DOI: 10.31082/1728-452X-2019-205-206-7-8-35-40
УДК 632.938:001.895:615.371:579.841.93

TOWARDS NEW HUMAN BRUCELLOSIS VACCINE: SELECTING IMMUNIZATION MODALITY

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There is no licensed, protective and safe vaccine against human brucellosis.

Objective. The main purpose of this work is to evaluate mucosal immunization route of vaccine candidate for human brucellosis in laboratory animals.

Material and methods. We used tetravalent vaccine formulations utilizing recombinant influenza A virus subtype H5N1 expressing *Brucella* proteins Omp 16, L7/L12 and Omp19 or Cu-Zn SOD with N-terminal 80 or 124 amino acids of the NS1 protein. Vaccine formulations administered to guinea pigs via mucosal sites including respiratory mucosa (nasal), conjunctiva and oral mucosa (sublingual). Animal body weight changes were recorded weekly. Blood samples were collected from guinea pigs of the control and experimental groups on days 0, 21 and 42 upon the prime and boost immunizations to detect antibodies against influenza A virus subtype H5N1 using hemagglutination-inhibition assay (HIA).

Results and discussion. The highest antibody response to influenza A virus subtype H5N1 in guinea pigs detected when vaccine formulations were administered at nasal route upon prime-boost immunization. Animal deaths and body weight loss were not observed over 42 days.

Conclusion. The data obtained indicate that intranasal immunization of guinea pigs showed a detectable accumulation of antibodies after prime-boost immunization.

Keywords: human brucellosis, mucosal vaccine, influenza viral vector.

For reference: Bugybayeva DA, Ryskeldinova ShZh, Kydyrbayev ZhK, Kozhamkulov YM, Inkarbekov DA, Shvecov RY, Tabynov KK. Towards new human brucellosis vaccine: selecting immunization modality. *Meditsina (Almaty) = Medicine (Almaty).* 2019;7-8(205-206):35-40 (In Russ.). DOI: 10.31082/1728-452X-2019-205-206-7-8-35-40

Т Ұ Ж Ы Р Ы М

АДАМ БРУЦЕЛЛЕЗІНЕ ҚАРСЫ ВАКЦИНАНЫ ЕГУ ӘДІСІН ТАҢДАУ

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Қазіргі таңда адам бруцеллезіне қарсы тиімді және қауіпсіз лицензияланған вакцина шығарылмаған.

Зерттеудің мақсаты. Адам бруцеллезына қарсы вакцина сынамаларын мукозалды иммунизациялау жолдарымен зертханалық жануарларда бағалау.

Материал және әдістері. Бұл жұмыста біз NS1 80 және 124 ақуызының позициясында Omp16, Omp19, L7/L12 немесе Su-Zn SOD бруцеллез ақуыздарын экспрессиялайтын тұмау вирусының А типіне жататын H5N1 субтипінің рекомбинантты векторы негізінде дайындалған төртвалентті вакцина формуляциясын қолдандық. Вакцина формуляциялары үш жолмен енгізілді, оның ішінде көз, ауыз және мұрын қуыстары арқылы. Зертханалық жануарлардың салмағы апта сайын 42 күн бақылауда болды. 0, 21-ші және 42-ші күндері бақылау және эксперименттік топтардың жануарларынан қан сарысу үлгілері жиналды және гемагглютинацияны тежеу (ингибирлеу) талдауын (ГТТ) қолдана отырып, H5N1 тұмауы вирусына антиденелердің түзілуі анықталды.

Нәтижелері және талқылауы. Нәтижесінде төрт валентті вакциналық құрамаларды теңіз

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Поступила 15.08.2019

шошқаларына мұрын қуысы арқылы прайм-буст иммунизация жасаған кезде, олардың қан сарысуларында тұмау вирусына қарсы антиденелер титрі басқа иммунизация жолдарынан жоғарырақ болды. Теніз шошқалардың салмағын бақылаудың бүкіл кезеңінде, бақылау немесе эксперименталдық топтарда жануарлардың салмағының артқаны байқалды.

Қорытынды.Осы жұмыстың қорытындысы бойынша мұрын қуысы арқылы прайм-буст иммунизация жолы тұмау вирусына қарсы жоғары антиденелер титрін көрсетті.

Негізгі сөздер: адам бруцеллезі, мукозалды вакциналар, тұмау вирус векторы.

Р Е З Ю М Е

ВЫБОР СПОСОБА ВВЕДЕНИЯ ВАКЦИННОЙ ФОРМУЛЯЦИИ ПРОТИВ БРУЦЕЛЛЕЗА ЧЕЛОВЕКА

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Бруцеллез - хроническая инфекционная болезнь животных и человека. В настоящее время нет лицензированной, эффективной и безопасной вакцины против бруцеллеза человека. Один из способов получения вакцины основан на использовании конструированных вакцинных векторов, в частности рекомбинантные вирусы гриппа. В проекте по созданию новой человеческой противобруцеллезной векторной вакцины используются гриппозные вирусные векторы, экспрессирующие бруцеллезные иммунодоминантные белки.

Цель исследования. Оценка мукозальных способов введения вакцинных формуляций на морских свинках.

Материал и методы. В данной работе мы использовали четырехвалентную вакцинную формуляцию, полученную на основе рекомбинантного гриппозного вирусного вектора типа А субтипов H5N1, экспрессирующих бруцеллезные белки Omp16, Omp19, L7/L12 или Su-Zn SOD в позиции белка NS1 80 и 124. Вакцинные формуляции вводили тремя способами: интраназально, конъюнктивально и орально. В течение 42 дней проводили наблюдения за изменением веса у лабораторных животных.

Результаты и обсуждение. Полученные данные показывают, что интраназальная иммунизация морских свинок вызывает заметное накопление антител после прайм-буст иммунизации. В течение всего периода наблюдения за весом животных отмечался прирост массы всех морских свинок контрольной и опытной групп, и все животные остались живы.

Вывод. При выборе способа введения вакцинной формуляции интраназальное введение дало более высокую детекцию антител после прайм-буст иммунизации.

Ключевые слова: бруцеллез человека, мукозальная вакцина, гриппозный вирусный вектор.

Для цитирования: Бугыбаева Д.А., Рыскелдинова Ш.Ж., Кыдырбаев Ж.К., Кожамкулов Е.М., Инкарбеков Д.А., Швецов Р.Ю., Табынов К.К. Выбор способа введения вакцинной формуляции против бруцеллеза человека // Медицина (Алматы). – 2019. – №7-8 (205-206). – С. 35-40

Brucellosis is a bacterial zoonotic disease that affects human health and animal productivity worldwide. Brucellosis is caused by pathogenic *Brucella* spp., of which *B.abortus*, *B. melitensis*, *B.suis* are potentially pathogenic to humans [1]. Still of these, *B. melitensis* is thought the principal cause of most acute and severe form of the disease in humans worldwide [2]. Although continuous progress is claimed to control the spread of human brucellosis, human cases of the disease are found around the world every year. The number of brucellosis reported is likely to be underestimated [3]. The highest prevalence of human brucellosis currently remains in Latin America, the Middle East, Africa and Asia areas.

In humans, the disease can be severely disabling causing significant economic costs associated with medical treatment and lifelong disability. Commonly localized disease manifestations are osteoarticular, including sacroiliitis and peripheral arthritis with clinical signs including fatigue, fever, anorexia,meningitis, and pneumonia. Having long incubation periods up to 6 months, in the absence of medical treatment the disease symptoms persist longer, for years [4].

Brucellosis in humans often occurs through consumption of nonpasteurized dairy products from infected animals, but infection can also occur through the direct contact with aborted animals' body fluids or tissues [5]. *Brucella* is considered a significant cause of laboratory-acquired infections. The features that facilitate *Brucella* are driven by its easy transmission, low infecting dose for humans and different infection routes in which the *Brucella* can enter human body, for example via respiratory and oral mucosal surfaces, conjunctivae or breaks in epidermis [6]. Transmission by aerosol route and minimum infection dose suggests features to use it as biological weapon. *Brucella spp.* is a Centers for Disease Control and Prevention

(CDC) second highest priority bioterrorism category B agent, because it considered moderately easy to spread across, can lead to moderate morbidity and low mortality and requires from the CDC to utilize specific enhancements to diagnose and disease surveillance [7].

Currently, there is no licensed vaccine for human brucellosis. Previously used vaccines *Bacillus abortus* S19 and *B. melitensis* Rev.1 are now considered unsafe for vaccination as can cause brucellosis. Moreover, different *Brucella* fractions, including *B. melitensis* PI and *B. abortus* sodium dodecyl sulphate fraction have been considered as potential human vaccines, but they found to be highly reactogenic and caused pain at the place of injection [8]. Therefore, the demand for safe and efficient vaccine against brucellosis remains.

Previous studies utilizing recombinant influenza A virus subtype H5N1 or H1N1 expressing *Brucella* proteins Omp 16, L7/L12 and Omp19 or Cu-Zn SOD from the open reading frame (ORF) NS1 (non-structural protein of influenza A viruses) gene have shown to be a new vector vaccine candidate to control brucellosis in different species susceptible to influenza [9]. These studies used A/Puerto Rico/8/34 (H1N1) strain to obtain influenza A virus vectors coding 80 or 124 aminoacids at N-terminal domain of its NS1 protein. Multiple roles of NS1 are in regulating mechanism of virus replication, inhibition of host immune responses and conducting cellular signaling pathways. Most of the amino acid substitutions are localized in the region at positions 80 and 124 [10].Two major glycoproteins on the surface haemagglutinin (HA) and neuraminidase (NA) was taken from A/chicken/Astana/6/05 (H5N1, with deletion of HA cleavage site) or A/New Caledonia/20/99 (H1N1) [11, 12].

On the basis of previous studies [9,11,12] using influenza virus vectors utilizing *Brucella* proteins, in this study two tetravalent vaccine formulations has been used.

Main **aim of the study** was to identify the route of immunization within mucosal sites including respiratory mucosa (nasal), conjunctiva, oral mucosa (sublingual) for these vaccine formulations using guinea pig as an animal model.

MATERIAL AND METHODS

Preparation of vaccine formulations

Two tetravalent influenza viral vector-based vaccine formulations in H5N1 subtype expressing the *Brucella* proteins *Omp16, Omp19,* L*7/L12* or *Cu-Zn SOD* at position 80 (Flu-NS1-80-Omp16-H5N1, Flu-NS1-80-Omp19-H5N1, Flu-NS1-80-L7/L12-H5N1, FluNS1-80-SOD-H5N1) and position 124(Flu-NS1-124-Omp16-H5N1, Flu-NS1-124-Omp19-H5N1, Flu-NS1-124-L7/L12-H5N1, FluNS1-124-SOD-H5N1) were used in this study. These influenza viral vectors type A were obtained as described previously [13]. Vaccine samples were produced in 10-day-oldchicken embryos at 34 ± 0.5ºCfor 48 h. After incubation period, the embryos were cooled to 2-8ºC and the allantoic fluid was collected. Separately produced allantoic suspensions containing *Omp16, Omp19,* L*7/L12* or *Cu-Zn SOD* Brucella antigenic genes, inserted in influenza viral vector (IVV) H5N1 (titer of about 8.0 log10 EID50/ml) were pooled in a single suspension in a 1:1 ratio to obtain the 4-valent vaccine formulation. Vaccine formulations were aliquoted into vials and stored at 2 - 8 ºC for no more than two days until used.

Immunization protocol

Conventional male guinea pig animal models (n=35) at 4-6 weeks of age were randomly assigned into seven groups (n=5 per group). Control group - non-treated (mock) guinea pigs were administrated PBS via nasal route at volume 200 µ (half volume in each nostril). Guinea pigs on six experimental groups vaccinated with tetravalent vaccine formulations at positions 80 and 124 administered via conjunctival - 50µL (half volume in each conjuctival sac), nasal - 200µL (half volume per each nostril), sublingual - 200 µL routes twice (prime and boost immunization) at an interval of 21 days. Group I and IV - immunized by conjunctival route; Group II and V- immunized by nasal, and Group III and VI -immunized through sublingual routes. Experimental groups from I to III delivered with vaccine formulations at 80 positions, and group IV-VI immunized with vaccine formulations at 124 positions. The body weights of each group of guinea pigs were recorded weekly. Table shows immunization details used in this study.

Detection of hemagglutinin inhibiting antibody response to influenza virus vectors

Antibody response to IVV subtype H5N1 in guinea pigs was determined at pre-and post- vaccination point on days 0, 21 and 42 by hemagglutination-inhibition assay (HIA). The native blood samples were obtained at saphenous vein into serum separator tubes (BD Vacutainer, the UK) following published method [14]. Serum was collected after prime and boost immunizations. HIA was performed according to the WHO protocol [15]. The inactivated influenza viral vectors subtype H5N1 was titrated for agglutination of 1% chicken red blood cell suspension and a dilution containing four hemagglutinating units used in the assay.

Statistical analysis

Differences in weight gain or loss were compared using two-way ANOVA. Serum antibody titers data were performed using one-way ANOVA with the Tukey's multiple comparisons test. All statistical analysis data was obtained using Graph Pad Prism Software version 6.01 for Windows. *p*-Values < 0.05 were considered significant.

Table 1 - **Summary of immunization route, vaccine formulations and experimental groups**

Immunization route of animals	Vaccine formulation	Biological name
Conjunctival (CONJUNC)	80-Omp16+L7/L12+Omp19+Cu-Zn SOD	Group I
Nasal	80-Omp16+L7/L12+Omp19+Cu-Zn SOD	Group II
Sublingual (SL)	80-Omp16+L7/L12+Omp19+Cu-Zn SOD	Group III
Conjunctival	124-Omp16+L7/L12+Omp19+Cu-Zn SOD	Group IV
Nasal	124-Omp16+L7/L12+Omp19+Cu-Zn SOD	Group V
Sublingual	124-Omp16+L7/L12+Omp19+Cu-Zn SOD	Group VI
Nasal	-	Control

Animal use

This study was carried out in accordance with the recommendations in the national and international laws and guidelines on animal handling. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Research Institute for Biological Safety Problems (RIBSP) of the Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan (approvals 0418/04). Guinea pigs maintained at Biological Research Facility at the RIBSP and were housed on 12 light/12 dark cycle in cages under controlled environmental conditions. Animals were monitored twice daily and were fed *ad libitum* with standard diet that included pelleted chow (Ssniff, Spezialdiäten GmbH) and had no water restrictions.

RESULTS AND DISCUSSION

General states of guinea pigs

After 2 weeks of acclimatization, guinea pigs were treated with vaccine formulations at week 0. Body weight changes of animals recorded on weeks 7, 14, 21, 28, 35 and 42 upon prime and boost immunization. We used one control group - non-treated (NT) and six experimental groups treated with different vaccine formulations shown in Table 1. A gradual increase in body weight was observed in all seven groups during the study. There were no animal deaths during this study. The two-way ANOVA with repeated measures interaction the day and biological groups as variable showed that during the 7 wk experiment, differences in body weights between the non-treated control group and experimental groups were not significant.

Vaccine formulations administered through nasal route generated detectable HAI titer

Route of vaccine delivery to appropriate anatomical site can affect localization of vaccine and induce protective immune response. Upon prime immunization, in guinea pigs antibody titers to influenza viral vectors coding amino acids in positions 80 and 124 were relatively low in range between 1:40 and 1:80, but observing antibody titers in position 80 when vaccine formulation delivered at conjunctival and nasal sites. No vaccination data available in animals of control and sublingual groups to influenza virus A.

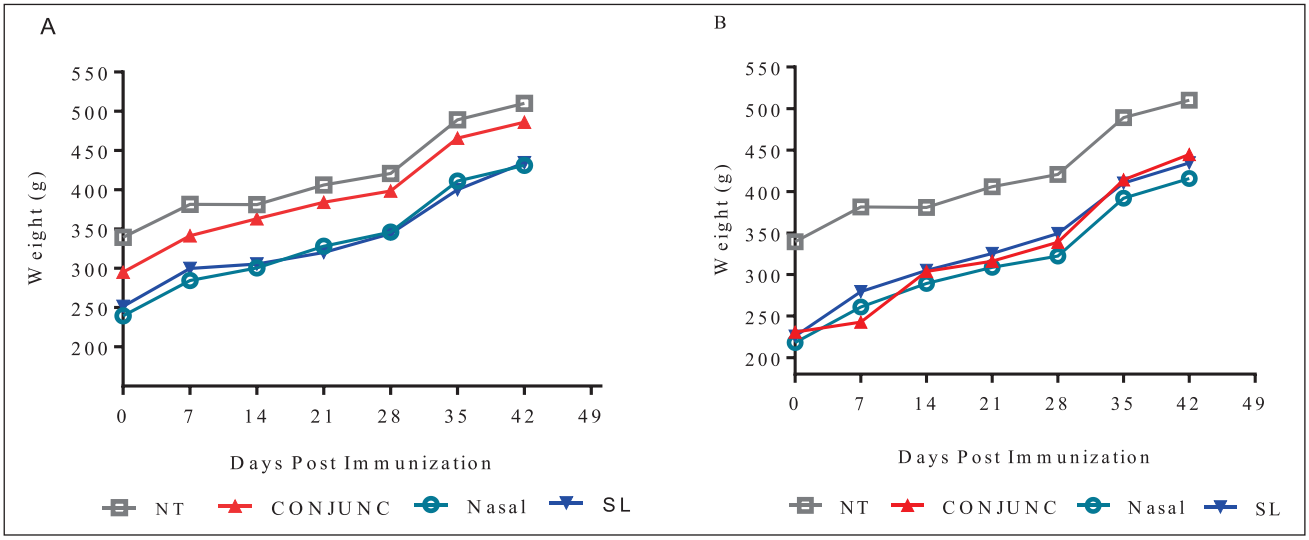

Figure 1 - Body weight growth of the four groups of guinea pigs after prime and boost immunization with vaccine formulations. ORF NS1 at position 80 (fig.1, A) and 124 (fig.1, B)

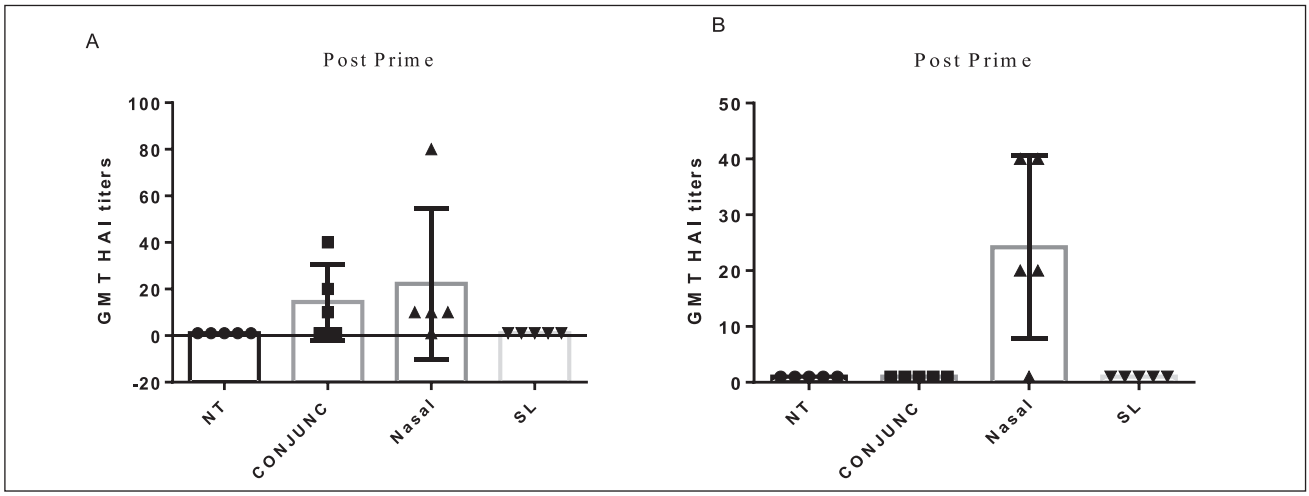

Figure 2 - Hemagglutination inhibition titers of after prime immunization of guinea pigs on day 21at position 80 (A) and 124 (B). NT-control group treated with saline, CONJUNC- conjunctival, SL- sublingual; the data is presented as geometric mean titer (GMT) ± standard error (SE)

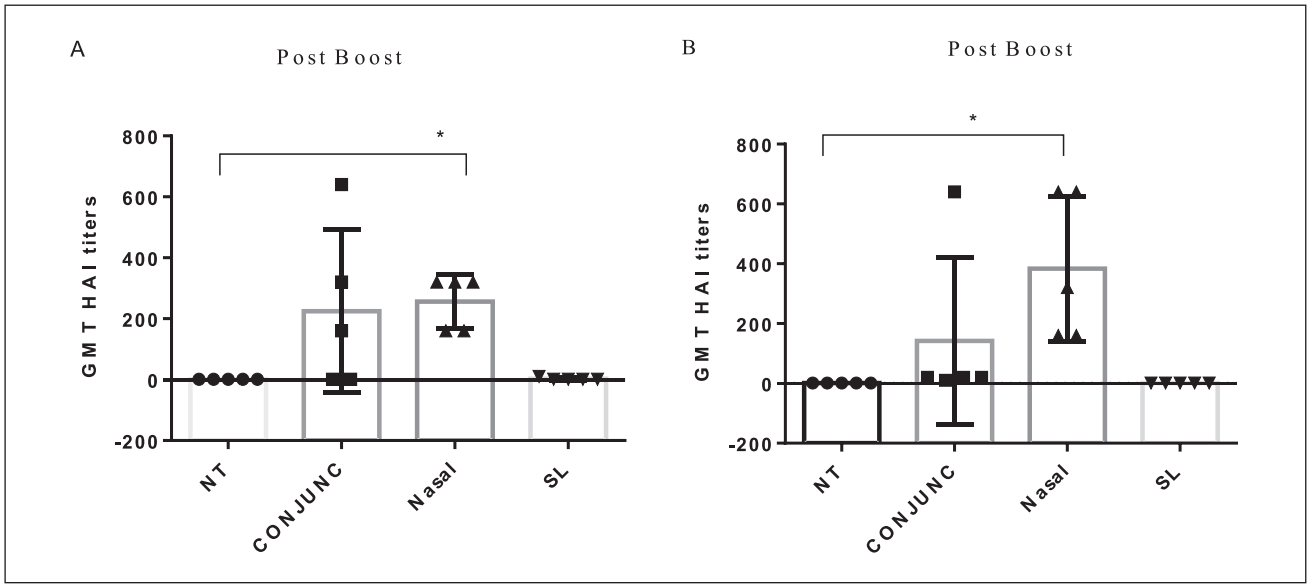

Figure 3 - Antibody titers after boost immunization at position 80 and 124. The data is presented as geometric mean titer (GMT) ± standard error (SE).*P = 0.01; statistical analysis was conducted using a one way ANOVA (Tukey's multiple comparisons test)

Both after prime and boost immunization on day 42, animals treated through nasal route resulted post vaccination titers of <1:160 and 1:640 at positions 80 (HIA, GMT 242.5) or 124 (HIA GMT 320.0) amino acids of the NS1 protein.

CONCLUSION

In this study, we used two tetravalent vaccine formulations developed on the basis of influenza A virus vectors type H5N1 (IVV) at position 80 and 124 of NS1 protein. This recombinant IVV codes *Brucella* immudominant proteins Omp16, Omp19, L7/L12 or Cu-Zn-SOD. Successful vaccination has several parameters that may impact vaccine efficacy [16]. It is important to determine appropriate vaccine delivery route to elicit antibody response adequately. We tested two tetravalent vaccine formulations at position of protein at 80 and 124 via mucosal administration (conjunctival, nasal and sublingual) at prime and boost immunization. Choice of the strategy over the other approaches is determined in influenza virus vectors tropism that is replicated in the respiratory epithelium. Within 7 weeks, general states of guinea pigs were observed in terms of weight growth and animals' general behavior. There were no abnormal findings. We detected antibody titers for influenza virus A in hemagglutination inhibition assay. Intranasal route of immunization with tetravalent vaccine formulation at position of protein 80 generated higher antibody titers applied at prime and boost immunization than other administration routes. These studies demonstrated that when highly pathogenic H5N1 influenza vaccine administered via intranasal route, the vaccine generated better protective immunity against influenza virus in mice [17]. In future studies, we will apply challenge studies to test protectiveness of these vaccine formulations in guinea pigs. Although conceptually prime vaccination against brucellosis in human may be preferable, prime and boost strategies in recombinant vaccines may require to be increased their antigenic activity.

Research transparency

Research did not have a sponsorship. The authors are absolutely responsible for presenting the release script for publication.

Declaration about financial and other relations

All authors took part in elaboration of article conception and writing the script. The release script was approved by all authors. The authors did not get the honorary for the article.

Conflict of interest

The authors declare no conflict of interest.

This work was supported by a grant from the Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan (grant no. AP05131463).

REFERENCES

1 Mandell GL, Bennett JE, Dolin R. Principles and practice of infectious disease. Brucellosis. Philadelphia, PA: Churchill Livingstone; 2000. P. 575

2 Cloeckart A, Vizcaino N, Paquet J, Bowden R, Elzer P. Major outer membrane proteins of Brucella spp.: past, present and future. *Veterinary Microbiology*. 2002;90:229-247. PMID: 12414146, DOI: 10.1016/S0378-1135(02)00211-0

3 Pappas G, Akritidis N, Bosilkovski M, Tsianos E. Brucellosis. *The New England Journal of Medicine*. 2005;352:2325–36. PMID:15930423, DOI:10.1056/NEJMra050570

4 Tuon FF, Gondolfo RB, Cerchiari N. Human-to-human transmission of Brucella - asystematic review. *Tropical Medicine and International Health*. 2017;22(5):539–46. PMID:28196298, DOI: 10.1111/tmi.12856

5 Baldi PC, Giambartolemi GH. Pathogenesis and pathobiology of zoonotic brucellosis in humans. *Revue of* International Office of Epizootics. 2013;32:117–25. PMID:23837370, DOI: 10.20506/rst.32.1.2192

6 Noviello S, Gello R, Kelly M, Limberger RJ, DeAngelis K, Cain L,Wallace B, Dumas N. Laboratory-acquired brucellosis. *Emerging Infectious Diseases*. 2004;10:1848–50. PMID:15504276, DOI:10.3201/eid1010.040076

7 Centers for Disease Control and Prevention. Bioterrorism

agents/diseases. Available from: https://emergency.cdc.gov/agent/agentlist-category.asp

8 Hadjichristodoulou C, Voulgaris P, Toulieres L, Babalis T, Manetas G, Goutziana G, Kastritis I, Tselentis I. Tolerance of the human brucellosis vaccine and the intradermal reaction test for brucellosis. *European Journal of Clinical Microbiology*. 1994;13:129–34. DOI: https://DOI.org/10.1007/BF01982185

9 Tabynov K. Influenza viral vector based Brucella abortus vaccine: a novel vaccine candidate for veterinary practice. *Expert Review of Vaccines*. 2016; 15(10):1237-9. PMID: 27356589, DOI: 10.1080/14760584.2016.1208089

10 Greenspan D, Palese P, Krystal M. Two nuclear location signals in the influenza virus NS1 nonstructural protein. *Journal of Virology*. 1988;62:3020-6.PMID: 2969057

11 Tabynov K, Sansyzbay A, Kydyrbayev Z, Yespembetov B, Ryskeldinova S, Zinina N, Assanzhanova N, Sultankulova K, Sandybayev N, Khairullin B, Kuznetsova I, Ferko B, Egorov A. Influenza viral vectors expressing the Brucella OMP16 or L7/L12 proteins as vaccines against *B. abortus* infection. *Virology Journal*. 2014;11:69. PMID: 24716528, DOI:10.1186/1743-422X-11-69

12 Tabynov K, Kydyrbayev Z, Ryskeldinova S, Yespembetov B, Syrymkyzy N, Akzhunusova I, Sansyzbay A. Safety of the novel vector vaccine against Brucellaabortus based on recombinant influenza viruses expressing Brucella L7/L12 and OMP16 proteins, in cattle. *Journal Vaccines and Immunology*. 2014;1:101. PMID: 29023541, DOI: 10.1371/journal.pone.0186484

13 Mailybayeva A, Yespembetov B, Ryskeldinova S, Zinina N, Sansyzbay A, et al. Improved influenza viral vector based Brucellaabortus vaccine induces robust B and T-cell responses and protection against Brucellamelitensis infection in pregnant sheep and goats. *PLOS ONE*. 2017;12(10). DOI: https://DOI.org/10.1371/journal.pone.0186484

14 Birck M, Tveden-Nyborg P, Lindblad M, Lykkesfeldt J. Non-terminal blood sampling techniques in guinea pigs. *Journal of visualized experiments*. 2014;92:e51982. PMID: 25350490, DOI: 10.3791/51982

15 WHO Global Influenza Surveillance Network. Manual for the laboratory diagnosis and virological surveillance of influenza.WHO. 2011. P. 28-59. Available from: https://www.who.int/influenza/gisrs_laboratory/manual_diagnosis_surveillance_influenza/en/

16 Zhang L, Wang W, Wang S. Effect of vaccine administration modality on immunogenicity and efficacy. *Expert Review of Vaccines*. 2015;14(11):1509–23. PMID: 26313239, DOI: 10.1586/14760584.2015.1081067

17 Ichinohe T, Ainai A, Tashiro M, Sata T, Hasegawa H. Poly-I:poly C12U adjuvant-combined intranasal vaccine protects mice against highly pathogenic H5N1 influenza virus variants. *Vaccine*. 2009; 27(45):6276–9. PMID: 19840660, DOI: 10.1016/j.vaccine.2009.04.074