Technical Opinion no. 2146/2009

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Applicant: Fort Dodge Saúde Animal Ltda.
CNPJ: 43.5880.045/0001-31
Address: Fort Dodge Saúde Animal Ltda., Avenida Luiz Fernando Rodrigues, 1701, Vila Boa Vista, 13064-789 Campinas, SP. Telephone: (19) 3745-6061, Fax: (19) 3745-6189.
Matter: Requests opinion on biosafety of a genetically modified organism for the purpose of import, transport, storage and marketing.
Previous summary: 1751/2009, Published in the Federal Official Gazette nº 39, of February 27, 2009.
Meeting: 127th CTNBio Regular Meeting, held on October 15, 2009.
Decision: GRANTED.

Summary: CTNBio, following examination of a request for Technical Opinion on biosafety of a biologic risk class I genetically modified organism aimed at activities of import, transport, storage and marketing, decided to GRANT the request under the terms of this Technical Opinion. Mr. Matusalém Pereira Santos, Chairman of Fort Dodge Saúde Animal Ltda. Internal Biosafety Commission, requests CTNBio a technical opinion on biosafety of a genetically modified organism to be used as vaccine for birds. The request encompasses the activities of import, storage and marketing by the company in Brazil of the product styled Poulvac E. coli – live vaccine against Escherichia coli. The product shall be imported ready and finished, being its purification and packaging conducted in the United States (State of Iowa, U.S.A.) by Fort Dodge Animal Health, to be used for vaccination of four-week birds. The company submitted the necessary documentation related to the request. In the context of Law nº 11,105/05, regulated by Decree nº 5,591/2005, the Commission found that experimental protocols and other foreseen biosafety measures comply with CTNBio rules and applicable legislation, which aim at securing biosafety of the environment, agriculture, and human and animal health.

TECHNICAL OPINION

(1) Technical grounds

Bacterium Escherichia coli may be found as animal flora in the bowels of humans, mammals and birds. The bacterium pathogenic forms for birds, causing bacillosis, are specific of such group and were never found contaminating mammals. Non-pathogenic forms, as well as pathogenic ones, are eliminated in birds' feces.

Colibacillosis is one of the main causes for mortality and morbility in chickens and turkeys, causing significant economic losses to the poultry industry. Colibacillosis in poultry is associated to the avian pathogen Escherichia coli. The disease is caused by a limited number of serotypes, the majority of which of type O78. Currently, live attenuated vaccines for chicken against Escherichia coli are not marketed. The vaccine developed by Fort Dodge Animal Health was produced from lineage E. coli aro EC 34195, where gene aroA is deleted. Null mutants of aroA need, for their growth, aromatic supplements such as tyrosine, phenylalanine, tryptophan and p-aminobenzoate (PABA). The need for PABA, a metabolite that is not found in tissues of vertebrates, results an attenuated in vitro growth of the biological agent.

Characterization of the GMO

The parental organism used was strain Escherichia coli EC34195, a wild strain isolated from a clinical case of bird colibacilosis, an OI78 serotype strain. The strain colonizes the liver, spleen and caecum of SPF chicks that had their sensibility pattern for antibiotics characterized.

Deletion of gene aroA was the genetic change performed in the receiving organism. This gene codifies 5-shikimate-3-phosphate synthase, an enzyme of the common aromatic biosynthetic pathway. The loss of gene aroA to the receiving organism results in mandatory requisite of aromatic metabolites, including tyrosine, phenylalanine, tryptophan, p-aminobenzoate (PABA) and 2,3-dihydroxybenzoate (DHBA).

To delete the gene, an intermediary vector was used, produced by PCR, in digestion with restriction enzymes and link to commercial plasmid followed by transformation into DH5*f*Ñ maxicompetent cells for the construction of the aroA version to be deleted. The version was removed from the plasmid by enzymatic digestion, cloned to a suicidal vector, also commercial, and transformed into K12S17*f*Ü competent cells. The cells were then conjugated with the parental strain, leading to deletion of the aroA gene. This way, the GMO strain did not receive any marker gene, though it may be differentiated from the parental strain by PCR and restriction digestion, since the gene, when deleted, remained outside the reading frame and came to contain two stop codons in addition to two restriction sites for Srf1 and BglII. The strain is positive by PCR test for fliC, fimC, esgA and rpos, but negative for iss, ths, hlyE and papC.

Virulence of the master seed

(a) For living animals

The genetically modified organism was developed to be used as a live vaccine. Efficacy studies of the vaccine produced from the master seed were conducted by spraying with large drops that showed minimum immune dose of 1.6 x 106 UFC/dose. Based on this, security studies were conducted with the target animal. The master seed was tested in chicken, either by oral administration or by spraying with large drops in a real title of 1.7 x 107 UFC/dose of bird. The animals were observed for twenty-one days. There were no clinic signs of colibacilosis disease or mortality. PCR analyses were conducted using primers (aroA1 and aroA4) described in Annex 4 of the application documentation, both for the master seed Escherichia coli aro-EC34195 and the working seed n+5 (level of the highest passage) in order to characterize genetic stability of the vaccine. The results indicated that Escherichia coli aroA-EC34195 was genetically stable at the highest passage.

A virulence reversal study was also conducted and noticed that after a series of backpassages (3) the vaccine became avirulent. The conclusion was that the GMO strain failed to revert into virulent after 3 passages and did not disseminate to other control birds.

Necropsies were conducted after seven days of inoculation and liver, spleen, heart and air bags of both, inoculated and control chicks, were collected. No GMO strain was isolated in the controls. A second experiment was conducted with post-21 day necropsy, which also resulted in no clinical signs and no mortality, and no vaccinal strain was found in post-necropsy, confirming that the master seed organism is avirulent in chicken.

(b) For non-target animals

Virulence of the master seed was tested in mice by an intraperitoneal way of 1.5 x 107 UFC or intracerebral injection of 1.0 x 106 UFC of Escherichia coli aroA EC34195 for BalbC animals. Inoculated mice failed to show adverse reactions after the procedure. Mother seed virulence was also tested in hogs by oral administration of 3.55 x 108 UFC

and no adverse reactions appeared during the 21 days after inoculation.
Virulence for humans was not tested. However, two other attenuated live vaccines against other poultry bacteria constructed with the same logic of gene aroA deletion are in use in the field for some time with no notice of adverse effects.
Phenotypical stability/recombination potential
The majority of poultry already carries Escherichia coli of types 01, 02 and 078 as part of their native flora. The likelihood of recombination between vaccinal strain and wild strain converting the vaccine into a virulent strain is held as very low. These serotypes are not pathogens recognized by humans. The vaccinal strain was phenotypically characterized by electronic microscope, serum agglutination, motility, hemagglutination and morphology of colonies and no modification became evidenced after several passages. The probability of recombination between the viral strain and wild bacteria is held as very low (about 10-13).
Assessment of risk to human and animal health
Data on tests made with chicken, hogs and mice were submitted where the applicant showed that there are no harmful effects to animal health. The safe history of this vaccine is known in the United States since 2006.
Regarding human health, human exposition will be limited to employees in poultry vaccinations and will be minimal, since jet applications will be conducted in closed vaccinators (acrylic boxes). Besides, the limited replication of the vaccinal strain in immunized birds and into the environment coupled with the absence of detection of the vaccinal strain in contact with neighboring birds indicate a limited potential of spreading the vaccine into the environment.
Another aspect vouching for the security of human health is that even an accidental exposure is not a source of concern since deletion of the aroA gene existing in the vaccinal strain guarantees limited replication in tissues of mammals, given that the requisite of aromatic metabolites is inexistent in human tissues.
Assessment of risk to the environment
Vaccine Poulvac Escherichia coli was constructed from the Escherichia coli strain contained in environments where commercial birds are raised. The GMO vaccine does not contain new added genetic material when compared with its wild kindred. Therefore, introducing this vaccine does not imply introduction of any new genetic information into the environment. The vaccinal strain persists in the environment for a limited time and, hence, even in the unlikely case of an accidental release of the organism, no adverse effect is expected to the environment.
(2) Opinion
CTNBio is favorable to the granting of the request for commercial release of the product styled vaccine against bird colibacilosis – Poulvac Escherichia coli, considering that:
(1) The attenuated parental strain does not display virulence against humans and other tested mammals (hogs and rodents);
(2) Vaccine Poulvac Escherichia coli is produced at the facilities of Ford Dodge Health in the United States (Iowa) and has a safe history of use in that country;
(3) The product has its manufacture and marketing authorized by USA authorities according to the documentation submitted by applicant;
(4) In the construct of the genetically modified vaccinal organism, gene aroA was inactivated by genetic engineering, without adding other genes, making multiplication of the organism slow and dependent on nutrients that are absent in the host;
(5) There are no expected conditions for Poulvac Escherichia coli to establish in the environment;
(6) There are no reports of human diseases associated with this lineage of bacterium

Escherichia coli;
(7) The elements necessary for reversion of this vaccine in the parental organism are rare and such remote event may generate an organism with the characteristics of the parental one, which is already present in commercial poultry farms;
(8) Experimental data submitted by applicant on Poulvac Escherichia coli safety and construct are both accurate and sufficient to establish a risk assessment on the vaccine;
(9) Risks posed to public health, animal health and the environment are low.
Nineteen (19) members of CTNBio voted favorably to commercial release of the product.
For the foregoing, CTNBio reached a conclusion that the activity is not a potential cause of significant degradation to the environment and not harmful to human and animal health and this sector commission is favorable to the granting of the request.

Dr. Walter Colli
President of CTNBio

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