



Original Contribution

CLINICAL AND EPIDEMIOLOGICAL STUDIES ON EXPERIMENTAL *E. COLI* (EPEC-015:H-) INFECTION IN RABBITS – TRANSMISSION TO PROGENY

M. Lyutskanov, V. Petrov, G. Mihaylov

Department of Veterinary Microbiology, Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria

ABSTRACT

In our previous studies, an experimental *coli* infection was provoked in a group of growing rabbits. A referent strain that was highly pathogenic for weaned rabbits, *E. coli* serotype O15:H and belonging to the EPEC group, was used.

The possibility of transmission and maintenance of the infection in the progeny of these animals and some other epidemiological parameters (age-related susceptibility, duration of excretion, morbidity rate, death rate and lethality) were studied. The clinical course of the infection, the severity of clinical symptoms and the outcome of the manifested diarrhoeic syndrome were followed up.

It was found that infected parents transmitted horizontally the enteropathogenic *E. coli* strains to their progenies.

The parameters of the epidemic process, the clinical manifestation and the severity of the infection did not differ considerably among the different progenies. It was concluded that the severity of infection was rather due to the pathogenic potential of the individual EPEC strain.

Key words: diarrhoea in rabbits, EPEC, lethality, morbidity rate, mechanisms of transmission.

INTRODUCTION

The colibacteriosis in rabbits is one of the commonest diseases, causing significant losses in the post-weaning period. Aetiologically, it is related to a strictly determined *E. coli* pathovar, the so-called enteropathogenic *E. coli* (EPEC). The representatives of this group are able to adhere upon enterocytes, to injure the microvilli and to change the cytoskeleton, thus impairing the metabolism in enterocytes and finally causing an intestinal disorder [1, 2].

According to their pathogenicity for weaned rabbits, the serotypes of this pathovar are divided into 2 groups – highly pathogenic (*E. coli* O15:H- and O103:H2) and moderately pathogenic (O2, O20, O109, O128, O132, O153 etc.). In infection with highly virulent strains, depending on the housing and feeding conditions and the resistance of the host, the morbidity rate varies within 30 to 80%; the death rate

–between 60 and 80%, and cumulative death rate reaches 40-50% [3, 4, 5].

Apart from losses due to lethality, an important problem is the stunted growth of survived animals that is related to higher forage consumption per unit weight gain [5].

Very often, a part of the surviving rabbits remain carriers of EPEC thus providing an important source of infection for healthy farms, where they could be imported as replacement or breeder animals. The excretion of these pathogens by the carrier rabbits is more frequently sporadic and for that reason the possibility for diagnostics during the lifetime is limited.

The aim of the present study was, therefore, to investigate the possibility of maintenance of the EPEC infection in an infected farm, by horizontal transmission of sporadically excreted colibacteria from the doe to the progeny, to determine the parameters associated with the carrier status and excretion in the progeny as well as to analyse the severity of spontaneous outbreak of infection in the progeny.

* **Correspondence to:** M. Lyutskanov, Trakia University, Stara Zagora; E-mail: mihni57@abv.bg

MATERIALS AND METHODS

Experimental animals

The experiment was performed on White New Zealand rabbits, weaned at the age of 30 days and immunized against haemorrhagic disease. At the age of 45 days, they were infected with bacterial suspension of *E. coli* O 15:H- with a density of 3×10^7 c.f.u./ml.

Following this artificial infection, six females that manifested and survived the clinical infection were selected [6]. They were housed separately at room temperature (20-22°C) in two-storey metal cages with a slat floor. The rabbits received pelleted forage and had permanent access to drinking water.

The rabbits were bred at the age of 6 months. Prior to the mating it was found out that they excreted, but only occasionally and at low amounts *E. coli* (O15:H-) with faeces. After the weaning of little rabbits, they were bred again.

The progenies of these does were weaned at the age of 30 days, immunized against haemorrhagic disease and placed in heat-disinfected metal cages. The animals received pelleted forage without coccidiostatics and had permanent access to drinking water. Weaned rabbits were reared to the age of 70 days and those surviving after that age were slaughtered.

Several generations were followed accordingly: 37 rabbits from 1st generation, 25 – from the 2nd and 28 from the 3rd. The offspring of each doe were placed in separate cages, so that rabbits born by different does were not mixed.

E. coli strain

The experimental colibacteriosis was procured using *E. coli* strain U83/39 (O 15:H-) . The strain was used to prepare a bacterial suspension with a density of 3×10^7 c.f.u./ml that was applied at a dose of 2 ml via a sterile non pyrogenic feeding tube (2,0×3,0mm/25cm). The strain was kindly provided by Dr. J. E. Peeters, National Institute of Veterinary Research, Brussels, Belgium.

Bacteriological study

Prior to the first breeding, rectal swab specimens were obtained from all does every day for 7 consecutive days. The samples were cultivated aerobically on McConkey agar (*Difco*) and in broth at 37°C for 24 hours.

Rectal swabs were also obtained from all rabbits at weaning and examined for

infection of enterobacteria. Similar samples were obtained at the age of 35, 40, 45, 50, 55 and 60 days. Specimens from the content of small intestines, bowels and caeca of dead rabbits were studied for presence of coliforms.

The isolation was done on McConkey arap (*Difco*) and incubation in aerobic conditions, at 37°C for 24 hours. All lactose-positive colonies were submitted for biochemical identification by the semi automated system Crystal (Becton Dickinson). Hyperimmune anti- O:15 rabbit serum was employed for serotyping via slide agglutination.

The quantitative evaluation of excretion was done on the basis of four-degree scale of Peeters, J.E. et al. (1984a) [7] depending on the density of bacterial growth on agar after inoculation with calibrated loops. According to this scale, (-) meant lack of growth, (+) – presence of single colonies, (++) – presence of countable colonies; (+++) – detection of separable, non-countable colonies and (++++) – dense, confluent growth.

RESULTS

The bacteriological examination of does showed that the direct inoculation on solid nutrient medium showed no growth. The passage through a liquid nutrient medium resulted in positivity of specimens and *E. coli* was not detected every day and not at a time in all animals.

The results of the survey on the excretion of the challenge strain in faeces of rabbits from the 1st generation are presented on **Table 1**.

As early as the time of weaning, 16,2% of rabbits excreted the pathogenic *E. coli* strain in faeces. At that age, the degree of excretion among the carriers was rather weak, taking into account the fact that they were offspring of three different does. The infection was not clinically manifested.

Five days later, excretion of *E. coli* was already observed in 37,8% of rabbits and the degree of excretion increased but yet, without manifested diarrhoeic syndrome.

At the age of 40 days, 75,6% of rectal swabs specimens were positive for EPEC, 9 out of ten being with dense growth with confluent colonies (++++), 8 – with dense growth and separate colonies (+++), 8 – with multiple countable colonies (++) and 3 – with single colonies (+). Clinical signs were still not present.

At the age of 45 days, only 2 animals from this generation were not excreting the

EPEC strain with faeces. The degree of excretion in this period continued to increase, and in 3 rabbits, signs of slight intestinal disorder appeared.

At the age of 50 days, 100% of samples

were positive for *E. coli* O15H-. The excretion rate was high and the number of rabbits with diarrhoeic syndrome increased to 8 (21,6%). The same day, one of them died as a result of high-degree dehydration.

Table 1. Dynamics of excretion and clinical manifestation of coliinfection in rabbits from the first progeny, born by experimentally infected does

Age, days	Frequency of excretion		Degree of excretion				Clinical signs	
	number	%	(+)	(++)	(+++)	(++++)	number	%
30	6/37	16,2	4	2	-	-	-	-
35	14/37	37,8	3	7	4	-	-	-
40	28/37	75,7	2	10	7	9	-	-
45	35/37	94,6	-	2	11	18	3	8,1
50	36/36	100	-	3	5	25	8	21,6
55	30/30	100	-	-	3	27	14	37,8
60	24/24	100	-	-	-	30	20	54,1

Table 2. Dynamics of excretion and clinical manifestation of coliinfection in rabbits from the second progeny, born by experimentally infected does

Age, days	Frequency of excretion		Degree of excretion				Clinical signs	
	number	%	(+)	(++)	(+++)	(++++)	number	%
30	2/25	8	1	-	-	1	1	4,0
35	10/24	41,7	5	3	2	-	1	4,0
40	16/24	66,7	6	5	4	1	1	4,0
45	20/24	83,3	2	8	6	4	3	12,0
50	20/22	90,9	-	5	8	7	7	28,0
55	19/21	90,5	-	1	12	6	12	48,0
60	17/19	89,5	-	-	10	7	12	48,0

Within the period from the 50th to the 55th day, another 6 animals manifested diarrhoeic signs and out of the total number of 14 diseased animals, 6 new lethal issues were registered.

In the 5 days that followed, another 6 rabbits showed clinical signs of disease. All live animals (both healthy and diseased) continued to excrete substantial amounts of the *E. coli* O15:H- strain, used for the experimental infection of the does.

The clinical signs observed by us consisted in an initial softening of faeces that, on the day that followed, turned into weak diarrhoea with staining of the perianal hairs. Gradually, the clinical signs progressed and after 5–8 days, in some animals a severe diarrhoeic syndrome with dehydration, exhaustion and death occurred.

In this progeny, 20 rabbits were affected and 13 out of them died. The morbidity rate was 54,1%, the lethality – 65%, and cumulative death rate – 35.1%.

On **Table 2**, the data for the carrier status and the clinical manifestation of spontaneous coliinfection in the second generation of rabbits are shown. The group consisted of 25 animals. At weaning, it was

observed that only 2 animals excreted the pathogenic strain (8,0%), one of them showing signs of intestinal disorder as early as the 28th day of age. The same rabbit died 4 days after the weaning at the age of 34 days.

The percentage of carriers increased progressively and by the age of 60 days, 17 of survived rabbits excreted *E. coli* O15:H- in faeces (89,5 %). The carrier status became particularly intensive after the age of 50 days, i.e. about 3 weeks after weaning.

Clinical signs of colibacteriosis were exhibited in 12 rabbits (48%), and 7 out of them died up to the 63rd day. The morbidity rate was 48%, the lethality – 58,3%, and cumulative death rate – 28%.

The third generation consisted of 28 offspring. At weaning, 4 rabbits (14,3%) excreted the strain and gradually, up to the age of 50 days, the entire group excreted *E. coli* O15:H-. During the first 10–15 days the rate of excretion was low but then, its intensity increased.

In these rabbits, clinical signs appeared as late as the 47th day in 1 animal, but regardless of the later onset, the disease affected the group rapidly and by the 52nd day, clinical signs were present in 15 animals

(53,6%), and by the 60th day – in 17. The disease was lethal for 9 rabbits with period of illness of 4–9 days.

The morbidity rates, lethality and cumulative death rates for all three generations are summarized on **Table 4**.

The observed morbidity rate in offspring was within 48.0–60.7%, the lethality was > 50%, and the cumulative death rate – more than 30%.

DISCUSSION

The analysis of experimental epidemiological data revealed that in all three rabbit generations of does, subjected to an artificial infection, carrier status is affirmed prior to weaning and that was accompanied by permanent excretion of the challenge strain for more than a month after that period.

The absence of pathogen in the direct inoculation of rectal specimens from does was due to the incidental excretion and the low number of colibacteria released in faeces of adult rabbits. The cause could be the acid pH of caecal content that suppresses the development of some groups of bacteria, including *E. coli* [8, 9]. It is known that most healthy adult rabbits have no coliform bacteria in their alimentary tract and *E. coli* are found in notably lesser amounts than in other mammals [10]. It is also known that caecotrophs are richer in bacteria from solid faeces.

The onset of a positive carrier status and the substantial excretion in the period during and after weaning could be explained by the hypothesis of Prohászka [8], that due to the change of the diet and the big amounts of crude fibres in the new diet, the necessity of secretion of more hydrochloric acid in the stomach increases. As a result, in blood serum are accumulated excessive amounts of bicarbonates that could not be entirely eliminated with urine. This results in increased pH of caecal content, dissociation of volatile fatty acids and finally, in decrease or loss of antibacterial activity. Prerequisites for proliferation of bacteria, including the enteropathogenic *E. coli* coming from the environment, are created.

Apart from the favourable environment for bacterial proliferation, the rapid exchange of colibacteria in the groups of rabbits after the weaning could also be due to the depletion of the colostral and milk-derived immunity because it is known that the protection against EPEC is based on secretory IgA immunoglobulins.

These animals were not monitored for period long enough to determine whether the excretion in growing rabbits begins to decrease, but in another study of ours it was observed that by the age of 75–80 days, rabbits get free from colibacteria to a larger extent although the duration of positive carrier status in some individuals is probably higher.

The clinical manifestation of spontaneous colibacteriosis, caused by the enteropathogenic for rabbits strain *E. coli* U83/39 (O15: H-) did not differ by its severity, development and issue from those observed in other studies of ours as well as by other investigators [4, 6, 7, 11, 12]

The quantitative parameters of the obvious epidemiological process are similar to already reported ones [3, 4, 5]. The data showed that despite the number of the generation, the infection transferred from does to their offspring was easily realized horizontally, forming a high morbidity rate. The lethality and the cumulative death rates observed in our study were relatively high, thus evidencing that the challenge *E. coli* U83/39 (O15: H-) strain preserved or restored its pathogenicity without difficulty, and therefore the challenge caused by it results rapidly in a spontaneous infection.

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