EFFECTS OF SPONTANEOUS AND EXPERIMENTAL COLIBACTERIOSIS ON SOME HAEMATOLOGICAL AND BLOOD BIOCHEMICAL PARAMETERS IN WEANED RABBITS

V. PETROV¹, M. LYUTSKANOV¹ & D. KANAKOV²

¹Department of Veterinary Microbiology, Infectious and Parasitic Diseases; ²Department of Internal Diseases, Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria

Summary


The changes in some haematological and blood biochemical indices in rabbits with spontaneous colibacteriosis, caused by Escherichia coli (EPEC) serotype O15:Hu, as well as rabbits with colibacteriosis experimentally induced with the same strain, were followed out. Blood samples were obtained on the day of weaning (30 days of age), at 45 days of age, and from all animals with clinical signs of diarrhoea of intestinal origin. The following parameters were examined: total erythrocyte count, haemoglobin, haematocrit, mean corpuscular volume (MCV), total and differential leukocyte counts, blood creatinine, urea, aspartate aminotransferase (ASAT), and alanine aminotransferase (ALAT). In rabbits with spontaneous or experimental colibacteriosis, a reduction in the total erythrocyte count has occurred along with an increase in haematocrit and haemoglobin levels. Leukocytosis with pseudoeosinophilia was also observed. In cases of diarrhoeic syndrome, an increase in the levels of all examined biochemical indicators was observed in both groups, with the changes being manifested as early as the 45th day of age in rabbits born by infected does, before the disease’s clinical expression.

Key words: colibacteriosis, EPEC, haematology, rabbits

INTRODUCTION

One of the most common ways infectious diseases occur in otherwise healthy farms is through the introduction of clinically healthy vectors (Peeters, 1994). Coliinfection, etiologically related to enteropathogenic E. coli (EPEC) is a persisting problem in a number of countries (Cantey & Blake, 1977; Okerman et al., 1982; Peeters et al., 1984; Camgulhem, 1985).

During the post weaning period, colibacteriosis in rabbits is related to EPEC. The mechanism of infection is related to changes in the cytoskeleton of enterocytes in the jejunum, ileum, caecum and the colon – the so-called “attaching effacing lesions” with consequent impairment of their functions (Moon et al., 1983; Nataro & Kaper, 1998). EPEC do not penetrate into the enterocytes and do not cross the intestinal barrier. They do not secrete extracellular toxins.

The diagnostics of EPEC infection in rabbits, especially its differentiation from diseases with similar symptoms, is...
difficult. According to Peeters et al. (1985; 1988) it is necessary to confirm the isolates as belonging to certain O-serotypes, to prove their local adherence to enterocytes, as well as, according to Pohl et al. (1993), the presence of the eae gene, responsible for the synthesis of intimin.

On the other hand, diagnostics should be also supported by the changes of some blood laboratory parameters, especially if they reflect the pathogenetic features of the infection. This would reflect on the therapeutic approach too. That is why we aimed to follow out the pattern of change in some haematological and blood biochemical indices in rabbits before and after the clinical expression of spontaneous and experimental EPEC infection, and to find out whether some of them could be used as markers to distinguish risk groups, especially in recently weaned rabbits.

MATERIALS AND METHODS

Animals and experimental design

The experiments included 30 New Zealand White rabbits. All animals had been weaned at the age of 30 days and did not shed *Eimeria* oocysts. They were kept in disinfected metal cages (modules) with slatted floors, 3–4 rabbits per cage, at room temperature. The rabbits were fed freely with pelleted feed in accordance to their age, which did not contain a coccidiodicidal, and had constant access to drinking water. They were divided into three groups.

Group I (negative control) consisted of 6 uninfected animals, born by healthy mothers. Blood samples from this group were obtained on the day of weaning (30 days of age), as well as at 45 and 55 days of age.

Group II (spontaneous colibacteriosis) consisted of 12 animals born by mothers infected with *E. coli* U83/39 (O15:H-) strain. Preceding bacteriological examinations revealed incidental shedding of the causative strain by these animals (Lyutskanov et al., 2005).

Group III (experimental colibacteriosis) consisted of 12 rabbits born by mothers free from the *E. coli* strain O15:H-. Preliminary bacteriological tests on the animals from this group showed that they did not shed *E. coli*. At the age of 45 days, these rabbits were orally infected with a bacterial suspension of the *E. coli* strain U83/39 (serotype O15:H-), kindly provided by Dr. J. E. Peeters, National Institute of Veterinary Research, Brussels, Belgium. It had a density of $2 \times 10^7$ CFU/mL, applied at a dose of 2 mL with a tube (feeding tube – sterile non-pyrogenic, 2.0*3.0 mm/25 cm).

Blood samples from the last two groups were obtained on the day of weaning (30 days of age) and at the age of 45 days. Samples were collected from all rabbits that exhibited signs of colibacteriosis on the 3rd day after their onset.

Haematological analyses

Blood samples were obtained from *v. auricularis externa* in Na$_2$EDTA tubes. The erythrocytes (T/L), total leukocyte (G/L) counts, haemoglobin (g/L), haematocrit (L/L), and mean corpuscular volume (fL) were determined on an automated haematological counter Serono 150 plus VET with a diluting apparatus Serono 106 plus (Germany). The differential leukocyte counts were determined on blood smears stained by Romanovski-Giemsa.

Blood biochemical analyses

For the examination of the plasma levels of urea, creatinine, ASAT and ALAT,
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Table 1. Changes in some haematological parameters in healthy rabbits, spontaneously infected and experimentally infected with *E. coli* O15:H - rabbits (mean±SD)

<table>
<thead>
<tr>
<th></th>
<th>Erythrocytes (T/L)</th>
<th>Haemoglobin (g/L)</th>
<th>Haematocrit (L/L)</th>
<th>Mean corpuscular volume (fl)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Controls</strong></td>
<td></td>
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<tr>
<td>day 30 (n=6)</td>
<td>5.37±0.48</td>
<td>100.83±6.18</td>
<td>0.30±0.03</td>
<td>63.55±1.71</td>
</tr>
<tr>
<td>day 45 (n=6)</td>
<td>5.45±0.32</td>
<td>101.33±11.27</td>
<td>0.32±0.03</td>
<td>60.53±1.81</td>
</tr>
<tr>
<td>day 55 (n=6)</td>
<td>5.64±0.54</td>
<td>102.17±5.32</td>
<td>0.31±0.02</td>
<td>62.48±1.79</td>
</tr>
<tr>
<td><strong>Spontaneous infection</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>day 30 (n=12)</td>
<td>5.57±0.61</td>
<td>99.6±4.95</td>
<td>0.34±0.036</td>
<td>60.92±0.72</td>
</tr>
<tr>
<td>day 45 (n=12)</td>
<td>5.19±0.18*</td>
<td>105.6±35.19*</td>
<td>0.32±0.02</td>
<td>58.05±1.26</td>
</tr>
<tr>
<td>diarrhoea (n=7)</td>
<td>4.87±0.22*</td>
<td>108.67±14.97*</td>
<td>0.33±0.02*</td>
<td>56.87±3.28*</td>
</tr>
<tr>
<td><strong>Experimental infection</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>day 30 (n=12)</td>
<td>5.97±2.76</td>
<td>94.11±8.54</td>
<td>0.30±0.009</td>
<td>59.01±0.75</td>
</tr>
<tr>
<td>day 45 (n=12)</td>
<td>6.61±4.43</td>
<td>98.22±7.04</td>
<td>0.29±0.007</td>
<td>55.59±1.01</td>
</tr>
<tr>
<td>diarrhoea (n=9)</td>
<td>5.90±0.16</td>
<td>104.83±2.15</td>
<td>0.32±0.008*</td>
<td>53.35±1.27*</td>
</tr>
</tbody>
</table>

*P<0.01 vs 30 days of age; # P<0.01 vs 45 days of age.

blood was obtained in tubes containing heparin. After centrifugation (1500×g; 15 min) the plasma was separated and the analysis was done immediately on a biochemical analyser BA-88, Mindray.

**Bacteriological tests**

The presence of *E. coli* was proved through bacteriological tests of rectal swab samples from the animals exhibiting diarrhoea, as well as from the contents of the small intestine, colon, and caecum of the dead rabbits. All samples were incubated on McConkey agar (Difco) at aerobic conditions, 37 °C for 24 h. Identification of the re-isolated EPEC was done through the semi-automatic system Crystal (Becton Dickinson). Serotyping with specific anti-O15 antiseraum was performed via slide agglutination.

**Statistical analysis**

Statistical processing of results was done by ANOVA (Microsoft Excel), while the significance of the differences was established through the LSD test.

**RESULTS**

The bacteriological examination of all samples from the rabbits with diarrhoeic syndrome confirmed the presence of *E. coli* with the biochemical and O-serotype features of the U83/39 (O15: H-) strain.

In rabbits born by mothers infected with the reference EPEC strain (group II), the first clinical signs were observed at the age of 45 days. The symptoms included anorexia, adynamia, varying extents of diarrhoea leading to dehydration and progressive exhaustion. Within this group, 7 animals exhibited signs of disease.

In the rabbits experimentally infected with the same strain (group III), the first clinical signs were observed on the 5th day after infection, with the symptoms being the same as those found in rabbits born by infected mothers. Within this group, 9 animals exhibited signs of disease.
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Haematological parameters

The data presented in Table 1 indicate that no significant changes in the erythrocyte counts, mean corpuscular volume, haemoglobin, and haematocrit could be observed throughout the entire experimental period in the control group.

In the rabbits born by infected mothers (group II), the total erythrocyte counts...
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The changes in the activity of blood ASAT and ALAT were also interesting. In rabbits born by infected does (group II), a significant increase in the activities of these enzymes was established at the age of 45 days. During the clinical expression of the EPEC infection, they continued to rise in group II and were significantly (P<0.01) higher than the levels in controls at the age of 55 days (Table 3). In experimentally infected rabbits (group III), a sharp and significant (P<0.01) increase in the activity of enzymes was established only after the disease was clinically expressed (Table 3).

DISCUSSION

The entheropathogenic E. coli attach to epithelial cells of the small intestine, yet do not penetrate within or pass through the intestinal barrier. They do not release extracellular toxins. It seems therefore reasonable for a local infection like this not to cause major general changes in the host homeostasis.

The discovered deviations in the blood picture, consisting of a lower erythrocyte counts with lower MCV, however, delineate the characteristics of developing anaemia. Over the course of our experiments, the occurrence of diarrhoea was accompanied by a moderate increase in the haematocrit caused by dehydration. These results correspond with the findings of Okerman (1989), who has assumed that toxic substances are accumulated and absorbed during EPEC infections, leading to damage to the endothelium of the small vessels, followed by disseminated intravascular coagulation and finally, signs of anaemia. This thesis is supported by the discovered haemorrhagic inflammation of the caecum, with these changes being more severe in the animals experimentally infected with the E. coli O15:H- strain and milder in the cases of natural infection, studied during epidemic outbreaks in industrial rabbit farms.

A decrease in Hb and MCV after weaning was also reported by Vachkova et al. (2007). They compared some hematological indices in rabbits weaned at ages of 21 and 35 days to establish a slight decrease in these parameters during the first week after weaning for both experimental groups. The authors assumed that the changes in the blood picture were due to the post weaning stress. During our studies, however, we found similar changes only in the rabbits born by infected mothers or after the clinical expression of colibacteriosis in the experimentally infected group. This fact allowed us to affirm that the changes were not caused by the weaning stress, but rather by the influence of EPEC.

The results of our studies showed that an increase in the count of leukocytes and the percentage of pseudoeosinophils was evident at the occurrence of diarrhoea, as well as a slight decrease in eosinophil and lymphocyte percentages, compared to their values before the clinical expression of colibacteriosis. They corresponded to the results of Okerman (1989) reporting leukocytosis, pseudoeosinophilia and lymphopenia in rabbits infected with 6 out of 8 strains (including those belonging to the O15:H- serotype).

The deviations in kidney function detected in experimentally infected or born by infected mothers rabbits, expressed as increased blood creatinine and urea, were similar to those established by Prohaszka & Baron (1981) and Okerman (1989).

Okerman (1989), while examining urine from clinically ill animals, found a low pH (5–6.5), proteinuria, erythrocyte cylinder casts and renal epithelial cells,
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This gave us reason to assume that they were a consequence of the relationships between EPEC and the host. This influence is probably related to endotoxins (LPS), released by EPEC, the impaired barrier and absorptive function of the intestinal wall, which allows for the passage of toxic substances from the intestinal content into the blood with consequent damage of kidney and liver functions.

The results of our research indicate that the clinical expression of EPEC infection is related to anaemia and relative leukocytosis, accompanied by pseudoeosinophilia. These deviations, however, are not strongly expressed and are not specific only to the EPEC infection. At the same time, the functioning of the kidneys and the liver are significantly impaired leading to a significant increase in blood creatinine, urea, ASAT and ALAT.

Despite our results, which point out that EPEC in rabbits influenced the liver and kidney functions, such changes could be observed in a number of other diseases as well, which does not allow us to conclude that they are specific for the EPEC infection only. Therefore, their detection in newly weaned rabbits could suggest the circulation of enteropathogenic E. coli in pig and rabbit intestines. Infection and Immunity, 1, 1340–1351.

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Correspondence:

Dr. Vladimir Petrov, Department of Veterinary Microbiology, Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, Trakia University, 6000 Stara Zagora, Bulgaria e-mail: vlado72@abv.bg

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