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Original Contribution

EXPERIMENTAL E. COLI (EPEC) INFECTION IN RABBITS –CLINICAL AND EPIDEMIOLOGICAL STUDIES AND ATTEMPT TO CONTROL WITH AN PHYTOBIOTIC

V. Petrov[1]*, M. Lyutskanov[1], A. Vachkov[1], I. Tsachev[1], G. Mihaylov[1] and S. Tanchev[2]

[1] Department of Veterinary Microbiology, Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, Trakia University, Stara Zagora; Bulgaria
[2]Department of Genetics, Reproduction and Breeding, Faculty of Agriculture, Trakia University, Stara Zagora; Bulgaria

ABSTRACT

PURPOSE: The aim of the study was to monitor and analyse some epidemiological parameters: time for appearance and duration of bacterial discharge, incubation period, duration of infection; the quantitative parameters: morbidity rate, death rate and lethality. Moreover, some clinical signs as severity of manifested symptoms, course and outcome of the disease were analysed in both groups comprising treated and untreated with the phytobiotic. Some productive traits as daily weight gain, feed conversion ratio and forage consumption per unit weight gain were followed up. METHODS: In recently weaned rabbits, aged 40–45 days, a diarrhoeic syndrome was experimentally reproduced via infection with a reference *E. coli* U83/39 (O15:H-) strain (EPEC pathovar). Attempts for control of the artificial infection using the phytobiotic Biomin® were performed. RESULTS: It was found that the phytobiotic Biomin® did not prevent the clinical manifestation of the *coli* infection. The effect of its application was observed as a delay of the start of discharge of enteropathogenic *E. coli* strains, reduction of the discharge period as well as prolongation of the incubation period in the treated group. CONCLUSIONS: The economic effect of Biomin® supplementation consisted of increased feed conversion ratio, resp. decreased forage consumption per unit weight gain.

Key words: diarrhoeas in rabbits, EPEC, phytobiotic

INTRODUCTION

Gastrointestinal diseases and particularly the diarrhoeic syndrome are the principal problems during the period before and after weaning of rabbits. The causes, which normally result in the manifestation of diarrhoea in most cases, are various. Often it is difficult to differentiate them into primary or secondary. One of the main predisposing factors is the unbalanced diet for the various age groups resulting in impaired antibacterial activity of volatile fatty acids (VFA).

This fact, according to a number of investigators, is responsible for the intensive colonisation of the intestinal tract by various microorganisms with pathogenic potential, whose effects manifest as diarrhoeic syndrome [1, 2].

Aetiologically, the involvement of different bacteria, viruses, protozoa and, more rarely, helminthes has been shown in both mono- and mixed infections [3, 4, 5, 6]. Some authors have emphasised on the lead role played here by spore-forming anaerobes – *Cl. spiroformae* (the so-called iota enterotoxaemia) or *Clostridium piliformis* (Tyzzer's disease) [7, 8, 9]. Others have implicated the *E. coli*, especially of the EPEC group [10, 11, 12, 13, 14, 15]. This is a specific pathovar whose representatives do not invade enterocytes and do not produce exotoxins, but change the cytoskeleton and the microvillous surface of enterocytes [16].

The aim of the present investigation was to monitor some principal clinical and epidemiological parameters of an experimental infection with a highly pathogenic EPEC strain and to study the potential of the phytobiotic product Biomin® P.E.P. 1000 (BIOMIN, Austria), as prophylaxis and control of the infection in

**Correspondence to:* V. Petrov, Department of Veterinary Microbiology, Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, Trakia University, Stara Zagora; Bulgaria

recently weaned rabbits.

MATERIALS AND METHODS

Experimental animals

The experiment was performed on 24 White New Zealand rabbits, aged 40–45 days, and another set of 24 rabbits aged 30 days. All animals were weaned at the age of 30 days and by weaning, their faeces were free from *Eimeria* oocysts and *E. coli.* The rabbits were housed in disinfected metal cages (modules) with a slat floor, 4 animals per cage at room temperature (20-22 ^{0}C). They were fed with pelleted forage without coccidiostatic *ad libitum* and had a permanent access to tap water.

E. coli strain

The experimental colibacteriosis was reproduced by an enteropathogenic *E. coli* U83/39 (O15: H-), kindly provided 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 3×10^7 c.f.u./ml.

Phytobiotic

Biomin® P.E.P. 1000 is manufactured by BIOMIN (Austria). It contains plant extracts, fructooligosaccharides, vegetable oils and fragrance. It is intended for stimulation of salivation, increased forage consumption, improved utilisation of carbohydrates and proteins and stabilisation of the resident intestinal microflora.

Experimental design

The experimental animals were divided into 4 principal groups, each consisting of 12 rabbits. Following weaning, they received pelleted feed supplemented with Biomin® P.E.P. 1000 at a dose of 1.5 kg per tonne feed. At the age of 45 days, they were infected orally with bacterial suspension of *E. coli* U83/39 (O 15:H-) at a dose of 2 ml via a sterile non pyrogenic feeding tube (2.0×3.0 mm/25cm).

The second group included also 12 rabbits, infected identically but receiving feed that did not contain the phytobiotic.

Group III comprised 12 rabbits. After weaning, they were fed with diet supplemented with Biomin® P.E.P. 1000, but were not experimentally infected.

Group IV consisted of 12 non-infected rabbits, but their diet was not supplemented with phytobiotic.

The first two groups served for determination of the preventive properties of Biomin® P.E.P. 1000, through monitoring of the following clinical and epidemiological parameters: beginning and onset of discharge, incubation period, duration of discharge, the quantitative parameters morbidity rate, death rate and lethality, severity of the clinical signs, development and outcome of the disease. The animals were observed until the age of 77 days.

The other 2 groups served for evaluation of the stimulating effect upon the growth and the utilisation of the forage containing phytobiotic. The observation period lasted 30 days.

Bacteriological study

Rectal swab specimens were obtained from all animals prior to the infection for coliform status. Similarly, discharge of the challenge strain was investigated by post infection days 1, 6, 11, 16, 25 and 31.

Specimens from the content of small intestines, bowels and caeca of dead rabbits were studied for presence of coliforms.

All samples investigated for occurrence of *Enterobacteriaceae* representatives were cultivated aerobically on McConkey agar (*Difco*) at 37^{0}C for 24 hours. The identification of EPEC re-isolates was done by the semi-automated system Crystal (*Becton Dickinson*). The serotyping with specific anti O15 antiserum was done by slide agglutination.

Pathoanatomical study

A complete necropsy was performed on each dead animal.

STATISTICAL ANALYSIS

The data were statistically processed by the nonparametric method for determination of χ^2 (Chi-square). The epidemiological parameters, morbidity rate, death rate and lethality were compared by non-parametric comparison of percentages using the t- criterion of Student (*Statsoft 1994–2000 Inc*.)

RESULTS

The data about the appearance, duration and the degree of voiding of the challenge *E. coli* strain in infected rabbits (**Groups I** and **II**) are presented on **Table 1**.

It was observed that prior to the infection, none of experimental animals has discharged colibacteria in the faeces.

Twenty-four hours after the infection, three rabbits that did not receive phytobiotic (**Group II**) began to discharge single colibacteria of the strain used.

Table 1. *Data from the bacteriological examination of rectal swabs*

Group	***Infec-tion***	***Day 1***	***Day 2***	***Day 3***	***Day 4***	***Day 5***	***Day 6***	***Day 11***	***Day 16***	***Day 25***	***Day 31***
Group I	-	-	-	++	++	++++	++++	++++	++++	++	-
№ 1	-	-	-	-	++	++	+++	++++	++++	+++	-
№ 2	-	-	-	+	+	++	++	++++	++++	dead	dead
№ 3	-	-	-	-	-	-	-	-	-	+++	-
№ 4	-	-	-	+	+	+++	++++	++++	++++	++	+
№ 5	-	-	-	++	++	++++	++++	++++	++++	dead	-
№ 6	-	-	+	++	+++	++++	++++	++++	dead	+++	dead
№ 7	-	-	-	-	+	++	+++	++++	++++	dead	+
№ 8	-	-	-	-	+	++	++++	++++	dead	++	dead
№ 9	-	-	-	+	++	+++	++++	++++	++++	++	-
№ 10	-	-	-	+	++	+++	++++	+++	++++	++	-
№ 11	-	-	-	+	+++	++++	++++	++++	++++		-
№ 12											
Group II	-	-	-	+	+	++++	++++	++++	++++	++	-
№ 1	-	-	+	+	+++	++	++	++++	dead	dead	dead
№ 2	-	+	++	+++	++	+++	++++	++++	++++	++	-
№ 3	-	-	++	++	+	++	++	++++	++++	+++	+
№ 4	-	-	-	+	++	++	++++	dead	dead	dead	dead
№ 5	-	-	-	+	+++	++++	++++	++++	++++	++	-
№ 6	-	-	+	++	++++	++++	++++	++++	++++	++	-
№ 7	-	+	++	+++	++++	++++	++++	++++	++++	dead	dead
№ 8	-	+	++	++++	+++	++++	++++	+++	++++	++	-
№ 9	-	-	-	+	++	+++	++++	++++	++++	+++	+
№ 10	-	-	-	-	++	++++	++++	++++	++++	++	+
№ 11	-	-	-	+	+++	+++	++++	++++	++++	dead	dead
№ 12											

Legend: (+) – single colonies (10-15)
(+ +) – countable colonies
(+ + +) – non-countable colonies
(+ + + +) – dense, confluating growth
(-) – no growth

Table 2. *Comparative clinical and epizootological data for both infected groups of rabbits, treated with Biomin® P.E.P. 1000 and untreated.*

Parameters	***Group I –treated***	***Group II –untreated***
Total number	12	12
Diarrhoeic syndrome	7	8
(++++)	3	3
(+++)	1	2
(++)	1	1
(+)	2	2
(-)	5	4
Dead	3	4
Morbidity rate %	58.33	66.67
Death ratey %	25	33.33
Lethality %	42.86	50.00

*Legend***:**
(-) – no signs of diarrhoea
(+) – slightly pulpous, amorphous faeces
(++) – pulpous to semiliquid consistency of faeces without staining
(+++) – liquid consistency with insignificant staining of perianal area
(++++) – aqueous consistency and considerable staining

In rabbits supplemented with Biomin® P.E.P. 1000 (group I) discharge was present 24 hours later (post infection hour 48) but only in one animal.

Up to the 4th day after the challenge, 79,2% of all infected animals from both groups began to discharge the challenge strain in a lesser or higher degree. In the group that received Biomin® P.E.P. 1000, the percentage of discharge at that time was 66,7%, vs 91,7% in rabbits without phytobiotic in the diet.

Between post infection days 5 and 16, all rabbits discharged the challenge strain with the exception of one rabbit from the group receiving phytobiotic, that remained free during the entire period of the study.

By post infection day 25, the bacteriological study of specimens obtained from rabbits that survived the experimental coli infection showed that all dischargers of the challenge strain by the previous time interval continued to release the strain, but at a significantly lesser extent.

By post infection day 31, the challenge *E. coli* U83/39 (O15: H-) strain was detected in 20,5 % of animals. In animals that received Biomin® P.E.P. this percentage was 16,7%, whereas in non-supplemented rabbits – 25,0%.

The first clinical signs appeared 6 days after the infection in 4 rabbits that did not receive the phytobiotic (**Group II**). They consisted of voided soft faeces and a slight staining of the anal area. Between post infection days 7 and 10, another 3 rabbits from the same group manifested similar symptoms. In **Group I**, a weak diarrhoeic syndrome appeared for the first time in one animal by post infection day 8.

By day 14 after the challenge, 8 rabbits from **Group II** and 5 rabbits from **Group I** manifested signs of diarrhoea. The clinical picture was manifested by diarrhoea with brownish, pulpous to aqueous faeces without blood, staining of the hair in the anal, perianal area and the hind legs, decreased to lack of appetite, adynamia, progressive dehydration and exhaustion. The disease was lethal in 7 experimental animals – 4 from **Group II** (between post infection days 11–16) and in 3 from **Group I** (between post infection days 16 and 18).

Up to the 18th day of the infection, signs of intestinal disorder with a various severity appeared in a total of 8 animals from **Group II** and 7 animals from **Group I**.

After the 18th day, the clinical signs gradually disappeared and by day 24 were not present in any of the surviving rabbits. The development of recovered animals however was not stunted.

The data from the development of clinical symptoms, the severity of the course and the issue of the reproduced coli infection, as well as the epidemiological quantitative parameters, are shown on **Table 2**.

The necropsy of dead rabbits revealed staining in the perianal area with aqueous faeces mixed with mucus. The gastric content was pulpous, covered with whitish, sticky and adhering mucus. The small intestine was semi-empty and filled with gas. The ileal wall was apparently thinned. The caecum was filled with liquid, green-brownish content, mixed with gas bubbles. In the other abdominal organs, no gross changes were observed.

***Table 3**. Effect of supplementation of the diet of weaned rabbits with Biomin® P.E.P. 1000.*

Parameters	***Group III - experimental (Biomin® 1,5 kg/t)***	***Group IV – control (no Biomin®)***
Number of rabbits	12	12
Weight of the group at weaning by day 30 (kg)	5,580	5,630
Average individual weight at weaning (kg)	0,465	0,469
Weight of the group by day 60 (kg)	18.120	17,800
Average individual weight by day 60 (kg)	1.510	1,483
Forage consumption (kg)	33.750	33,100
Average forage consumption per individual (kg)	2.813	2.758
Average daily consumption per individual (g)	93.77	90.67
Average daily weight gain (g)	34.83	33,80
FCR – feed conversion ratio	2,69	2.72

Table 3 presents the data about the effect of Biomin® P.E.P. 1000 on some principal economic traits in healthy rabbits. It could be seen that, from the background of an

initial difference in the average weight of the group and the individual weight in favour of animals that did not receive the phytobiotic, its supplementation in the diet of experimental rabbits results in overcoming the difference within 30 days and better values of traits vs controls by the end of the period.

This change in the group weight resulted from the increased daily weight gain – 34,83 g in the group whose diet was supplemented with Biomin® vs 33,80 g in rabbits that did not receive the product, although the difference was not significant (p>0,05). The forage consumption in **Group III** was 2.813 kg/rabbit that was higher by 0.055 kg/rabbit compared to the control **Group IV**, taking also into account the difference in the feed conversion ratio (FCR) –2.69 and 2.72 in the control and the experimental groups, respectively.

DISCUSSION

The successful reproducing of artificial infection in the majority of animals in the study evidences the pathogenic potential of the EPEC E. coli U83/39 (O15: H-) strain. The observed high lethality in both infected groups suggested the marked virulence of the strain in these animals. EPEC isolates with various pathogenic potential have been reported [11, 12, 15, 17, 18]. Isolates with high virulence against recently weaned rabbits were also reported by Cantey et al. [11] – strain, described as RDEC-1, (O15:H-) as well as from Camguilhem et al. [17] – (B10 [O103:H2]) and Peeters et al. [20] – (U83/39 [O15:H-].

In experimental infection with those strains, the authors observed appearance of diarrhoea after 4 to 9 days following oral infection, the course of the infection for 4–8 days and lethality in30 to 90% cases.

Our data with regard to the appearance of discharged bacteria, the onset and the severity of clinical signs, the development and the issue of the artificial *E. coli* infection with the U83/39 [O15:H-] strain, as well as the gross morphological changes in the intestinal tract corresponded to data reported by other authors [11, 17, 19].

Thus, the role of enteropathogenic in the pathology of recently weaned rabbits is confirmed, together with their potential role in the aetiology of widely enzootically prevailing enteropathies.

The insignificant differences between rabbits supplemented or not with the phytobiotic Biomin® P.E.P. 1000 with regard to the parameters morbidity rate, death rate and lethality, as well as the severity of observed clinical signs showed the relatively weak preventive effect of the preparation against this type of infection. It could be however stated that the rabbits that received the product with their diet, began to discharge the challenge strain a little bit later and manifested the signs of the disease later. The differences in morbidity rates, death rates and lethality percentages were insignificant.

With regard to the observed economic traits, our data point out the advantages of the diet supplemented with the phytobiotic Biomin® P.E.P. 1000 – a higher average daily weight gain, a higher weight of the group, a more rapid development due to the increased forage consumption and its better utilisation.

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