Research Institute for Biological Safety Problems

A NOVEL INFLUENZA VIRAL VECTOR BASED *BRUCELLA ABORTUS* VACCINE AT THE STAGE OF IMPLEMENTATION INTO PRACTICE

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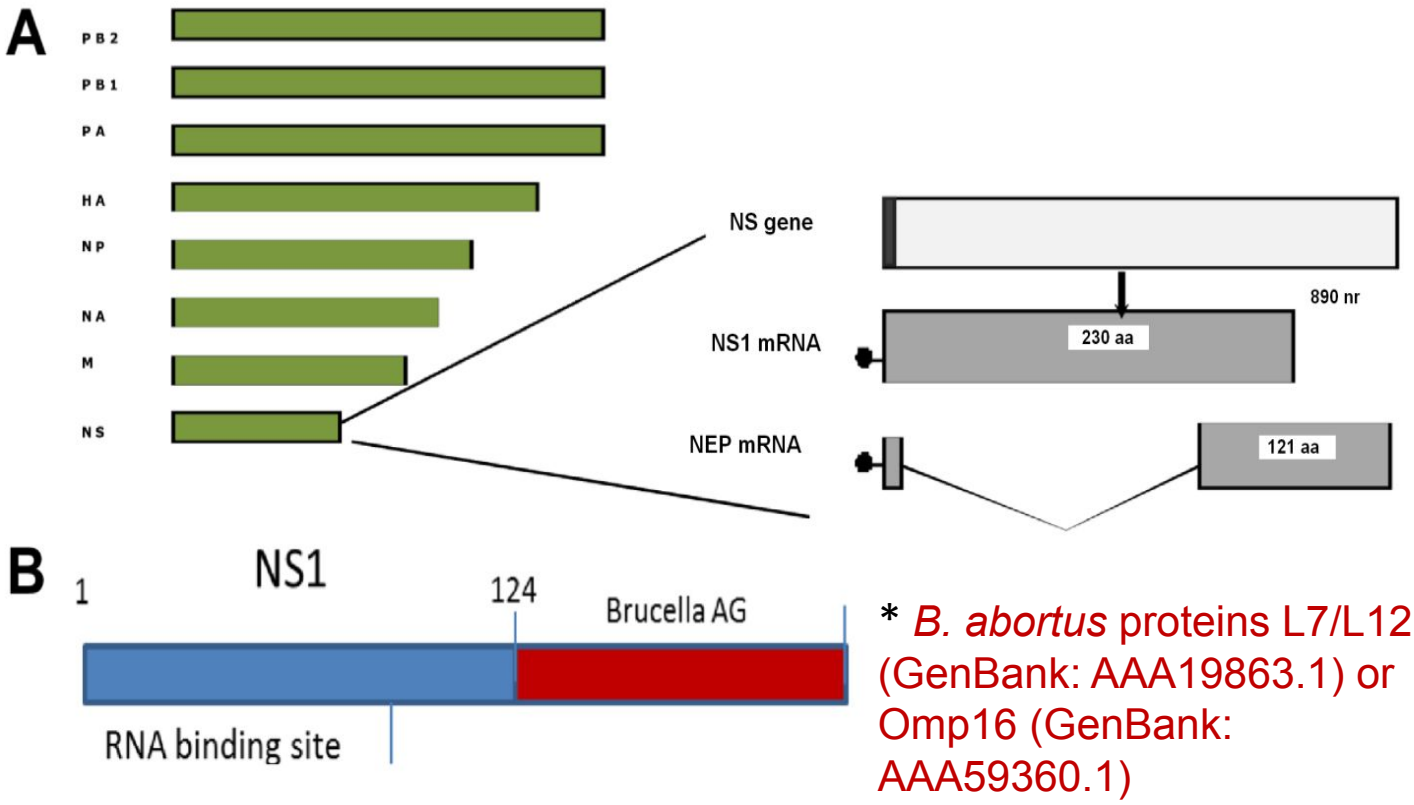
CONSTRUCTION OF INFLUENZA VIRAL VECTORS



* *B. abortus* proteins L7/L12 (GenBank: AAA19863.1) or Omp16 (GenBank: AAA59360.1)

Four influenza viral vectors of the subtypes H5N1 or H1N1 expressing the *Brucella* proteins L7/L12 or Omp16 were obtained by a reverse genetics method: Flu-NS1-124-L7/L12-H5N1, Flu-NS1-124-Omp16-H5N1, Flu-NS1-124-L7/L12-H1N1 and Flu-NS1-124-Omp16-H1N1

Schematic representation of the influenza virus *NS1* gene (A) and recombinant chimeric *NS1* genes of recombinant influenza A viral vectors of the subtypes H5N1 and H1N1 containing the genetic sequences of the *Brucella* proteins L7/L12 or Omp16 (B)

TECHNICAL CHARACTERISTICS OF THE VACCINE

- The vaccine is a mixture of recombinant strains of the influenza virus expressing brucellosis antigens (Omp16 and L7/L12) accumulated in 9-11 day old chicken embryos (CE) and lyophilized with stabilizing medium
- The vaccine is packaged in 1-10 ml into ampoules or vials
- As a solvent we use 10-20% adjuvant Montanide Gel 01 (Seppic, France), packaged in 5-500 ml in vials
- Depending on the subtype of influenza viral vectors included in the formulation, the vaccine is marked as "Vaccine 1" (a mixture of influenza viral vectors subtype H5N1) and "Vaccine 2" (a mixture of influenza viral vectors subtype H1N1)

CONSUMER CHARACTERISTICS OF THE VACCINE

- The vector vaccine is used for double immunization of cattle (young animals aged 6 months or more, including bulls, heifers or cows, including pregnant) first by "Vaccine 1" (prime vaccination), and then through 21-28 days "Vaccine 2" (booster vaccination)
- The vaccine can be used in the Brucella-free, Brucella-infected or in farms (in conjunction with other antiepizootic measures) at the stage of recovery from brucellosis.
- Method and dose of administration of vaccine for cattle in Brucella-free farms: subcutaneously in the neck region in a volume of 1.0 ml. In Brucella-infected farms: subcutaneously in the neck region in a volume of 1.0 ml with simultaneous conjunctival administration of the vaccine in a volume of 0.5 ml (0.25 ml per eye)

THE MAIN PROPERTIES OF THE DEVELOPED VACCINE

- The vaccine is a live vaccine based on influenza viral vectors, and induces a strong Th-1 immune response in cattle;
- Vaccinated cattle do not form *Brucella* agglutinogen antibodies, making it easy to differentiate vaccinated animals from infected animals;
- As the truncated NS1 protein (interferon antagonist) influenza viral vectors has limited replicative capacity (influenza viral vectors subtype H5 was further attenuated by exchanging its polybasic cleavage site with a trypsin-dependent sequence); this attenuated vaccine cannot cause disease in cattle or humans;
- The influenza viral vectors are not shed by vaccinated animals into the environment and cannot be transmitted to other animals or humans;

THE MAIN PROPERTIES OF THE DEVELOPED VACCINE (continuation)

- The vaccine is genetically stable, as it retains all of its basic biological properties including attenuation markers and does not lose the *Brucella* protein inserts after repeated passage in its culture system, chicken embryos;
- The vaccine in vaccinated cattle provides formation of long-term protective immune response which lasting at least 12 months after booster vaccination;
- The vaccine is able to provide cross-protection against *Brucella melitensis* infection in pregnant heifers;
- The vaccine can be used in all sex and age groups of cattle, regardless of the status of pregnancy in animals, both in Brucella-free and Brucella-infected farms.
- In the presence of a production site for the production of dry preparations based on the use of chicken embryos, the vaccine can be easily and on a large scale produced

PUBLICATIONS IN INTERNATIONAL PEER-REVIEWED JOURNALS

1. Mailybayeva A, et al. **Improved influenza viral vector based Brucella abortus vaccine induces robust B and T-cell responses and protection against Brucella melitensis infection in pregnant sheep and goats. PLoS One. 2017;12(10):e0186484. (Impact factor-2.8)**
2. Tabynov K, et al. **First evaluation of an influenza viral vector based *Brucella abortus* vaccine in sheep and goats: Assessment of safety, immunogenicity and protective efficacy against *Brucella melitensis* infection. *Vet Microbiol*. 2016;197:15-20. (Impact factor-2.5)**
3. Tabynov K., et al. **Simultaneous subcutaneous and conjunctival administration of the influenza viral vector based *Brucella abortus* vaccine to pregnant heifers provides better protection against *B. abortus* 544 infection than the commercial B. abortus S19 vaccine. *Vaccine. 2016;34(42):5049-52.* (Impact factor-3.6)**
4. Tabynov K. **Influenza viral vector based Brucella abortus vaccine: a novel vaccine candidate for veterinary practice. *Expert Rev Vaccines.* 2016*;15(10):1237-9*. (Impact factor-4.2)**
5. Tabynov K, et al. **Prime-booster vaccination of cattle with an influenza viral vector *Brucella abortus* vaccine induces a long-term protective immune response against *Brucella abortus* infection. *Vaccine*. 2016. 34:438-444. (Impact factor-3.6)**
6. Tabynov K, et al. **Safety of the novel influenza viral vector *Brucella abortus* vaccine in pregnant heifers. *Ciência Rural*. 2016. 46(1):114-118. (Impact factor-0.4)**
7. Tabynov K, et al. **An influenza viral vector *Brucella abortus* vaccine induces good cross-protection against *Brucella melitensis* infection in pregnant heifers. *Vaccine*. 2015. 33(31):3619-23. (Impact factor-3.6)**
8. Tabynov K, et al. **Novel vector vaccine against *Brucella abortus* based on influenza A viruses expressing *Brucella* L7/L12 or Omp16 proteins: Evaluation of protection in pregnant heifers. *Vaccine*. 2014. 32(45):5889-92. (Impact factor-3.6)**
9. Tabynov K, et al. **Novel influenza virus vectors expressing Brucella L7/L12 or Omp16 proteins in cattle induce a strong T-cell immune response, as well as high protectiveness against *B. abortus* infection. *Vaccine*. 2014. 32(18):2034-41. (Impact factor-3.6)**
10. Tabynov K, et al. **Influenza viral vectors expressing the *Brucella* OMP16 or L7/L12 proteins as vaccines against *B. abortus* infection. *Virology Journal*. 2014. 11: 69. (Impact factor-2.18)**

COMPETITIVE ADVANTAGES

Parameters	Names of vaccines			
	Flu-BA	*B. abortus* S19	*B. abortus* 82	*B. abortus* RB51
Causes abortion in pregnant cows	No	Yes	Yes	Yes
Secrets through milk	No	Yes	Yes	Yes
Causes Orchitis and Infertility in Bulls	No	Yes	Unknown	Yes
Dangerous to human health	No	Yes	Yes	Yes
Allows to differentiate infected animals from vaccinated	Yes	No	No	Yes
Grafting volume	1.0-1.5 ml	4.0 ml	5.0 ml	2.0 ml
Vaccination efficacy	57.2-100%	66.6-95%	50-87.5%	50-65%
Cross-protection against *B. melitensis* infection	Yes	Yes	Unknown	No
Market price	0.76 USD/dose	0.19 USD/dose	0.27 USD/dose	2.17 USD/dose

FIELD AND REGISTRATION TRIALS

- According to Order of the Chairman of the Committee of Veterinary Control and Supervision of the Ministry of Agriculture of the Republic of Kazakhstan No. 76 dated 05.24.2018, between May 17 and November 23, 2018, it was conducted field and registration trials of our vaccine.
- It has been established that the vaccine in its physical and immunobiological properties fully meets the requirements of the regulatory document.
- The vaccine is harmless and protective in heifers after a double immunization
- In late December 2018, it is expected to receive a registration certificate for the vaccine, and its inclusion in the State Register of Veterinary Medicines of the Republic of Kazakhstan

CONCLUSION

- Thus, since the introduction of the latest commercial *B. abortus* RB51 vaccine in the United States 20 years ago, we have for the first time developed and offer to commercialize a completely new vaccine against bovine brucellosis, which has better properties (by safety profile) in comparison with commercial preparations

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