon. Atrophy, vacuolization, ulceration and desquamation of the resorption epithelium were found out (Fig. 3). Submucosal oedema and blood vessel hyperaemia were observed in both small and large intestine (Fig. 4). Clusters of polymorphonuclear lymphocytes were

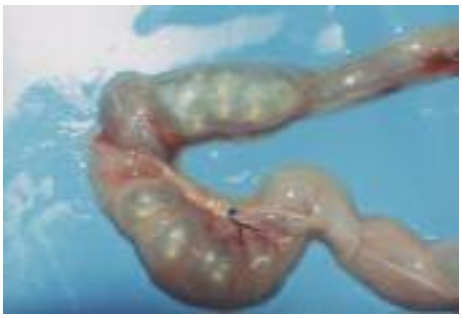

Fig. 1. Ileum of rabbit No 3 from group I (infected with *E. coli* U83/39 serotype O15:H- and untreated). Lethal outcome – by post infection day 12. A thinning of ileal wall is visible.

Fig. 2. Ileum of rabbit No 5 from group I (infected with *E. coli* U83/39 serotype O15:H- and untreated). Lethal outcome – by post infection day 14. The content of the caecum is liquid, mixed with gas.

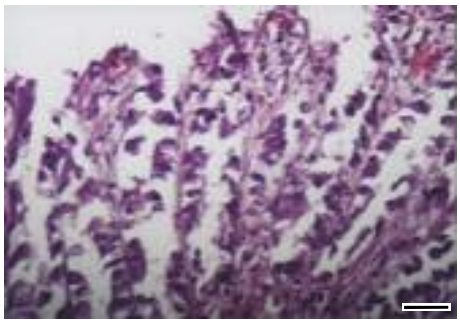

Fig. 3. Ileum of rabbit No 3 from group I (infected with *E. coli* U83/39 serotype O15:H- and untreated). Lethal outcome – by post infection day 12. Atrophy and desquamation of the resorption epithelium is seen. H/E, bar = 50 μm.

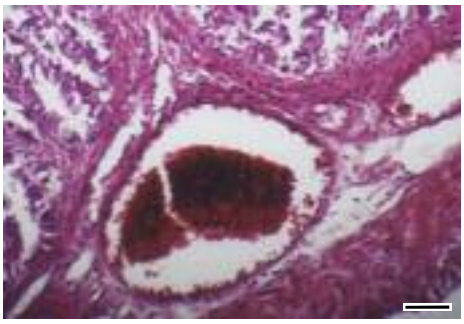

Fig. 4. Ileum of rabbit No 5 from group I (infected with *E. coli* U83/39 serotype O15:H- and untreated). Lethal outcome – by post infection day 14. Blood vessel hyperaemia in the submucosa is observed. H/E, bar = 50 μm.

observed in some areas of lamina propria. The cup cells of the colon were empty of their mucous content. No macro- or microscopic changes between dead rabbits from both groups were observed.

The bacteriological study of liver, spleen, kidneys, lungs and cardiac blood was negative. The aerobic inoculation of duodenal, jejunal, ileal and caecal content evidenced a lactose positive growth. The tests for isolate identification showed fermentation of glucose with gas formation, no formation of hydrogen sulfide or urea, no utilization of citrate on Simons's citrate medium and a positive indole reaction. The serological typization via slide agglutination with O *E. coli* antisera showed that isolates belonged to the O15 serotype.

The result of the parasitological examination by the method of Fuleborn for presence of *Eimeria* oocysts was negative.

DISCUSSION

The reproduction of the artificial infection in the majority of experimental animals showed that the pathogenic potential of the EPEC strain *E. coli* U83/39 (O15: H-) was preserved.

The high lethality, especially in untreated rabbits suggested a high virulence of the used strain for this animal category. Other isolates, belonging to the EPEC pathovar with various pathogenic potential have been described (Cantey and Blake, 1977; Varga and Pesti, 1982; Camguilhem *et al.*, 1986; Peeters *et al.*, 1988; Finazzi *et al.*, 2000). The highly pathogenic strains are particularly important to weaned rabbits: such as the RDEC-1 (O15:H-) strain isolated by Cantey and Blake (1977) as well as isolates B10 (O103:H2) studied by Camguilhem *et al.* (1986) and the U83/39 (O15:H-) strain, isolated by Peeters *et al.* (1988) and used in the present study.

In experimental infection with those strains, the diarrhoea appeared on the average 4–9 days after oral challenge, the diseases developed for 4–8 days and the lethality was between 30–50% to 90%. The infection was always accompanied by severe morphological alterations in ileal

enterocytes (attaching and effacing lesia, A/E).

Our results, related to the time of appearance and the severity of clinical signs, the development and the outcome of the artificial *E. coli* U83/39 (O15:H-) infection, as well as the macroscopic and histopathological changes were similar to those observed by Cantey and Blake (1977), Peeters *et al.* (1984), Camguilhem *et al.* (1986). Thus, we confirmed the role of EPEC for the pathology of weaned rabbits and its potential participation in the aetiology of the commonest enzootic enteropathies in some countries.

The differences between rabbits supplemented with acidifyer and non-supplemented ones by respect to the morbidity rate, lethality and death rate, the severity of clinical manifestation and the outcome of the infection provided strong evidence for the protective effect of the tested product. Treated rabbits become infected later, less animals were affected, the severity of the diarrhoea syndrome was weaker and the outcome – more favourable. This substantiated the necessity of the introduction of such products in prophylactic and metaphylactic programmes of intensive rabbit breeding.

Interpreting our experimental data, it could be concluded that the used *E. coli* U83/39 serotype O 15:H- strain was capable to induce a disease after artificial infection at a dose of 2 mL and density of 2.7×10^7 cfu/mL without additional alkalization of the stomach content. The application of the acidifyer Biotronic P® did not competely prevent the clinical manifestation of diarrhoea in 5–7–week old rabbits. The supplementation of the diet of rabbits with Biotronic P® at 2.5 kg/tonne resulted in significant decrease in lethality and the cumulative death rate in artificially provoked intestinal coliinfection using the EPEC strain U83/39 serotype O15:H-. The course of the disease in treated rabbits was milder and with a more favourable outcome compared to untreated animals.

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