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A FATAL MIXED BACTERIAL INFECTION IN GUINEA PIGS – CLINICAL SYMPTOMS, MICROBIOLOGY AND PATHOMORPHOLOGY CASE REPORT

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ABSTRACT

The paper describes a case of an acute lethal infection in guinea pigs, demonstrated with watery to bloody profuse diarrhea, respiratory paralysis and death. Microbiological tests indentified several isolates: *Clostridium novyi (oedematiens) (C. novyi), Escherichia coli (E. coli), Enterococcus* sp. and *Bacillus cereus (B. cereus*). Materials from internal organs were obtained for pathohistological examination. History data, microbiology and histopathology were evidential for toxin-forming pathogenic microorganisms, imported by food and/or probably turned to virulent endogenous bacterial strains, causing the death of the animals.

Key words: C. *novyi*, E. *coli*, *Enterococcus* sp., B. *cereus*, lethal infection, guinea pigs.

Introduction

The occurrence of acute and chronic spontaneous or food born bacterial infections is a serious problem in the research practices that can compromise the normal course of experiments. Although the compliance with the necessary rules for means of transport, housing and feeding of laboratory animals, it is possible to import ubiquitous pathogens via food or animal bedding. Especially dangerous are some pathogenic spore-forming soil microorganisms that can contaminate concentrated and rough forages, fruit and vegetables both in harvesting and improper storage.

This article presents a case of an acute infection in guinea pigs bred as laboratory animals, graduated lethally. Several weeks after their purchase the animals begin to show signs of depression, food and water refusal. Symptoms characteristic for toxicoinfection – cachexia, fever with drop of temperature below normal, spiking of hair, dehydration, watery to bloody profuse diarrhea, difficult breathing, bluish mucous membranes and subsequent respiratory paralysis were noticed for a period of 2-3 days. The disease was fatal to all animals within several days after the onset of the clinical signs. Materials from dead animals were taken for microbiological, parasitological and pathohistological examinations and clarification of the cause of death.

The aim of this study was to clarify the cause of the acute lethal infection in guinea pigs reared in laboratory conditions.

Materials and methods

Animals. 24 male and female bi- and tri-colored guinea pigs, 5 and 6 months of age, with an average weight of 0,336 kg ± 0,139 kg were grown for breeding and experimental purposes in accordance with the requirements and Regulation № 15/ 03.02.2006 regarding laboratory animals and animal welfare. The room temperature of 21-22ºC and relative humidity of 40-60% were constantly kept. Specially designed spacious cages were used; three animals in each were kept.

Sawdust was purchased commercially (MINIPAK® Ltd., Bulgaria) and was regularly replaced. For animal feeding was used full 20-component mixture for rodents (Scalar Ltd., Bulgaria) with the addition of apples, carrots, white cabbage and pumpkins. Drinking water was provided freely through specially designed drinkers. Cleaning, washing and disinfection of inventory were performed every 3 days. 1% Ecocid®S (KRKA, Slovenia) for disinfection was used, afterwards surfaces were washed thoroughly with clean drinking water.

Microbiological studies:

Organs. For the isolation, identification and differentiation of microorganisms were made cultures from materials of small and large intestines and livers of all 24 deceased animals, after sterilization of the used surgical instruments.

Nutrient medium. Selective and differential nutrient media for different groups of microorganisms were supported by BUL BIO – National Center of Infectious and Parasitic Diseases (NCIPD) Ltd. – Sofia and Scharlau—Antisel, Bulgaria.

The nutrient-infused liquid medium for isolation used was Tarozzi's liver broth. As solid nutrient medium were used agar in Tsaysler, Mueller – Hinton agar with 5% blood and Endo agar supplied on individual agar plates. Incubation of the cultures was performed at 37°C for 24-72 h at aerobic and anaerobic conditions (by anaerob pack with palladium catalyst – H_2+CO_2 – BUL BIO NCIPD Ltd. – Sofia). The isolation for spore-forming microorganisms was performed under required specific conditions and heating at 80 °C for 20 min. The cultural features identification of the isolated bacteria was accomplished by microscopic examinations of native, Gram and Pfeiffer stained preparations, and biochemical properties testing using Polymicrotest (BUL BIO NCIPD Ltd. – Sofia). Additional samples were performed on reactivity to oxidase, catalase, ect (Scharlau – Antisel, Bulgaria). Isolation and identification of bacteria was conducted in accordance with International definers Bergey (Holt et al.,1994) and according to Logan and Berkeley, 1984 (Logan and Berkeley, 1984).

Parasitological studies. Faecal samples were evaluated by four parasitological methods:

1. Fulleborn flotation method (saturated sodium chloride solution with a specific gravity of 1.20) – to detect light and moderate helminth eggs, and protozoa oocysts.

2. Faust flotation method (zinc sulphate solution with a specific gravity of 1.40) for the detection of heavy helminth eggs and cysts of giardia.

3. Faecal smears stained with Lugol 's iodine for an express detection of protozoa (particularly vegetative forms of giardia).

4. Examination of faecal smears stained by a modified Ziehl–Neelsen method for a detection of *Cryptosporidium* sp.

Pathohistological studies. Materials from lungs, heart, liver, kidneys, spleen, stomach and urinary bladder were fixed in 10% buffered formalin (pH 7), dehydrated and paraffin embedded, cut into 5-10μm slides, dewaxed in xylene and stained with Hematoxylin-Eosin (H&E) using standard histological techniques. Studies were performed on a microscope Leika DM 500B, Wetzlar, Germany and photomicrographs were made.

Results

Microbiology and parasitology. The coproscopic examinations provided by the all four methods were negative for a presence of intestinal parasites.

The isolated microorganisms from the tested materials are presented in Table 1. The determining of the bacteria species that microscopically and culturally were assigned to the genus *Bacillus*, was accomplished by biochemical tests, the results of which are presented in Table 2. Based on the biochemical characteristic the isolate was classified to belong to the species *B. cereus.*

Table 1: Microorganisms isolated from small and large intestines, and livers.

Organ	Isolated bacterial species
Intestines	*C. novyi, E. coli, Enterococcus* sp., *B. cereus*
Liver	*C. novyi, E. coli and Enterococcus* sp.

Table 2: Biochemical characteristics of the isolated bacteria.

Test (enzyme, substrate, end product)	Strain activity
nitrate reductase	+
urease	-
catalase	+
oxidase	-
β-galactosidase (ONPG)	+
L-lysine decarboxylase	-
L-ornithine decarboxylase	+
L-arginine dihydrolase	-
L-phenylalanine deaminase	-
indole	-
hydrogen sulfide	-
VP	-
D-glucose	+
lactose	-
saharose	+
L-arabinose	-
L-rhamnose	-
mannitol	-
adonitol	-
meso-inositol	-
sorbitol	-
esculin	+
sodium malonate	-

Gross-pathology. During the macroscopic examinations as major changes were established variations in the color and texture of lungs, liver and kidneys of some animals, catarrhal gastritis, enteritis and colitis with hemorrhagic and necrotic alterations, and vigorously gas ballooning of intestines. The lungs were spotted with hemorrhagic abnormalities. The livers and kidneys had softer texture and were diffusely paler in color. Because of the poor condition of the bowel and macroscopically visible changes – catarrhal-hemorrhagic inflammation and gases, materials from them were not taken for histological examination. The perineal area of the corpses was dirty with loose stools and blood. The rest of the internal organs were unchanged in size, shape, color and texture.

Pathohistology. The alterations in the pulmonary histoarchitecture were evidential for severe respiratory distress and fatal subsequent hypoxia. Histologically were observed spasms of the terminal bronchi and bronchioles (Fig. 1a, b), peribronchial obstructive atelectasis (Fig. 1b), engorgement and thickening of small peripheral pulmonary blood vessels, especially arteries and interstitial edema, erythrocyte diapedesis and leukocyte extravasation in alveolar septa.

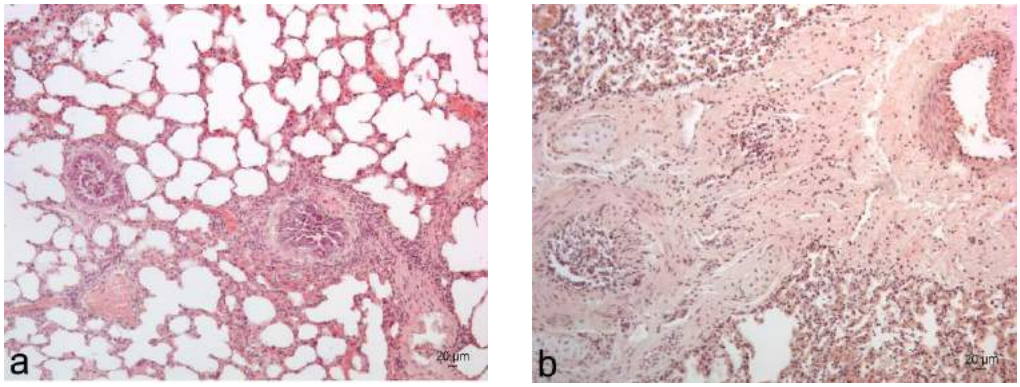

Figure 1: Lungs, guinea pig: spasm of the terminal bronchi (a, b), thickening of small pulmonary arteries and collapse of alveoli adjacent to bronchovascular bundles (b), H&E.

The liver pathology consisted mainly of narrowed sinusoids, hepatocellular damage forming multifocally necrotic parenchyma in the centrilobular and periportal areas (Fig. 2a). In the kidneys were observed focal degenerative alterations of the epithelium covering the proximal and distal convoluted tubules. The tubule walls were unstructured with detached from the basal membrane apoptotic pyknotic epithelial cells (Fig. 2b), as well some renal tubules were filled with protein and cellular detritus. Generally, most of the renal corpuscles had greatly expanded zone between the capsular (parietal) epithelium and the podocytes, forming the glomerular (visceral) epithelium of the Bowman's capsule (Fig. 2b). Disintegration and pyknosis of cells were characteristic finding in spleens of the most of the guinea pigs. The microscopic examinations of the stomach revealed hyperemia and edema of the mucosa with neutrophilic and lymphocytic infiltrates. The hearts and urinary bladders were comparatively pathologically unchanged.

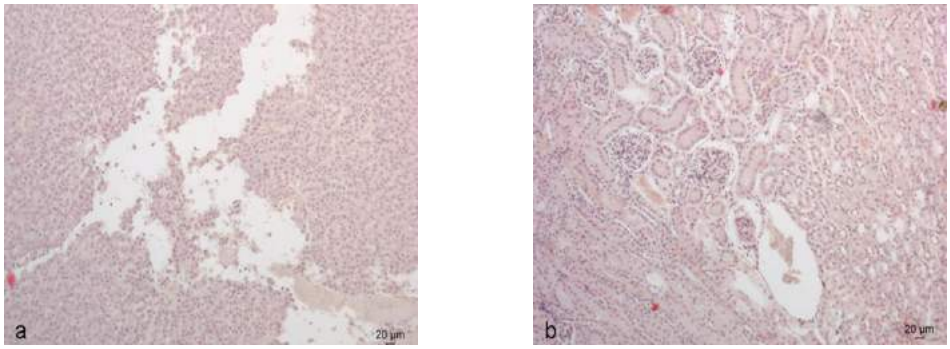

Figure 2: a) liver, guinea pig: narrowed sinusoids, periportal hepatocellular necrosis, H&E; b) kidneys, guinea pig: focal degeneration and pyknosis of the epithelium covering the convoluted tubules and expanded zone between the capsular and visceral epithelium of the Bowman's capsule, H&E.

Discussion. According to the data by the anamnesis and underlying microbiology, conclusions made turn to probably imported by raw food toxin-forming pathogenic microorganisms, leading to inflammation of the gut (allowing passage of toxins and macromolecules to portal vein circulation, sensibilisation and intoxication of the guinea pigs. Along with that, a passage from own microbiome non virulent endogenous strains of bacteria to virulent ones and the complex action of all toxins was also possible, causing the severe reaction and death of the young animals. The histopathological findings attesting necrotic changes in parenchyma organs and bowel, congestion of the lungs with bronchospasm and death by hypoxia, also support this view. The massive cell death in the liver and kidneys was a further leading reason to toxicity of the macro-organisms. The findings of obliteration and spasm of the terminal bronchi leading to collapse of the lung parenchyma, hypoxia and suffocation of animals, may be provoked additionally by release of prostaglandins, which sensitized to contraction the smooth muscle tissue there.

The data from the microbiology were indicative of anaerobic infections involving *C. novyi*, a Gram positive obligate anaerob of the *Clostridia* class, family Bacillaceae. *C. novyi* is classified into three types-A, B and C, while some authors define *C. haemolyticum* as Type D, by the toxins emitted. Different types cause diseases in humans and animals. Factor for that may be disadvantageous conditions for macro-organism and the spore forming ability of clostridia (Schultz et al., 2001), which helps passage via the intestinal tract and blood, and localization in different tissues and organs. There, the bacteria begin to produce highly potent exotoxins (A to D) with lethal, necrotic, oedematous and hemolytic effect, causing acute toxemia and species specific and non-specific diseases (Hatheway et al., 1990; Duran and Walton,' 1997; Taylor, 1999). *C. novyi* type A and B are isolated in cases of sudden death in pigs (Duran and Walton,' 1997; Taylor, 1999). Both types produce alpha toxin with oedematous action (Bette et al., 1989) inducing morphological changes in all cell types, particularly endothelial and breakage of the cytoskeleton of the cells (Müller et al., 1992). As a result, the integrity of the capillaries is disrupted and lead to edema and diapedesis in the surrounding tissues, similar to the observed in lungs by us. Beta toxin of *C. novyi* is similar to the alpha toxin of *C. botulinum* (Hofmann et al., 1995). In our case, the manifestation of the symptoms both from the gastrointestinal and respiratory tract, as well as the histological findings in kidneys and livers were perhaps due to more than one toxin of *C. novyi*, together with the toxins of the other isolated pathogenic bacteria.

The severe gastroenteritis and the isolation of *E. coli* from the internal organs, especially from the liver is evidential for colisepticemia. *E. coli* is a Gram negative, facultative anaerobe normally distributed in the lower section of the intestinal tract. Some strains of *E. coli*, however, can cause serious food poisonings in humans and animals ("*Escherichia coli*". CDC National Center for Emerging and Zoonotic Infectious Diseases. Retrieved 2012-10-02.; Vogt and Dippold, 2005). The blowing agent is cytolisin – a hemolytic toxin that plays a role in the pathogenesis of these diseases (Ludwig et al., 2004). It is possible that some pathogenic strains of *E. coli* were imported alimentary or non-pathogenic strains passed to virulent, amid poor general condition of the animals, because of the mixed bacterial toxicoinfection.

The other identified bacteria *Enterococcus* sp. and *B. cereus* probably had a minor role in the development of the pathological events observed. *B. cereus* is a Gram positive facultative anaerobe of the genus *Bacillus*, family Bacillaceae and like the other representatives can form protective endospores. Some of the strains are used as probiotics in animal feed (Ryan and Ray, 2004), while others are pathogenic and cause food poisoning, accompanied by headache, vomiting and severe

diarrhea (Kotiranta et al., 2000). The virulence of strains is determined by a synthesis of the four toxins – emetic toxin (ETE) and three different enterotoxins: HBL (haemolytic), Nhe (non-haemolytic) and EntK (Guinebretière et al., 2004). The pathological effect is due to the formation of pores in the cell membrane, loss of electric potential and cell death.

Enterococci have long been regarded as belonging to the genus *Streptococcus*, but after analyzing of the genome, they were separated in a genus *Enterococcus* (Schleifer and Kilpper-Balz, 1984). Enterococci are normal inhabitants of the intestinal tract, but sometimes cause chronic infections of the urinary tract and prostate, bacteremia, bacterial endocarditis and meningitis (Fisher and Phillips, 2009;Ryan and Ray, 2004; Devriese et al., 1991).

The provided data of our results confirm the high sensitivity of guinea pigs especially to *Clostridium* sp. infections, which is why they are the best laboratory animals in research on such diseases.

Conclusion

As a result of the complex microbiological, parasitological and pathological examinations of the bodies of the guinea pigs was established bacterial toxicoinfection, involving anaerobic bacteria *C. novyi*. By these results the high sensitivity of this species to *Clostridium* infections was confirmed.

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