INFLUENCE OF IMMUNE STRESS FACTORS ON THE PRESENCE AND SHEDDING OF *Campylobacter jejuni* IN POULTRY

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**Abstract:** The nature and pathogenesis of infective diseases depend on development and functioning of immune system. Weakening of an organism or its total disfunctioning does not disturb health condition of an animal, but provide condition for development of microorganisms which under other conditions would not appear. Campylobacter belong to a group of microorganisms that, as comensals, may be found in nature. In all the animals their presence is registered. Infection of animals and humans with these microorganisms is not simultaneously clinically and pathomorphologically manifested campylobacteriosis. Having in mind that there is a problem of insufficiently explained problems regarding campylobacter in animals (a large number of infected animals, but with few clinical findings), the subject of our research was to follow how *Campylobacter jejuni* spreads in an organism and is shed in infected experimental conditions and to follow simultaneous immunization provided by vaccination against infective bursal disease (IBD). The objective of this paper is to determine how vaccination against IBD influences colonization and transition of *Campylobacter jejuni* in organs, as well as to notice if there is a difference in shedding of this microorganism into the environment. In the experiment we used referent strains of *Campylobacter jejuni* and commercial vaccine (Nobilis Gumboro D 78, monovalent liophylisated live vaccine). The isolation of the inoculated strain was done on selective media, and for identification Api Campy strips were used. Immunology response was confirmed with AGID test.

In the research it was detected that in the experimental group, vaccinated an infected with *C. jejuni*, *C. jejuni* could be isolated in parenchymatous organs (liver and spleen) 3 days earlier than in a group only infected with this microorganism.

**Key words:** *Campylobacter jejuni*, immunity, parenchymatous organs, transmission, shedding
Introduction

The nature and pathogenesis of infective diseases in many ways depend on the development and functioning of immune system (Newell, 2002; Aiello, 1998). This opinion is supported by the fact that some, especially viral infections, directly influence immunology response by immunomodulation and reduce its effect. The weakening of an organism protection or total dysfunction of an immune system do not disturb health condition of animal, but provide a condition for development of microorganisms which, under other circumstances, would not cause a disease (Stojanov et al., 2008).

Campylobacter belong to a group of microorganisms that, as comensals, may be found in nature (Newell, 2002; Wassenaar and Blaser, 1999), so they are present in almost all animals. The infection with these microorganisms does not necessarily cause the occurrence of campylobacteriosis, a diseases with clinical and pathomorphological signs. Clinical signs of this disease may be found, as already mentioned, when some factors disturb homeostasis (Wassenaar and Blaser, 1999), and this can be recognized as the primary factor of the disease, i.e. the organism becomes diseased and it cannot fight against microflora which is not obligatorily pathogen. This way of understanding could contribute to the development of a new approach in diagnosing and treating, because the cases of infective diseases, first of all bacterial diseases, could indicate that there is another, primary factor which provides comensal microorganisms to provoke clinical symptoms of a disease.

Having in mind that there is a problem of insufficiently explained problems regarding campylobacter in animals (a large number of infected animals, but with few clinical findings), the subject of our research is to follow how Campylobacter jejuni spreads in an organism and is shed in infected experimental conditions and to follow simultaneous immunization provided by vaccination against infective bursal disease (IBD). The objective of this paper is to determine how vaccination against IBD influences colonization and transition of Campylobacter jejuni in organs, as well as to notice if there is a difference in shedding of this microorganism into the environment.

Materials and Methods

For the experiment one day old chickens of Arbor Arcers were used. Two groups were formed and each consisted of 30 chickens. Before the experiment, the birds were tested with AGID test on the presence of IBD maternal antibodies. It was determined that there were no antibodies. The experimental group of chickens was vaccinated with commercial vaccine (Nobilis Gumboro D78, monovalent liophylisated live vaccine). Three days after vaccination both the experimental and
control group were orally (p/o) infected with a referent strain of *Campylobacter jejuni*. *Campylobacter jejuni* was isolated from the digestive tract (caecum and jejunum) and parenchymatous organs (liver and spleen) of the sacrificed animals. The first control on the presence of *Campylobacter jejuni* was carried out three days after the infection. For isolation Columbia agar (bioMerieux) + 5% defibrinated sheep blood and (Campylosel) containing antibiotics (bioMerieux) for restriction of unwanted flora *(Quinn et al., 2002)*. Microaerophilic conditions in Mcintosh jar for anaerobiosis were provided by Generbox microaer (bioMerieux). *Campylobacter jejuni* was confirmed by API Campy strips (bioMerieux) and a software was used for reading the results *(Biberstein and Zee, 1990)*.

**Results and Discussion**

According to *Saif (2003)* campylobacteriosis in poultry, caused by *Campylobacter jejuni*, is characterized with intensive tension and intestine enlargement in distal parts of digestive tract with characteristical necrotic changes on liver tissue. However, according to this author clinical form of campylobacteriosis most often is accompanied by other factors. The aim of examining the influence of immunosuppressive factors of campylobacteriosis was to determine if it is possible to provoke campylobacteriosis in controlled conditions. *Arnold and Holt (1995)* discovered that in the experimental animals, where immunosuppression was provoked by chemicals (by ciklofosfamide), characteristic changes on liver were red spots. There is a need for better understanding of the influence of immunosuppression of campylobacteriosis.

In our paper we examined immunosuppressive effect of IBD virus. There are more authors *(Saif, 2003; Naglić and Hajsig, 1993; Aiello, 1998)* that report about immunosuppression that occurs in infective bursal diseases (Gumoboro) and causes reduced number of B cells in bursal follicles and their apoptosis. The virus provokes disorders in humoral, but also cell immunity, however the consequences are not severe and do not last long. The infection with this virus causes reduced immune response, primarily humoral response to different bacterial, viral and parasitic diseases. *Sharma et al. (2000)* determined that clinical and subclinical infection with IBD causes immunosuppression so humoral and cellular immunity is disturbed.

Disorders in immunology system create conditions for development of bacteria that otherwise would not have an effect, but now populate and spread within the organism.

Immunization is one of the best way for controlling the infectious inflammation of bursa (IBD). In his work *Saif (2003)* points out that vaccines designed in different way may have unwanted effect when speaking about IBD prophylaxis. Attenuated vaccines made out of virulent virus strains may cause a
collapse of protective role of neutralization maternal antibodies of a virus and cause atrophy of bursa and induce immunosuppression in chickens old 1 day to 3 weeks.

In Table 1 are displayed the results of examining the influence of IBD live vaccine on spreading of *Campylobacter jejuni* through digestive tract up to parenchymatous organs.

**Table 1. Results of examining *C. jejuni* in parenchymatous organs**

<table>
<thead>
<tr>
<th>Day</th>
<th>Experimental group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infection with <em>Campylobacter jejuni</em> + <em>Nobilis Gumboro D78</em></td>
<td>Infection <em>Campylobacter jejuni</em></td>
</tr>
<tr>
<td></td>
<td>Parenchymatous. org. liver + spleen</td>
<td>Parenchymatous. org. liver + spleen</td>
</tr>
<tr>
<td></td>
<td>Digest. tact caecum + jejunum.</td>
<td>Digest. tract caecum + jejunum.</td>
</tr>
<tr>
<td>day 3</td>
<td>-</td>
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</tr>
<tr>
<td>day 6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>day 9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>day 12</td>
<td>+</td>
<td>-</td>
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<tr>
<td>day 15</td>
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<td>+</td>
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<tr>
<td>day 18</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>day 21</td>
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<td>+</td>
</tr>
</tbody>
</table>

The obtained results show that in the experimental group, vaccinated with attenuated vaccine, *Campylobacter jejuni* was isolated 3 days earlier comparing to the control group. Subler *et al.* (2006) investigated what is the influence of induced immunosuppression of chicken on colonization and shedding of *Campylobacter jejuni*. Immunosuppression of the experimental chicken was induced by infection with IBD virus, after which suspension of *C. jejuni* was administered. For observing/following the level of colonization and shedding we used a group of chickens which were administered only suspension of *C. jejuni*. The authors discovered that in the group of chickens where immunosuppression was induced, *C. jejuni* colonized the intestine faster, so shedding of this microorganism was more intensive and bursa became pathoanatomically smaller. They concluded that this research should be directed towards the preparation of vaccine against IBDV which would not cause immunosuppressive effect.

Our examination also proved that in the experimental group of chickens *Campylobacter jejuni* was detectable earlier than in the control group.

The importance of live vaccine applied to chicken and its influence on their health status and vitality has been reported in the work of Ezekoli *et al.* (1990). He compared the reaction of chickens vaccinated with live vaccine against infective inflammation (IBD) to the unvaccinated chickens. It was detected that in vaccinated chicken mortality was higher and large lymphocyte exhaustion was noticed.
Butter et al. (2003) point out that immunosuppression in vaccinated chickens with the vaccine against IBD may be used for determining its effect during immunoprophylaxis. Their research pointed on a possibility of designing a recombinant vaccine which would contain IBD protein parts and would provoke immune response with a lower immunosuppressive effect, but better protection than the protection that provide existing vaccines.

Influence of IBD virus on infections caused by different microorganisms, like for examples colibacillosis, Mitra et al. (2004) described in their paper and pointed that infection with IBD virus causes immunosuppression and is favorable for the cases of secondary bacteria infections.

According to Wassenaar and Blaser (1999) spreading of campylobacteriosis in organisms depends, on one hand, from their ability to avoid host defense mechanism, but, on the other hand, their virulence depends on the characteristics of bacteria as well on immunology reaction of the organism. These investigations have shown that colonization of digestive tract and spreading of Campylobacter jejuni on parenchymatous organs of the infected chicken depends on the immunocompetence of a host.

**Conclusion**

The obtained results point that shedding of Campylobacter jejuni through digestive tract of the infected chicken vaccinated with live attenuated vaccine against IBD was possible after 3 days. Shedding of C. jejuni in the experimental group, that was not vaccinated, was a bit later. These results may indicate that vaccination influenced this phenomenon, because temporary weakening of immunity provided a possibility of faster colonization and faster beginning of C. jejuni shedding.

In the same the spreading of C. jejuni from intestine to parenchymatous organs could be explained. It was also noticed that in the groups that were vaccinated against IBD this bacteria could be isolated sooner than in unvaccinated group.

This investigation could support the opinion that there is a need for a new approach in diagnostics and therapy of some infective disease. Clinical symptoms could, in some cases, indicate the presence of other primary factors that provide conditions for other diseases which would not occur if the homeostasis was not disturbed.
Uticaj imunostresnih faktora na prisustvo i izlučivanje Campylobacter jejuni kod živine

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Rezime


Istraživanjem je utvrđeno da je 3 dana ranije izolovan Campylobacter jejuni iz parenhimatoznih organa (jetra i slezina) ogledne grupe koja je vakcinisana i inficirana sa C. jejuni u odnosu na grupu infusediranu samo spomenutim mikroorganizmom.

References


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