HISTOPATHOLOGICAL INVESTIGATIONS OF RABBIT COLIBACTERIOSIS, CAUSED BY ENTEROPATHOGENIC ESCHERICHIA COLI (O15:H–)

HISTOPATOLOŠKA Istraživanja kolibakterioze kod kunića izazvane enteropatogenom bakterijom Escherichia coli (O15:H–)

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The histopathological alterations in the intestinal tract of recently weaned rabbits with experimental and spontaneous Escherichia coli (O15:H–) infection were followed. A considerable shortening and thickening of well epithelialized intestinal villi were observed, whose tips, after a Warthin-Starry staining, were profusely colonized with coliform bacteria. The observed pathological pattern was a permanent finding in such infections and could be used as a pathognomic feature in the differential diagnosis of spontaneous diarrhoeic syndromes. The adhesion of colibacteria to enterocytes, together with the data from the bacteriological studies (isolation, identification, determination of the O-serogroup affiliation and the biochemical behaviour) allowed the assignment of isolates to the group of enteropathogenic E. coli (EPEC). Only when required, more detailed diagnostical procedures as PCR, could be performed.

Key words: colibacteriosis, enteropathogenic E. coli (EPEC)

Introduction / Uvod

During the last three decades, a number of investigators have emphasized the role of enteropathogenic Escherichia coli (EPEC) as a leading bacterial agent in diarrhoeic diseases in rabbits during the first post weaning weeks. This group of colibacteria, categorized as a pathovar, is distinguished with the absence...
of toxin production and invasiveness. The pathogenic effect of EPEC is realized through alterations in the cytoskeleton of enterocytes [30, 16, 17].

The epidemiology of the disease, the methods of diagnostics, the alternatives for control and eradication have been investigated [18, 4, 23, 24].

Via both scanning and transmission electron microscopy, it was evidenced that the most important micromorphological changes consisted in the adhesion of smaller or larger groups of colibacteria on the enterocytes and the M-cells on Payer's patches. As a result of this adherence and the consequent changes in the cytoskeleton of the intestinal epithelium, the intestinal villi become considerably shortened and the intestinal mucosa – thinner [14, 22, 31, 9].

This kind of studies are still limited, and in Bulgaria, have not been performed up to the present. That is why our objective was to follow the light microscopic features of the intestinal tract in rabbits with experimental and spontaneous E. coli (O15:H-) infection.

Materials and methods / Materijal i metode ispitivanja

Animals and experimental design / Životinje i eksperimentalni plan

In this study, 30 rabbits were included, divided into two groups as follows:

Group I included 15 animals with experimental colibacteriosis provoked by the highly pathogenic E. coli U83/39 (serotype O15: H-) strain.

Group II consisted of 15 rabbits, offspring of does, experimentally infected during a previous experiment with the same E. coli strain. Part of these animals exhibited signs of spontaneous intestinal diarrhoea.

All animals from both groups, participating in the experiment, were weaned at the age of 30 days and did not shed Eimeria spp. oocysts at the time of weaning.

E. coli strain / Soj E.coli

The experimental colibacteriosis was induced by an enteropathogenic E. coli U83/39 (O15: H-) strain, kindly provided by Dr. J. E. Peeters, from the National Institute of Veterinary Research, Brussels, Belgium. It served to prepare a bacterial suspension with a density of $3 \times 10^7$ c.f.u./ml.

Bacteriological studies / Bacteriološka istraživanja

Rectal swab samples were analyzed for detection of the causing agent in the rabbits from group I as well as for the determination of the presence of enteropathogenic E. coli in the rabbits with spontaneous disease (group II) that manifested an intestinal indigestion. The routine bacteriological techniques for isolation and classification of enterobacteria as well as of identification of colibacteria were utilized [29]. The O-serogroup affiliation of E. coli isolates was determined [29].
Histological studies / Histološka istraživanja

After euthanasia of rabbits by the 3rd-4th day of the clinical manifestation of the disease, ileal loops were obtained for a histological study after ligation from both ends, filling and placement in 10% buffered formalin for 72-96 h. From them, rings with a height of 5 mm were prepared, embedded in paraffin, 6-8 μm sections were cut and stained with haematoxylin/eosin and according to Warthin-Starry [11], using routine histological techniques.

The observations were performed on a transmitted light NU-2 microscope.

Results and discussion / Rezultati i diskusija

In animals with experimental E. coli U83/39 (O15: H-) infection, a considerable shortening and thickening of well epithelized intestinal villi was observed (Fig. 1), whose tips, after Warthin-Starry staining, appeared massively colonized with coliform bacteria (Fig. 2) and at numerous sites, their propria was infiltrated with pseudoeosinophils (Fig. 3).

In rabbits, offspring of EPEC-infected does, apart from the histopathological changes specific for colibacteriosis, a weaker or more severe infection with Eimeria spp. was also present (Fig. 4).
According to the present studies in rabbits with experimental and spontaneous E. coli (O15:H-) infection, the histological alterations due to EPEC are related to the atrophy of intestinal villi, vacuolization, increased amount of cytoplasm, impaired structure of the superficial epithelial layer of the intestinal mucous coat, presence of desquamated cells in the lumen. These injuries according to a number of authors take place gradually and consecutively, occurring initially at a cell level [30, 16, 17].
According to Milon (1996) this process occurs in three stages:

- Attachment / Vezivanje. Initially, the bacteria are loosely attached to enterocytes via plasmid-determined fimbrial adhesins, called bundle forming pili (BFP).

- Effacement / Brisanje. The alterations in the cytoskeleton are resulting from the action of several functional proteins, synthetized after the attachment. A number of chromosome genes, located adjacently and forming the so-called “pathogenicity island” or "locus of enterocyte effacement" (LEE), are responsible for their synthesis.

- Cytoskeleton reorganization / Citoskeletna reorganizacija. The actin undergoes a change from filamentous into globular and this results in an altered enterocyte cytoskeleton. The change consists in the formation of pedestal-like structures and effacement of the microvilli.

On the other side, it was shown that the changes in membrane proteins blocked the transport systems of the colonized enterocyte, impairing the absorption of water, the excretion and absorption of ions (Law 1994). The histopathological picture of this process is manifested through the rounding of epithelial cells and subsequent disorganization of the entire superficial layer of enterocytes in affected areas.
In some cases, ovoid bacteria on the epithelial surface, but not in the cell's cytoplasm or the deeper layers of the mucous coat, could be observed.

In some parts of the lamina propria, weak to moderate oedema and infiltration with pseudoeosinophils was observed. Also, hyperaemia and mucosal haemorrhages were noticed [5, 20, 21].

Similarly to our studies, after the special Warthin-Starry staining and in other reports in EPEC involvement in rabbits using scanning electron microscopy, small or bigger clusters of colibacteria, tightly attached to the surface of enterocytes and particularly on enterocytes, covering the Payer’s patches without presence of diffuse adherence, have been observed [31, 9, 22, 14]. It seemed that this pathological pattern was a permanent finding in such infections and could be used as a pathognomic feature in field cases, indicative of the need for further diagnostic studies.

The detection of Eimeria spp. oocysts in some animals is most probably a co-infection, due to the lack of application of coccidiostatics, but it could be supposed that the simultaneous Eimeria spp. infection aggravated the pathological features of colibacteriosis.

References / Literatura

HISTOPATOLOŠKA ISTRAŽivanja KOLIBAKTERIOZE KOD KUNIĆA IZAZVANE ENTEROPATOGENOM BAKTERIJOM ESCHERICHIA coli (015:H-)

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Praćene su histopatološke promene na intestinalnom traktu tek zalučenih kunića sa eksperimentalnom ili spontanom infekcijom bakterijom Escherichia coli (O15:H-). Zapažena su znatna skraćenja i zadebljanja dobro epitelizovanih intestinalnih resica, na čijim vrhovima su uočljive bogato kolonizirane koliformne bakterije, posle bojenja po Vartin-Stari metodi. Uočena patološka šema predstavlja konstantni nalaz kod takvih infekcija i mogla bi se upotrebiti kao patognomska karakteristika kod diferencijalne dijagnoze spontanih sindroma dijareje. Adhezija kolibakterija na enterocite, zajedno sa podacima iz bakterioloških studija (izolacija, identifikacija, određivanje pripadnosti O-serogrupi i bioheimijsko ponašanje) omogućilo je da se odredi da izolati pripadaju grupi enteropatogene E.coli (EPEC). Detaljnije dijagnostičke procedure, kao što je PCR, mogu se vršiti samo ukoliko je neophodno.

Ključne reči: kolibakterioza, enteropatogena E. coli (EPEC)