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THE IMMUNE RESPONSE INDUCED BY E. COLI IN CHICKENS AND AVAILABLE VACCINES

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Abstract

The paper aimed to present the available vaccines for E.coli in chicken and discuss the immune response induced by E.coli. It is based on statistical data provided by already published articles and data interpreted from specialised journals. Avian pathogenic E. coli (APEC) represents the most economically significant disease which has a negative impact on the industry of broilers. In many countries, a common practice during the last decades has been to administer antibiotics in order to suppress infection with APEC, but lately market pressures, by trade and statutory restraints, have limited the use of antibiotics and this has led to the development of specially designed E.coli vaccines, to stimulate an immune response against pathogenic E. coli, besides vaccination against primary respiratory and immuno-suppressive pathogens. Chickens display different mechanisms to protect against and to combat pathogenic infection. Typically APEC is inhaled or ingested, by crossing the mucosal barrier; it cannot penetrate the skin, which generally acts as a protective element. In broilers, the source of infection are, usually, the contaminated drinking water or the inhaled dust, laden with APEC. Antimicrobial drugs remain an important tool in reducing of incidence as of mortality, associated with this disease, but a vaccine-based approach for the disease control remains highly desirable, which is why the focus of this paper will be to analyze and criticize each available type of E.coli vaccine, including the advantages and disadvantages of each, in terms of preparation, efficiency, costs and other important factors. The latest developments in molecular biology have created new vaccine strains effective against APEC, like the modified gene-deleted vaccines which stimulate both, tissue (cellular) associated immunity and humoral (circulating antibody) immunity. The first commercial E. coli vaccine for chickens was licensed by the US Department of Agriculture in 2006, so this paper will also try to focus on the newest research, directed after this year.

Key words*: E.coli vaccines, immune response*

INTRODUCTION

E. coli can enter the body by various routes, all of which can lead to colibacillosis. Avian pathogenic E. coli (APEC) represents the most economically significant disease for poultry, which has a negative impact on the industry of broilers. Two major problems currently make it difficult to control poultry colibacillosis, namely, the lack of a dependable method to identify the pathogenic strains of E. coli and the not totally effective available vaccines, also vaccines against E. coli are not widely used, and this may be due to the large variety of serogroups involved in field outbreaks. Septicemic disease may be rapidly fatal or chronic, manifested by debilitation, diarrhea and respiratory distress. Other pathological features are pericarditis, synovitis, salpingitis and panophthalmitis (Hirsh and Zee., 1999).

Avian colibacillosis is a complex syndrome characterized by multiple organ lesions with airsacculitis and associated pericarditis, perihepatitis and peritonitis. Environmental factors as well as the constitution of poultry or initial viral infections, influence the outcome of APEC-infections. Experimental studies have shown that the respiratory tract, mainly the gas exchange region of the lung and the interstitium of the air sacs are the most important sites of entry for avian pathogenic E.coli (Ewers et al., 2005).

E. coli vaccines induced mucosal and humoral immunity, but were not effective in the

prevention of E.coli infections, due to highly serotype diversity.
Researchers have proposed different types of vaccines in attempts to induce protective immunity against colibacillosis in chickens. The earliest studies involved evaluation of killed organisms, subunit vaccines and live vaccines. (Maas, 2013).
This paper will discuss, in the results and discussion section, the benefits of live, attenuated, inactivated, subunit, recombinant, conjugate and toxoid vaccines.

MATERIALS AND METHODS

Specific articles were selected from hundreds of articles analysed in PubMed, Google Academic databases, NCBI, Research Gate, vet research, Biomed central, and Science Direct. The research method followed three main steps.
1-scientific databases research of the relevant articles concerning E. coli available vaccines.
2- Analysis and selection of the relevant data
3- Extraction and summarization of the results. When necessary and available, the data have also been statistically processed and interpreted.

RESULTS AND DISCUSSIONS

Respiratory tract infection often presents itself with one or more birds sneezing, having a runny nose and foamy running eyes. Septicemic disease course may be rapidly fatal or chronic, manifested by debilitation, diarrhea and respiratory distress. Other infections are pericarditis, synovitis, salpingitis and panophthalmitis (Hirsh and Zee., 1999). In severe cases these birds can have swollen sinuses, stop eating and, in extreme cases, die. Chicken respiratory infections are usually spread by direct contact between infected and uninfected chickens, but also by contact with objects that infected chickens have sneezed or coughed on, such as transport coops or clothing can carry the infectious organisms from place to place, too. Escherichia coli infections are widely distributed among poultry of all ages and categories. They are primarily related to poor hygienic conditions, to neglected technological requirements or to previous respiratory and immunosuppressive infections. A common sequel of navel infections is the local or diffuse peritonitis. Bacteriophages are viruses that can infect and kill bacteria. Pathogens that cause respiratory or enteric disease typically enter via mucosal surfaces and stimulate a local immune response, involving production of the mucosal secretory immunoglobulin A (sIgA), the most important immunoglobulin involved in the immune response of mucous in many species (McGhee et al., 1992).
Despite the results of many studies, carried to determine the efficacy of the bacteriophage to prevent an Escherichia coli respiratory infection in broiler chickens, the data proved that this can protect birds from a respiratory challenge with E. coli, the adding the bacteriophage to the drinking water did not protect the birds against E.coli challenge. (Huff et al, 2002).
Avian colibacillosis is an infectious disease of birds caused by Escherichia coli. Avian colibacillosis has been noticed to be a major infectious disease in birds of all ages. This disease has an important economic impact on poultry production worldwide. The majority of economic losses results from mortality and decrease in productivity of the affected birds.
Two major problems currently make it difficult to control poultry colibacillosis, namely, the lack of a reliable method to identify the pathogenic strains of E. coli and the limited efficacy of the available vaccines, limiting the use of vaccines against E. coli, and this is due also to the huge variety of serogroups identified in outbreaks. (Hirsh and Zee., 1999).
Avian colibacillosis is a complex syndrome characterized by multiple organ lesions with airsacculitis and associated pericarditis, perihepatitis and peritonitis. Environmental factors as well as the biological status of poultry and/or previous chronical/unapparent viral infections, influence the outcome of APEC-infections. Experimental studies have shown that the respiratory tract, principally the gas exchange region of the lung and the interstitium of the air sacs are the most important sites of entry for avian pathogenic E.coli (Ewers et al., 2005).
Compared to bacterins and subunit vaccines, live vaccines are attractive because they are much cheaper than purified proteins, and can be used for mass immunization via aerosols,

feed, or drinking water (Peighambari, et al, 2012). Immunogenicity (SC injection) of an oil-emulsified E. coli (O1:K1) bacterin with an aqueous-phase-to-oil-phase ratio of 1:4 in chickens was evaluated by Panigrahy M. in 1984 (Maas, 2013).

The great diversity among APEC strains limits the possibilities of vaccination, and vaccines are not used on a large scale. Vaccines based on killed or attenuated strains have been tested experimentally. Generally, they induce a satisfactory protection against infection with homologous strains, but lesser or none protection against heterologous. Passive immunization of young birds by the yolk antibodies is protecting for the next two weeks after hatching, if the birds are challenged with homologous strains. Vaccines based on virulence factors like fimbriae, also give a good homologous protection, i.e., against APEC possessing the same fimbriae.

The Mucosal Immune System (MIS) is the main inductor sites for the immune response induction in poultry, when the primary lymphoid organs are subjected to atrophy (20 weeks old). (Janeway et al, 2001).

Although have been reported many studies on bacteria isolation and methods of detection, host immune responses to APEC infection remain unclear (Li et al, 2016). For hosts, the innate immune response represents the first line of defense against enteric pathogens (Mogensen, 2009). When pathogens invade, the innate immune response manages the invasion by inducing programmed cell death and secreting pro-inflammatory compounds that direct immune cells to infection sites (Takeuchi and Akira, 2010). In that context, toll-like receptors (TLRs) serve as important pattern recognition receptors (PRRs) that can recognize various pathogenic organisms (Keestra et al., 2013).

Infection is associated with the induction of both, humoral and cell-mediated immune response. It is unclear whether such events are also induced by existing commercial vaccines. Passively immunity can be acquired either vertically via egg-yolk antibody or administering antibodies intravenously. Environmental factors as well as the biological status of the poultry or the presence of the previous viral infections influence the outcome of APEC-infections.

In poultry, immunity can be innate or acquired. Innate immunity refers to the natural or inherited ability to resist disease. Acquired immunity can be active or passive. Active immunity refers to an active immune response in a bird as a result of recovery from the disease or as a response to exposure to vaccine antigen. The bird produces its own immune cells and/or antibodies to provide protection. Active acquired immunity is divided into non-cellular (humoral) and cellular components (Janeway et al, 2001).

In the last few years, emphasis has been made about the epithelium intestinal biology, the defence mucosa mechanisms and the regulation of induction and expression of immunity, which is reflected in the productive performance of the broiler farm. It is because these poultries grow in intensive condition and receive vaccines to induce immunity (Peralta, 2016).

Mucosal immunity is short-lived and mucosal immunogens do not induce a memory response (Keestra et al., 2013). However, others contend that the induction of mucosal memory response to certain proteins is possible (Vajdy and Lycke, 1995). Generally, it is assumed that eliciting of T and B cells in mucosal tissues such as GALT by microbial antigen presented to that site, leads to priming of B cells.

Antimicrobial drugs remain an important tool in reducing both, incidence and mortality associated with this disease, but a vaccine-based approach for the disease control remains highly desirable, which is why the focus of this paper will be to analyse and discuss each available type of E.coli vaccine, including the advantages and disadvantages of each, in terms of preparation, efficiency, costs and other important factors.

The latest developments in molecular biology have created new vaccine strains effective against APEC, like for example modified gene-deleted vaccines which stimulate both tissue (cellular) immunity and humoral (circulating antibody) immunity. The first commercial E. coli vaccine for chickens was licensed by the US Department of Agriculture in 2006 (Ferguson et al., 1995).

The great diversity among APEC strains limits the possibilities of vaccination, and vaccines are not used on a large scale. Several vaccines based on killed or attenuated strains have been tested experimentally. In general, they induce a satisfactory protection against infection with homologous strains, but protection against heterologous strains is less efficient. In vaccines against enteric infections, gut antibodies can provide antibacterial protection by two mechanisms: direct action against the bacteria or combination with the bacterial products. The first mechanism can result in immobilization, agglutination or prevention of mucosa adherence. The combination of bacterial products like toxins or enzymes can cause inactivation and can help in the destruction by proteolytic enzymes (Ferguson et al., 1995; Smith and Beal, 2008). Previous exposure to the same antigen (Ag) or cross-reaction can guide the mucosal immune response (MIR).

Attenuated vaccines mainly induce better MIR than the inactivated one (Meeusen, 2011). The new generation of orals avian vaccines include genes that guide the vaccines to dendritic cells, as these cells have an important function in the antigenic presentation mechanism, leading to cellular or humoral immunity (De Geus and Velverde, 2013; Parra et al., 2013). Live vaccines are widely used throughout the world because they are commonly effective when mass applied and relatively economical.

Inactivated vaccines or killed vaccines used in poultry are generally whole bacteria or virus preparation, delivered with an adjuvant designed for subcutaneous or intramuscular injection. A study conducted by D.R. Anuruddhika Dissanayake and T.G. Wijewardena (2016) determined the LPS core specific antibody titters of chickens immunized with a single dose of heat killed rough mutant E. coli strains comprising of LPS core types R1, R2,

R3 and R4. Thus, the heat killed mixture of rough mutant E. coli strains can be used as a vaccine to enhance LPS core specific antibodies in chickens. They are frequently used in laying hens, to stimulate long-lasting immunity toward specific antigens. Most inactivated vaccines, however, induce a weaker immune response than do live vaccines. Compared to bacterins and subunit vaccines, live vaccines are attractive because they are much cheaper than purified proteins, and can be used for mass immunization via aerosol, feed, or drinking water.

Live virus vaccines usually induce high levels of immunity, long-lasting in the host. Instead of using the whole bacterial body, subunit vaccines include only those selected antigens, that better stimulate the immune system. In some cases, these vaccines use epitopes—the very specific parts of the antigen that antibodies or T cells recognize and bind to. Because subunit vaccines contain only the essential antigens and not all the other molecules that make up the microbe, the risk of side reactions to the vaccine are lower.

Recombinant vaccines are made using live viruses or bacteria, as a vector to host and transport the gene coding for the protective antigen of a second infection agent, for which immunity is desired. Recombinant technologies provide a very safe and economical way for large-scale production of subunit vaccines (Ribot et al., 2006). The technology facilitates identification, extraction, amplification and modification of DNA/RNA fragments encoding the antigenic part of a pathogen for recombinant expression. The first step is the identification of a suitable antigen with the ability to elicit a protective, long lasting immune response. Recombinant vaccines can be divided into two major groups: DNA and antigen-based vaccines.

DNA based-vaccines are a new type of vaccine which started to evolve in the late 1990s. They can achieve both humoral and cell-mediated immunity and are very similar to live-vaccines, and have the safety associated with inactivated or vector vaccines. DNA vaccines can be used successfully in poultry for avian diseases, but they still have technological and economical challenges to overcome, so are not very cost-effective (Ribot et al., 2006). The main disadvantages of DNA vaccines are the need for large amounts of DNA to achieve protective immune response in large animals (Brun et al., 2011)

The use of DNA as vaccine is a new and promising approach in vaccination since it is safe (no infectious antigen is involved), specific, easy and economical. The term refers

to induction of immune response against a protein(s) expressed in vivo (vaccine recipient) subsequent to the injection of plasmid DNA, therefore mimicking the live pathogen in immune system induction. The plasmid DNA is carrying an antigen-coding sequence under a host-specific promoter. This method is attractive due to its simplicity. It only requires plasmid construction, proliferation and extraction (Dertzbaugh, 1998).

Antigen-based vaccine (subunit recombinant vaccine) are also called protein based subunit vaccines and they present an antigen to the immune system without viral particles, using a specific, isolated protein of the pathogen. A weakness of this technique is that isolated proteins, if denatured, may bind to different antibodies than the protein of the pathogen. Recently, virus-like particles (VLPs) and subviral particles (SVPs) have received great attention due to their potential application in vaccine development as well as in drug targeting and gene therapy (Zhao et al., 2011). VLPs are composed of one or more recombinantly expressed viral proteins, which spontaneously assemble into supermolecular structure. VLPs have the same size and morphology as the parent virus, whereas SVPs are smaller in size or possessing a lesser degree of organization than the whole intact viral particle, because the parent virus comprises several different coat protein subunits while SVPs are chromomeric (Manzenrieder et al., 2011).

Conjugate subunit vaccines create a response against the molecules in the pathogen's capsule. These types of vaccines can prevent common bacterial infections. Producing conjugate vaccines in recombinant E coli is potentially easier and more cost-effective than alternatives, but the method suffers from low yields. A conjugate vaccine is a substance that is composed of a polysaccharide antigen fused (conjugated) to a carrier molecule. This enhances the stability and the effectiveness of the vaccine (Ree, 1985).

Toxoid vaccines are made from the bacterial toxins. The body learns how to fight off the bacterial's natural toxin once exposed to, through producing antibodies against. It has been possible to serologically demonstrate that the vast majority of the birds having an E. coli septicaemia had been infected with E. coli strains that contain fimbriae of the F11 type. Such fimbriae contain subunit proteins. In the past have already been cloned F11 fimbriae from a wild-type uropathogenic E. coli strain (Ree, 1985). Toxoid E.coli vaccines may contain, together with or instead of F 11 fimbriae, immunogenic sections of F11 fimbriae. Other E. coli antigens can be included, such as E. coli flagella toxins. (Ree, 1985)

CONCLUSIONS

Active immunization involves administration of vaccines containing antigenic molecules (or the genes controlling these molecules) derived from APEC. As a result, vaccinated animals should generate specific immune response and to develop prolonged, strong immunity against APEC.

When properly used, vaccines against APEC are highly effective in controlling infectious diseases. Several criteria determine whether a vaccine can or should be used. First, each APEC producing a disease outbreak must be identified and characterized. Although this appears self-evident, it has not always been followed in practice.

An ideal APEC vaccine for active immunization should confer prolonged, strong immunity in vaccinated animals, as well as rapid onset of immunity. It should not cause side effects, should be stable genetically and thermostable and, for poultry, should be economically affordable and proper to mass administration. It should allow discriminate between the post-infection immune response and the vaccinal one, so the vaccination and the eradication may proceed simultaneously. Modified live vaccines are more difficult to create for bacteria: bacteria have thousands of genes and thus are much harder to control. Scientists working on a live vaccine for a bacterium, however, might be able to use recombinant DNA technology to remove several key genes.

Avian pathogenic Escherichia coli (APEC) cause a wide range of economically significant infections in chickens. Control of these infections by antimicrobial drug is no longer possible due to high prevalence of multidrug resistance strains.

Involvement of large number of serotypes in these infections left none serotype specific vaccine as the only option. A study conducted by D.R. Anuruddhika Dissanayake and T.G. Wijewardena (2016) determined the LPS core specific antibody titters of chickens immunized with a single dose of heat killed rough mutant E. coli strains comprising of LPS core types R1, R2, R3 and R4. Thus, the heat killed mixture of rough mutant *E. coli* strains can be used as a vaccine to enhance LPS core specific antibodies in chickens.

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