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Review Article

The Rational for Autovaccines in Colibacillosis of Poultry" to "The Rational for Auto Vaccines in Poultry Colibacillosis

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Abstract

Colibacillosis is a rather important economic problem for poultry production, associated to Avian Pathogenic *Escherichia coli* (APEC) strains, which may cause several extraintestinal pathologies, such as airsacculitis and cellulitis in broiler chickens, and salpingitis and peritonitis in broiler breeders, leading to septicemic mortality. Control of morbidity and mortality in the outbrakes of colibacillosis may be performed with antibiotics and/or by vaccination. The use antibiotics is frequently ineffective as *Escherichia coli* (*E. coli*) is considered the largest reservoir of antimicrobial resistance, characteristic that may even transmit to other bacteria, turning the situation into a serious problem of public health. Vaccination may be the alternative solution but as many different strains arise, flock-specific autovaccines seem to be needed under several possible protocols, with live attenuated and/or inactivated vaccines from different strains that should be identified and characterized according to their virulence factors, within different flocks.

Keywords: Colibacillosis; Avian Pathogenic *Escherichia coli* (APEC); autovaccines; live-attenuated; inactivated; virulence-associated genes (VAGs)

Abbreviations

APEC: Avian Pathogenic *Escherichia coli;*

VAGs: Virulence-Associated Genes;

AFEC: Avian Faecal *Escherichia coli;*

O: Somatic;

H: Flagellar;

K: Capsular;

F: Fimbrial;

PF: Preventable Fraction

Introduction

Biosecurity of poultry farms stands as fundamental domain in order to allow the control of major infectious agents, which is fundamental for farm profitability and public health. Despite all the involved technological advances and extraordinary improvements at the good hygiene practices level, occurrence of infections, especially colibacillosis, is still a real problem. The infection is caused by APEC strains, responsible for considerable economic losses in the poultry industry [1, 2]. Recent studies even indicate an increase in the incidence of new cases in Europe [3]. Physiologically, *E. coli* colonizes the digestive tract of several animal species, including birds, establishing a commensal relationship with the host. In this situation *E. coli* strains are denominated Avian Faecal *Escherichia coli* (AFEC). However, APEC strains hold different genetic profile from AFEC, allowing them to cause disease in infected individuals, resisting the host immune system through several mechanisms. *Escherichia coli* contain a high number of antigens: i) somatic (O); ii) flagellar (H); iii) capsular (K); iv) fimbrial (F) and v) toxins [4]. Between the 180 O antigens many serotypes have been associated with avian colibacillosis, but in the field serotypes isolated from affected birds were significantly different from those isolated from healthy ones. Intestinal infection from healthy birds with strains from sick birds has shown to be frequent, but in asymptomatic chickens, 10-15% of intestinal coliforms may belong to potentially pathogenic serotypes. In the environment, the bacteria may persist for long periods, particularly in dry and dusty surfaces, and environmental isolates consist of a different population from pathogenic isolates. Contaminated food may be a mean of transportation of new *E. coli* serotypes from a flock to another and contaminated water is also a possible source of fecal-oral transmission [4].

Host infection by *E. coli* is promoted by a variety of factors coded by virulence-associated genes (VAGs) such as adhesins, invasins, toxins, iron acquisition systems and protectins, involved in colonization, adhesion, invasion and survival to host defenses [3,5]. As extraintestinal iron availability is low, to improve the capacity for iron fixation APEC strains developed several strategies for sequestering iron from host, like capture of iron from heme or heme-containing proteins, such as haemoglobin or hemopexin [6] by siderophore-encoding genes [7,8,9].

The current incubation period of colibacillosis is estimated in 1-3 days in severe infections and the installation of a septic situation occurs in 5-7 days post-infection. However, in disease, *E. coli* infection is often associated with other etiological factors, making frequently difficult to determine their individual contribution to global disease [4].

In order to minimize the damage caused by the presence of these strains, poultry industry is frequently forced to use antibiotics to avoid what otherwise would result in perfectly unbearable losses. This timely therapeutic approach may control the problem but it also becomes expensive for the farmer and in parallel with the economic impact on the industry, APEC are also considered as the largest reservoir of antimicrobial resistance which may transmit to other bacteria, via plasmids or other genetic material [10]. Feces and rodent secretions are also common sources of pathogenic strains and the intestinal tract from rodents appears as an appropriate environment for the transfer of resistance genes to susceptible strains [4].

The need for a more frequent use of antibiotics in order to control colibacillosis outbreaks, by possibly allowing residues in the food-chain and selecting resistant strains, with the consequent reduction in effectiveness of those essential drugs, may lead to dangerous implications for humans and animals [10]. These factors point to the interest in developing especially effective vaccines based on the specific pathogenic strains, which are circulating in each potentially affected poultry flock, allowing a more specific immunization and ensuring effective protection. Considering that APEC strains may contain many virulence factors and that the associated molecular basis is still insufficient, it is important to pursuit the characterization of the virulence genes from individual APEC strains, especially from broiler breeders. In fact, Kemmett et al. (2013) [11] have demonstrated that the development of systemic disease was linked to several genes and that one gene alone was not responsible for colibacillosis. A variation in the frequency of genes was also observed by the acquisition of new genes, resulting in high capacity of these bacteria to transmit genetic information via plasmids [2].

With regard to colibacillosis in birds, several susceptibility/resistance factors should be considered [4]:

A. of Susceptibility:
   - young birds and males;
   - immune depression;
   - viral, bacterial and parasitic infections (e.g. avian bronchitis in chickens and hemorrhagic enteritis in turkeys).
   - environmental and food stress.
   - toxins.
   - injuries (inflammation and gateway).
   - obesity.

B. of Resistance:
   - immune stimulation and immune competence;
   - good nutrition;
   - genetics;
   - adult birds and females;
   - moderate stress (contact of the immune system with microorganisms stimulates the defenses, leading to a state of immune "excitement", which installs a state of immune "readiness").
   - nonspecific moderate airway inflammation

increases the resistance to aerogenous infection by *E. coli*.
- vaccination against predisposing agents (e.g. infectious bronchitis) indirectly promotes resistance.
- socialization.
- healthy intestinal flora.

Another important feature was the observation of an inverse correlation between growth rate and resistance to colibacillosis. However, no correlation was found between fertility and early immunity acquisition against *E. coli* in breeders, which does not seem to make infective resistance and productivity as incompatible.

Discussion

The vaccines

Autovaccines, autogenous vaccines or flock vaccines are those whose antigenic mass results from cultures of organisms from the patient's own tissues or secretions. To produce autovaccine infectious organisms responsible for disease and/or economic loss, pathogens have to be firstly isolated for further preparation of the vaccine with the inclusion of the relevant antigenic determinants. These vaccines are also used in emergency situations, to control local outbreaks of different diseases [12]. However, in current practice this type of vaccines is often a last recourse, after several other treatment options commercially available, including vaccines, being tempted.

The best choice of microbial agents to immunize a given group of animals should depend on [13]:
- confirmation that those agents really exist in animals/environment;
- certification that the impairment is severe enough so that its occurrence cause significant damage, such as mortality, growth delay and decreased fertility, or at least greater than the cost of vaccination;
- severe damage to animal welfare;
- confirmation that the vaccine conveniently protects against the targets.

For a good vaccine-derived immunization a strong and long lasting protection and an anamnestic response should be expected and to produce an effective flock vaccine it will be necessary to involve different veterinary specialties, including pathology, microbiology and immunology.

Regarding the nature of the vaccines, two main alternatives may be equated: i) live attenuated vaccines or ii) inactivated vaccines. Both alternatives have advantages and disadvantages, as follows [12,14,15].

Advantages of live attenuated:
- require less antigenic inoculations and doses, may dispense adjuvants and present less risk of adverse reactions;
- provide rapid and prolonged protection (especially useful in outbreaks);
- relatively cheaper;
- may have a simpler application by the natural infective route.
- good induction of mucosal immunity;
- good for routine actions.

Disadvantages of live attenuated:
- less safe because of possible spread to other susceptible animals or gain in virulence (although it is unlikely);
- may serve contaminating microorganisms (if badly prepared);
- immune depressed animals can get sick, serving as a battery of dissemination for other agents;
- minor stability and susceptible to inactivation.

Advantages of inactivated:
- stable and easier to storage (allow the use of preservatives);
- the activity can be increased by the use of adjuvants;
- efficacy is less influenced by simultaneous antibiotic therapy;
- no residual virulence or contamination with other microorganisms.

Disadvantages of inactivated:
- shorter immunity;
- multiple doses are required (greater intervention);
- more antigenic mass is needed (higher cost);
- require adjuvants (more likely to cause adverse reactions);
- restricted to parenteral use;
- possible administration to laying hens.

Implementation and vaccine development

Flock vaccination is usually initiated using conventional/1st generation vaccines (derived from complete microorganisms). However, it should be convenient to evolve to 2nd generation vaccines, which may belong to one of three categories [15]: i) containing inactivated recombinant microorganisms or pure antigen derived from recombinant organisms; ii) containing living organisms with genetic deletions or heterologous genes or markers and iii) containing living expression vectors with heterologous genes for immunizing antigens. Most of these vaccines relay on the knowledge of the genome of the implicated microorganism, establishing a relationship between a given protein and the gene responsible for its coding. It is therefore important to determine the immunogenic protein profile and relate it to the conferred protection [15], since the simple determination of antibody titer may not correlate with the real effectiveness [14].

Regarding recombinant vaccines, even when live attenuated, lower immunogenic ability should be expected, requiring special immune potentiation [4]. However, other apparently simple details may also play important roles in the success rate of anti-*E. coli* vaccination, as for instance the dimension of the coarse spray droplets, since a particle size of less than 5 μm seems to be associated to a higher protective effect, by reaching the deep respiratory tract [16].

Actually, specific immunoglobulins (Ig) such as IgM, IgG and IgA have been detected in respiratory secretions upon infection or vaccination with different respiratory pathogens [17-21] which comes in favor of a good airway immunization against those agents.

Effectiveness of *E. coli* vaccines, either as bacterins, subunit vaccines and live vaccines have been demonstrated for the protection against colibacillosis, although when using mutant strains in live vaccines the protective result may not be as high as desired, since several important epitopes could be lost during that preparative process [22].

Vaccination Strategy

Two criteria should be central: i) the immune system is able to respond protectively against the involved agents and ii) the risks (and vaccine costs) do not exceed those of naturally occurring disease. It may not be cost effective vaccinating for the control of a rare or a low morbidity/mortality rate disease. Similarly, vaccination may not be the method of choice when other acceptable, effective and cost/benefit methods are available [15]. In this context, two vaccination categories may be considered: i) core vaccination (essential) and ii) non-core vaccination (not essential) [15,23].

The choice for inactivated vaccines, more stable and eventually safer under the microbiological point of view, will require for protection at least one revaccination after 2-4 weeks of primary immunization [15]. Resulting humoral immune response should then be evaluated, comparing the level of specific antibodies with the related morbidity/mortality rates, in order to establish the protective thresholds for the involved etiological agents [16].

For broiler breeder chickens reared in an environment where the pathogen to be immunized to is ubiquitous (e.g. *E. coli*) the administration of immunogens must occur for at least two times, 2-4 weeks apart, possibly associated to other management interventions (e.g. weighing and/or transfer) in order avoid unnecessary stress. However, regarding primary vaccination, maternal derived antibodies should be taken in consideration, since they gradually decline after 10 days post-hatching until the 3rd week of age. Then, a rise in antibody titer should be due to natural exposure. Progeny from breeder hens vaccinated against *E. coli* still present with detectable specific antibodies at the 2nd week post-hatching [24]. Hence, a vaccination protocol should be able to overcome that interference. Based on these results an effective vaccination plan should be established for the productive life.

As different strains of a same serotype may vary in their virulence the use of the same strain that has been found in the field should also play an important role in a vaccination system [16]. For an adequate microbiological characterization, pathogen isolation from animal and environmental samples, followed by phenotyping and genotyping, should be performed. Antibiotic sensitivity, as well as the characterization of fimbrial adhesins, an important virulence factor in *E. coli*, is also highly relevant [25,26].

Currently, it is not only the immunogenic bacterial mass that matters, but also the expression of the antigenic determinants that stimulate an effective immune response [27] (e.g. adhesins from *E. coli* fimbriae play a critical role by stimulating an effective immune response). If there is a good development of *E. coli* in culture but a reduced expression of adhesin antigens, the resulting vaccine may lead to an impaired protective effect, despite an appropriate protocol of administration.

Evaluation of vaccine efficacy

A current method to check the effectiveness of vaccination is to challenge vaccinated individuals with field agents. In the case of ubiquitous agents, infective pressure is present from the start and continues throughout the productive life and any gain in pathogenicity by increased virulence or an immune deficiency of the host, may result in increased morbidity/mortality.

To evaluate the effectiveness of a vaccine two main groups should be available, one vaccinated and one control. Then, during the evaluation period the Preventable Fraction (PF) should be determined [15]:

PF = (% dead in control - % dead vaccinated) / % dead in control

For the success of an immunization procedure, good health supported by good management, is crucial [23]. A positive impact on the immune competence has been demonstrated by the intake of vitamins A, D, E and C, as well as several oligoelements such as selenium, copper and zinc [28,29,30] but simple supplementation in vitamin E may also act as immune stimulant [12].

From *in vitro* preparation to *in vivo* administration

After obtaining the field strains of the involved pathogens, *in vitro* production scale should be adapted to the size of the flock to be immunized. Since the initial culture, preparation of an inactivated vaccine will take a minimum of 4 weeks and 2 more weeks should be added until being ready for administration, in order to allow the necessary sterility and residual toxicity testing [22]. Hence, the global production time for vaccine release will depend on the number of animals to be vaccinated, the isolated microorganisms and strains (its speed multiplication - generation time/growth rate, nutritional and environmental requirements) and number of administrations to be performed within the shelf-life of the vaccine preparation.

Besides the adequate strains to be identified and used for immunization, vaccine type (live attenuated or inactivated), routes of administration (drinking water, coarse spray or injection) and their calendar should also be object of detailed study, allowing to compare the protective results obtained with the type of immune response produced.

Fimbrial-related genes seem to be effectively associated with *E. coli* pathogenicity by the ability to adhere and invade the host cells, while iron uptake genes seem to be rather important as a virulence factor. In fact, recent results

from our research group (unpublished data) suggest that iron-uptake genes may play a significant role in the pathogenesis of colibacillosis. However, further research is required to evaluate the potential of these genes as promising antigens for an efficient vaccine against colibacillosis, in the context of a global protocol.

Conclusions

Upon recognizing *E. Coli* as an unambiguous productive impairment cause, several aspects should be clearly considered:

1. Pathogens have to be isolated for a vaccine preparation containing the relevant antigenic determinants.
2. Live attenuated and inactivated vaccines are frequently useful in a conjunction protocol.
3. Different routes of administration may show to be useful, according the type of vaccine, the age of the effective and the production system.
4. Recombinant vaccines should be considered when large effectives are to be immunized.
5. Adequate microbiological characterization of the pathogens by phenotyping and genotyping should be performed.
6. Fimbrial-related genes may show association with *E. coli* pathogenicity, while iron uptake genes seem to be rather important as a virulence factor.
7. Evaluation of vaccine efficacy should be successively determined, as changes in pathogenicity associated to genetic changes of this ubiquitous agent may occur.
8. Good vaccination efficacy should only be expected in healthy effectives.
9. Supplementation with vitamins A, D, E and C, as well as several oligoelements like selenium, copper and zinc, may help achieving a better immune condition.
10. All good management practices are still essential for a good health condition of the flocks, despite the use of any vaccine prophylaxis.

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