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EFFECTS OF SOME IMMUNOSUPPRESSIVE FACTORS ON CAMPYLOBACTERIOSIS OUTBREAKS IN POULTRY

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Campylobacteria are widely distributed in domestic and wild animals. Avian species are the most common host of campylobacteria, thus an important source for human infection caused by this organism. Campylobacteriosis in animals frequently proceeds without clinical symptoms, which may be due to the different virulence of the agent, as well as to the immune status of the infected animal. In this trial we investigated the effects of the vaccine against infective bursal disease (IBD) on occurrence and clinical manifestation of campylobacteriosis in conditions of controlled experimental infection with the strain Campylobacter jejuni. The immunosuppressive effects of a live attenuated vaccine against IBD on development of clinically manifest campylobacteriosis in perorally challenged chickens were assessed. The investigation was performed on 90 chickens in experimental conditions. The presence of Campylobacter jejuni was confirmed by re-isolation, while identification of the organism was performed on the basis of its morphological, cultural and biochemical characteristics. Specific antibody titer and humoral immune response to C. jejuni specific antibodies were monitored using the complement fixation reaction method. Clinical symptoms of the disease were observed in chickens infected with Campylobacter jejuni and vaccinated against infective bursal disease. Pathomorphological findings revealed changes in the intestines and liver, from which Campylobacter jejuni was isolated. C. jejuni specific antibodies were detected in the infected birds in the third week post infection, with titer values ranging from 1:4 to 1:16. Results of our research strongly imply an immunosuppressive effect of the IBD vaccine on development of campylobacteriosis, which is supported by the clinical and morphological findings, i.e. isolation of the agent and detection of specific antibodies.

Key words: campylobacteriosis, infective bursal disease, Campylobacter jejuni, immune response

INTRODUCTION

Campylobacter organisms are frequently isolated in various animal species. They are widely distributed both in animals and humans. The presence of campylobacteria was confirmed in all domestic and wild animals, as well as in their excreta. Campylobacteria are detected in reptiles, fishes, mollusks, as well as in waste- and surface waters, soil and mud (Dobbin *et al.*, 2005).

Campylobacteriosis is an infective disease of animals and humans characterized by changes in the gastrointestinal tract and reproductive disorders. It is widely distributed among animals causing abortions in sheep, mastitis and dysentery in cattle, acute enteritis in calves, lambs, pigs, dogs and cats, as well as hepatitis in poultry. The disease is characterized by increased body temperature, weakness, sleepiness, frequent diarrhea, colic, decreased growth and production performance (Aiello, 1998; Quinn, 2002; Saif, 2003).

Until the mid XX century there was a strong belief that human infections caused by campylobacteria are rare, and the organism was considered an opportune human pathogen. The researches dating from the last 30 years established the importance of Campylobacter species in the pathogenesis of human diseases. Human campylobacteriosis occurs sporadically, as small-scale family infections or epidemics in particular communities. Campylobacteriosis is characterized by poor general health status of the patient, recurrent high body temperature, tremor, headache, abdominal pain, vomiting and diarrhea (Otašević, 2002). Occurrence and severity of these symptoms is determined by the virulence of the organism and by the immune status of the patient. Results of recent studies revealed that campylobacter infections mostly affect the newborns and children, as well as patients with an immune deficiency syndrome or malignant diseases of the immune system. Furthermore, the infections frequently occur in patients with diabetes and alcoholics (Zonios *et al.,* 2004). Investigation of diarrhea in animals revealed that, besides the campylobacteria, the decreased levels of gamma globulin significantly contributes to such clinical condition. The problem of malnutrition in parents is associated with decreased immunoglobulin levels in the blood serum, resulting in immune deficiency in the offspring (Andres *et al.,* 2006).

Fusell (1998) emphasizes that immunosuppressive factors produce considerable losses in poultry production, which is manifested by increased mortality and decreased production parameters. The author points out the importance of identifying all stress factors that may induce immunosuppressive effects.

Saif (1991) established that IBD virus infection in chickens at an early age may induce disorders of humoral and cell-mediated immune response. Immunosuppression results from the direct lysis of B lymphocytes or their precursors, while effects of vaccine antigen on T lymphocytes are weaker and last shorter. Kim Jeong *et al.* in 1999 investigated the effects of the vaccine against infective bursal disease on the immune system of chickens, establishing that the primary immune response to antigens was reduced during the first six weeks after administration. Nowadays, research is focused on factors influencing the occurrence of some particular forms of campylobacteriosis in poultry

populations, in which colonization of various tissues with campylobacteria and prevalence of the infection is established. Considering that the presence of this organism in the infected animals not always results in a clinically manifest infection, the aim of our investigation was to determine whether vaccination against infectious bursal disease may affect clinically manifest campylobacteriosis in poultry, as well as to quantify the humoral immune response.

MATERIAL AND METHODS

The experimental trial was conducted in controlled conditions, using one day old chickens of Arbor Arcers breed. Prior to be introduced into the experiment the chickens were examined for the presence of *Campylobacter* species.

During the experiment three groups were formed, each consisting of 30 chickens.

– First (1.) experimental group was perorally inoculated with 1 x 10^8 cfu/mL field strain of Campylobacter jejuni.

– Second (2.) experimental group, was perorally immunized with Nobilis Gumboro D78 monovalent lyophilized live vaccine.

– Third (3.) experimental group, 30 chickens, was perorally inoculated with 1x10^8 cfu/mL field strain of Campylobacter jejuni and perorally immunized with Nobilis Gumboro D78 monovalent lyophilized live vaccine

Samples for bacteriological examination were collected from sacrificed birds, i.e. 2 chickens from each experimental group. Sampling was performed on day 10, 17 and 22 after challenge. Liver, cecum and distal portions of the large intestine were subjected to bacteriological examination.

Media for isolation of *Campylobacter jejuni*:

1. Columbia agar (bioMerieux) + 5% defibrinated sheep blood

2. Campylosel antimicrobial additive (bioMerieux) containing the following antibiotics: cefoperazone 3 mg, colistin 2000 U, vankomycin 2 mg, amphotericin B 0,4 mg (2 mL of suspended antibiotic mixture added to 200 mL Columbia agar).

3. Generbox microaer (bioMerieux) Gas-pack sachets providing microaerophillic conditions in the McIntosh jar for anaerobiosis.

Diagnostic sets for identification of *Campylobacter jejuni*

1. API Camp strips (bioMerieux) for biochemical identification of campylobacteria.

Samples for serological examination. Blood samples were collected before the investigation, with four subsequent samplings at 7 day intervals.

Clinical examination

The experimental birds were observed daily throughout the whole investigation period. Monitoring and assessment included their general health status and presence of gastrointestinal disorders such as: altered feces in the

litter, change of the feces (consistency, traces of blood or mucous), contamination of the cloacal region or feathers on the breasts.

Pathomorphological examination

Pathomorphological examination was performed on all birds sacrificed at scheduled time intervals throughout the investigation period. On opening the abdominal cavity liver and intestines were examined for the presence of pathological changes.

Isolation and identification of Campylobacter jejuni

Samples were directly inoculated onto the nutritive medium and placed in an anaerobiotic jar equipped with Gas-packs that provided microaerophillic conditions. All samples were inoculated and prepared as described above, and incubated for 48 hours at 37°C and 42°C. After incubation the inoculated media were read and suspect colonies were Gram stained and examined microscopically. Gram negative, spiral, oxidase positive organisms were biochemically identified by the use of API Campy identification system and the appropriate software (bioMerieux). The enzyme activities of *Campylobacter jejuni* were determined, i.e. urease, nitrate reduction, esterase, hippurate, gamma-glutamyl-transferase, chloride reduction to the triphenyl tetrazolium, pyrrolidonyl arylamidase, L-arginine arylamdase, L-aspartate arylamdase, alkaline phosphatase, H_2S production, usage of glucose, succinate, acetate, propionate, malate, citrate, as well as sensitivity to nalidixic acid, cefalosine and erythromycin.

Serological examination

Detection of *Campylobacter jejuni* specific antibodies was performed by the complement fixation reaction and a test-kit produced by Virion, Switzerland. The experimental chickens were examined for the presence of maternal antibodies against infective bursitis using the agar-gel immunodiffusion test (AGID-test). The blood samples were taken immediately before experimental infection, four times throughout the investigation period of five weeks, at seven day intervals.

RESULTS AND DISCUSSION

Prior to be challenged with *C. jejuni* the chicks were bacteriologically examined and confirmed "free from *Campylobacter* species". All birds were seronegative for *C. jejuni* specific antibodies, also.

Clinical examination of chickens from the first experimental group that was infected with a field strain of *C. jejuni* and chickens from the third experimental group infected with strains *C. jejuni* and vaccinated with Nobilis Gumboro D78 monovalent lyophilized live vaccine revealed changes in the cloacal region. Contamination of feathers in the cloacal region and watery diarrhea were observed in the investigated birds during the first two weeks of the experiment. In the same period, small quantities of hemorrhagic content were noticed in the feces. Foamy, watery feces with blood traces were observed in the litter (Figure 1).

Changes described above were not observed in the second experimental group vaccinated with Nobilis Gumboro D78 vaccine.

Figure 1. Droppings with traces of blood

According to data from the available literature, presence of *Campylobacter jejuni* in poultry may result in clinical signs of diarrhea, and the patho-anatomical picture may reveal intestinal changes affecting predominantly the jejunum and caecum, though changes may extend to the distal portions of the intestines, also (Saif, 2003). Pathoanatomical changes are manifested as distensions and constrictions of the intestines, as a consequence of the accumulated watery and mucous content. Hemorrhages are not necessarily associated with all cases of enteritis induced by campylobacteria. Recent studies revealed that clinically manifest campylobacteriosis occurs in newborn children and patients with immunodeficiency syndrome, as well as in all diseases or syndromes associated with immune system impairment (HIV infection, liver cirrhosis, ageing, pregnancy, diabetes mellitus, malignant diseases, autoimmune diseases, transplantation, splenoectomy) (Zonios *et al.*, 2004).

Pathomorphological examination of the first and second experimental group did not reveal any characteristic pathological changes in the liver and intestines. In the third experimental group (infected with strains *C. jejuni* and vaccinated with Nobilis Gumboro D78 monovalent lyophilized live vaccine) characteristic liquid foamy content was observed in the intestinal lumina. The intestines and caecum were tense, distended, and with obvious small hemorrhages in the mucosa (Figures 2, 3). Changes in the liver were manifested as red and yellow lines (Figure 4).

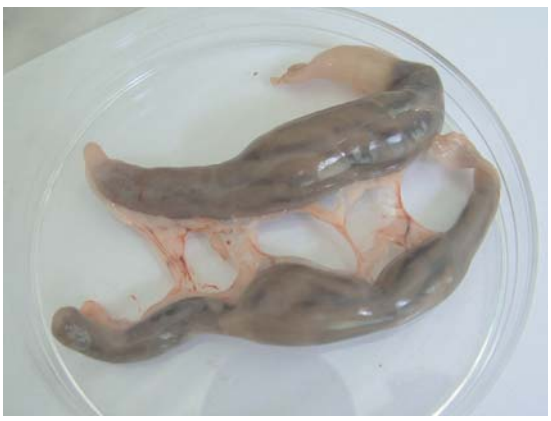


Figure 2. Changed caecums

In the firs experimental group infected with *C. jejuni* no changes in the liver and the intestines were observed.

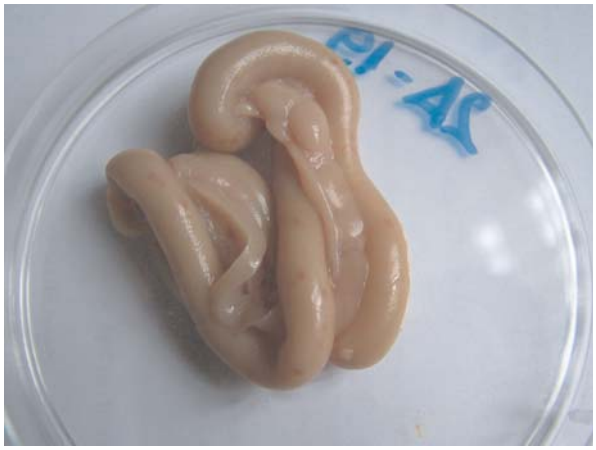


Figure 3. Changed caecums from the experimental groups

Figure 4. Changes on liver in infected chickens

Such clinical and pathomorphological changes may occur in chickens exposed to immunosuppressive agents. Administration of immunosuppressive substances results in the occurrence of clinically manifest changes, while pathoanatomical and laboratory findings imply campylobacteriosis. In a recent research Subler *et.al.* (2006) investigated whether infection with IBD virus may affect the colonization and shedding of *Campylobacter jejuni* in chickens. They established a significantly higher isolation rate of *C. jejuni* in chickens infected with *C. jejuni* and IBD virus, in comparison to the group infected only with *C. jejuni*. The same ratio was established with respect to colonization rate. Presence of *C. jejuni* in organs of chicks could be detected more rapidly in a group infected with

Campylobacter and IBD virus in comparison with the group infected only with *Campylobacter*.

Bacteriological examination of the organs (liver, caecum and intestines) of experimental chickens revealed the presence of *Campylobacter jejuni* in the first and third experimental group. Recovery of the inoculated organism confirmed successful challenge and colonization of experimental animals. Other researchers (Kazwala *et al.*, 1992) report on a strong dependence of colonization rate on the age of challenged chicks and on the size of the inoculum (cfu/mL). Younger birds undergo colonization more readily, and clinical symptoms get more pronounced with the increase of density of the inoculating suspension. Diergaardt *et al*. (2004) established that amount of 100-500 units of campylobacteria is sufficient to provoke the infection.

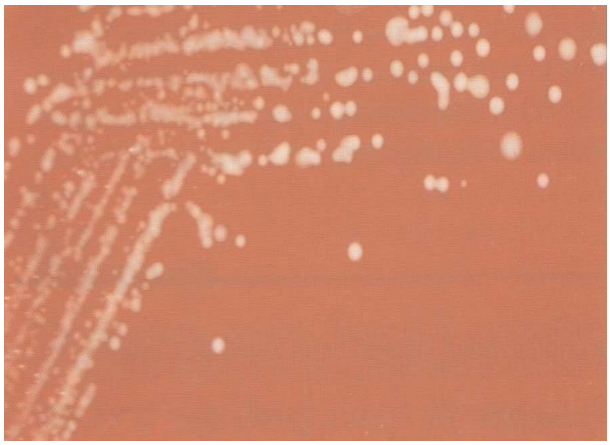


Figure 5. Colonies of *Campylobacter jejuni*

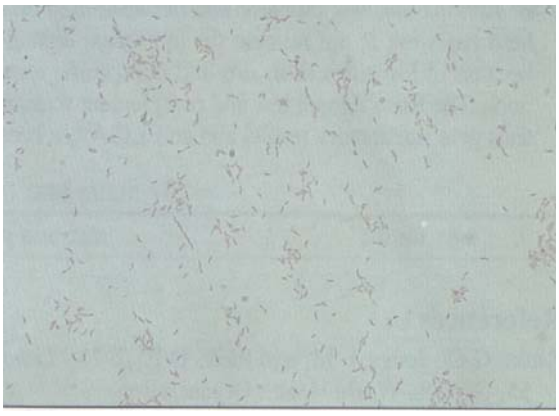
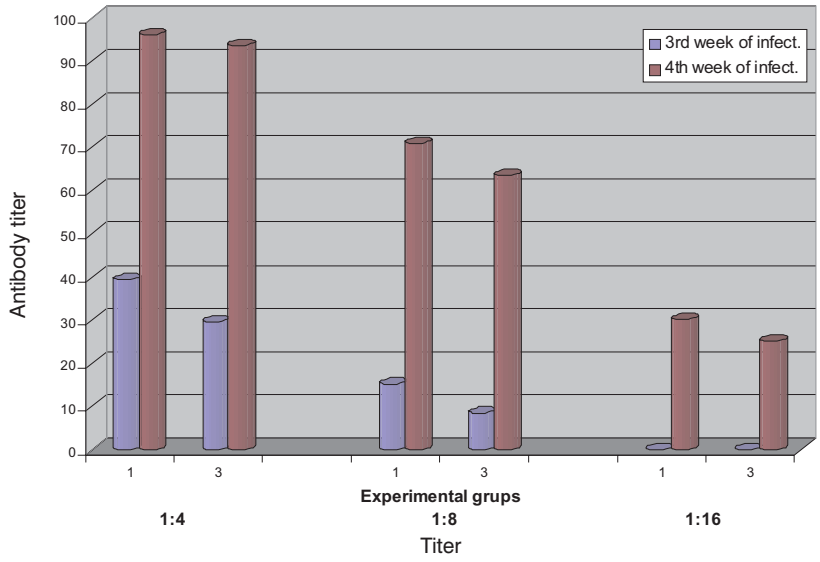


Figure 6. Smear of *Campylobacter jejuni*

C. jejuni-specific antibodies were detected in blood sera of experimental chicks from groups 1 and 3 three to four weeks after challenge with the field strain of *C. jejuni* by the method of complement fixation. Blood sera obtained from non-infected birds (group 2) vaccinated against IBD with Nobilis Gamboro D78 vaccine proved negative to *C. jejuni* specific antibodies.

Titer values ranging 1:8 and higher were considered to be a positive serological finding. Complement fixing antibodies detected in blood sera of chickens from the first and third experimental group in the third and fourth week post inoculation ranged from 1:4 to 1:8, and from 1:4 to 1:16, respectively.

Graph 1 displays median values for antibody titers in the first and third experimental group, obtained by the method of complement fixation reaction.



Graph 1. Comparative display of median titer value range in the 1. and 3. experimental group

Data on *C. jejuni*-antibody titer range obtained in our experiment after statistical analyzis reveal significant differences (probability rate 95%) between particular groups when analyzed by weeks; however, such differences were not evident if the groups are observed as a whole.

Statistical evaluation revealed significant differences between experimental group 1 and 3. The first experimental group, infected with *C. jejuni,* showed higher antibody titer values in comparison to the group infected with *Campylobacter* and vaccinated with IBD vaccine.

Saif (2003) reported on mechanisms and immunosuppressive effects of IBD virus and live monovalent vaccines against IBD on the immune system of infected

poultry, which was confirmed in our research. Furthermore, Subler *et al.* (2006) reported that immune system exposed to IBD virus is impaired, thus unable to prevent easy and rapid colonization, as well as prolonged shedding of *C. jejuni* in the infected chickens.

Different (mostly low) *C. jejuni* - antibody titer values determined in the experimental group immunized with the vaccine against IBD during this trial strongly suggest an impact on the immune system. In the third and fourth week post challenge this group exhibited a weaker antibody response to *C. jejuni* antigens as compared with Group 1 that was infected with campylobacter only, and was not immunized with Nobilis Gumboro D78 monovalent live vaccine against IBD. Differences between *C. jejuni* - antibody titer values recorded during this experiment imply that the vaccine affected the immune system and induced a weaker immune response to *C. jejuni*, contrary to the group infected with *C. jejuni* only. Clinical symptoms and pathomorphological findings observed in the experimental birds revealed that changes may be attributed to a concomitant action of the immunosuppressive effect of IBD vaccine and campylobacterial infection induced by challenge with the field strain *C. jejuni*.

We may conclude that campylobacteriosis outbreaks in poultry may occur in particular situations, most commonly as a consequence of cumulative impact of different adverse factors on susceptible birds. It is still not elucidated, whether clinically manifest campylobacteriosis is to be attributed only to the activity of some representatives of the genus *Campylobacter*, or if some other etiological factor existing in the infected animal has preceded the pathogenic action of this organism, providing conditions (as the primary factor) for pronounced pathogenic effects of *Campylobacter* species. Data from the literature, as well as results of our research, confirmed the ability of *Campylobacter* species to induce the clinically manifest disease in infected poultry; however, rarely as the primary etiological agent, and mostly after the action of various stress factors in susceptible individuals.

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ISPITIVANJE UTICAJA NEKIH IMUNOSUPRESIVNIH FAKTORA NA POJAVU KAMPILOBAKTERIOZE ŽIVINE

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SADRŽAJ

Kampilobakterije su kod domaćih i divljih životinja široko rasprostranjeni mikroorganizmi, a živina je jedan od glavnih nosilaca kampilobakterija i važan izvor infekcija ljudi. Klinički simptomi kampilobakterioze kod živine se relativno retko javljaju, a razloge treba tražiti u različitoj virulenciji uzročnika i u imunološkom statusu inficiranih jedniki. U ovom radu je ispitivan uticaj vakcine protiv infektivnog burzitisa živine na nastanak i kliničku pojavu kampilobakterioze, posle veštačke infekcije terenskim sojem *Campylobacter jejuni.* Ispitivanje je izvršeno na 90 pilića u eksperimentalnim uslovima. Prisustvo *Campylobacter jejuni* u organizmu veštački inficiranih pilića dokazivano je reizolacijom uzročnika, a identifikacija izolovanih bakterija vršena je na osnovu njihovih morfoloških, kulturelnih i biohemijskih osobina. Prisustvo titra specifičnih antitela i kretanje humoralnog imunskog odgovora na antigene *Campylobacter jejuni* kod pilića u ogledu praćeno je primenom reakcije vezivanja komplementa (RVK). Kod pilića inficiranih sojem *Campylobacter jejuni* i imunizovanih vakcinom protiv infektivnog burzitisa uočeni su klinički znaci bolesti. Patomorfološkim ispitivanjem utvrđene su promene na

crevima i jetri pilića iz kojih je izolovan *Campylobacter jejuni.* Specifična antitela za *C. jejuni* dokazana su kod inficiranih jedinki u trećoj nedelji od veštačke infekcije, a vrednosti njihovog titra su iznosile od 1:4 do 1:16. Rezultati ovih ispitivanja su potvrdili imunospresivno delovanje vakcine protiv infektivnog burzitisa koje je imalo za rezultat klinički manifestnu kampilobakteriozu. Infekcija je potvrđena na osnovu kliničkih simptoma i patomorfoloških promena, izolacijom uzročnika, kao i na osnovu vrednosti titra specifičnih antitela utvrđenih u uzorcima krvnih seruma oglednih pilića.