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ТҮЙІН

Бұл мақалада адамдар мен жануарлардағы трипаносомоздың эпидемиологиясы мен диагностикасының мәселелері қарастырылған. Трипаносомоз – қан мен лимфа жүйесіне әсер ететін трипаносома паразиттерінің әртүрлі түрлерінен туындаған векторлық ауру болып табылады. Тақырыптың өзектілігі Қазақстандағы жануарлардың трипаносомозға шалдығуының артуы және диагностиканың қиындығымен байланысты. Маңғыстау облысында трипаносомоздың эпизоотиялық жағдайын зерттеу бойынша алдын ала зерттеу жұмыстары жүргізілді. 100 бас түйеден және 95 бас жылқыдан қан сарысуларының сынамалары алынды. Жылқылардағы *Trypanosoma equiperdum*-ге антиденелер комплементті бекіту реакциясы арқылы анықталды. Түйе қан сарысуларындағы *Trypanosoma evansi*-ге антиденелердің болуы комплементті бекіту реакциясы мен қосымша формалин реакциясы арқылы анықталды. Серологиялық зерттеулерде түйе сарысуының 5 оң сынамасы комплементті бекіту реакциясында және 65 оң сынама формалин реакциясында анықталды. Нәтижелер патогеннің болуын және одан әрі зерттеу қажеттілігін растайды. Серопозитивті үлгілердің болуы әрқашан патогенді микроскопиялық анықтау арқылы расталмайтынын ескеру маңызды, бұл дәлірек диагностикалық әдістерді қолдану қажеттілігін көрсетеді. Бұл мәселелерді шешу өңір үшін түйе және жылқы шаруашылығының маңыздылығын ескере отырып, Қазақстанның ветеринариялық қауіпсіздігі мен экономикалық әлеуетіне әсер етеді.

UDC 619.612.017:636.2 ***DOI 10.52578/2305-9397-2024-2-1-270-278***
IRSTI: 68.41.35. 0403301

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CLINICAL TRIALS OF INACTIVATED CULTURE VACCINE AGAINST CAMELPOX ON NATURALLY SUSCEPTIBLE ANIMALS

ANNOTATION

In the development of inactivated culture vaccine against camelpox, the deposited strain of camelpox virus M-0001, isolated in one of the farms of Mangistau region Beineuovskiy district in 2020,

was used. For inactivation 0.05 % of final concentration of inactivant ß-propiolactone was used. To stimulate the immune response was used adjuvant Montanidae Gel01 in 1% concentration.

The true state of immunity of the livestock during immunoprophylaxis will be determined by the results of routine immunologic (serologic) monitoring and elimination of the emerging focus of infection. For the purpose of approbation of the culture inactivated anti-vax vaccine from the strain "M-0001" against camel pox in production conditions, the vivarium of SPE "Antigen" LLP was used as a base. Immunogenicity of the vaccine was tested on 3 camels, which were injected with the vaccine subcutaneously in a dose of 3.0 cm3 in the groin area. In vaccinated animals with inactivated culture vaccine against camelpox from the strain "M-0001" on the 0 day the antibody titer against smallpox showed negative results, on the 7 day it was 1:2 - 1:4, on the 14 day it was 1:4-1:8, on the 21 day the antibody titer was 1:8.

Key words: *smallpox vaccine virus, infection, pathogenicity, immune response, antibodies.*

Introduction. Kazakhstan is traditionally one of the world's leading camel milk producers and intends to take a leading position in the development of productive camel breeding in the global community [1-4].

The main factor limiting the increase of camel milk population and production to fully meet the needs of domestic and foreign markets is various diseases of bacterial and viral nature. One such disease is camel pox [5-8].

Camelpox is a species-specific disease causing fever, generalized depression, dyspnea and a smallpox rash on the skin. The disease is transmitted by inhalation of aerosols, skin abrasion, contamination of feed and water, and is presumably transmitted by insects. The disease causes high morbidity (100%) and high mortality (10%-50%), especially in young animals.

Economic losses in camel pox outbreaks include adult mortality (up to 2-4%), death of young stock (up to 10% and more), abortions among foaling camel mothers (up to 25-27%), costs associated with anti-epizootic, therapeutic, preventive and quarantine measures, as well as costs for additional feeding of sick and recovered camels [9-14].

Taxonomically, camelpox virus belongs to the species Camelpox, genus Orthopoxvirus, a member of the broad family Poxviridae, which unites viruses of humans, animals, birds, and insects [15-20].

Despite the improvement of the epizootic situation on camel pox in the Republic of Kazakhstan, the problem of livestock recovery has not been finally solved. It is necessary to clarify the reasons for long-term well-being and the reasons for the emergence of new cases of the disease in favorable farms. A characteristic modern feature is restructuring of livestock breeding, creation of farms and leased farms, increase of livestock in private farms of citizens. The danger of introduction of camel pox pathogens into favorable herds has increased.

The relatively recent epizootic of camelpox (2019-2020) in the Mangistau region of the Republic of Kazakhstan and the damage it caused to agriculture predetermines the relevance of studying the problems associated with this infection.

As a result of significant economic losses from camelpox outbreaks, research has been directed towards developing preventive methods to contain the spread of camelpox in the Republic of Kazakhstan and enzootic countries.

Unfortunately, effective control programs such as sanitary measures, quarantine of infected areas, restriction of camel movements, provision of clean drinking water, and prevention of skin abrasions are difficult to implement due to the camel's migration pattern and difficulties in fixing the animals, especially during the rainy season.

An effective way to control camelpox is through vaccination. Several camelpox vaccines, both inactivated and live attenuated, are available in different countries.

Materials and methods. When performing research work we were guided by virological, serological and molecular-biological studies, using generally accepted methods according to the ethical principles of experimentation on animals set forth in the legislation of the Republic of Kazakhstan (Rules for conducting preclinical studies, medical and biological experiments and clinical trials in the Republic of Kazakhstan, approved by the Order of the Minister of Health № 442 of July 25, 2007; ST RK 1613-2006 "Good Laboratory Practice. Basic Provisions").

During the sampling procedure of biological material from camels, strict observance of safety and personal hygiene measures is necessary. Trained specialists should be allowed to do this work, since humans are susceptible to the camelpox virus due to the kinship of the causative agent with the causative agent of smallpox.

When working with animals, PPE was used during the procedures of fixation, collection and packaging of biological material samples: overalls, goggles, respirator, double gloves, special shoes. Strict compliance with personal hygiene measures, biosafety principles, PPE donning and doffing techniques, further processing, collection and disposal of used materials is the key to preventing possible spread of infection.

Camel fixation. Fixation was carried out with specialists directly caring for the animal. The animal's legs and head were monitored, as it can strike, bite and spit, and samples were taken with the animal lying down, with legs tied with a rope at the elbow and knee joints, and jaws tied.

Sampling. Blood sampling from the jugular vein of a camel was performed from the middle third of the neck, having prepared the site of blood sampling by trimming the hair and treating it with 70% alcohol. A tourniquet or rope was applied below the vein puncture site to constrict the vein. The needle was inserted towards the head of the animal. A vacuum system designed for serum separation was used for blood collection. For serum separation, the tube was placed in an upright position and left overnight at 4°C or at room temperature for 1 hour.

Serologic diagnosis. The presence of antibodies in sera was determined by neutralization reaction (NR) with a constant dose of virus and different dilutions of serum. To perform the reaction, the tested sera were inactivated at 56℃ for 30 minutes and double dilutions were prepared from 1:2 to 1:16, 0.2 ml of each dilution.

Determination of reactogenicity (harmlessness) Determination of reactogenicity (harmlessness) and immunogenicity was carried out on the basis of LLP "Antigen".

Harmlessness test was conducted on 2 camels (1 of them control), 5 rabbits and 5 guinea pigs. The vaccine was administered intramuscularly to camels in the thigh region in the initial dilution of 6 cm^3. Rabbits and guinea pigs were administered the vaccine intramuscularly in a dose of 1 cm^3 each. The observation period is 10 days.

Determination of immunogenicity. Immunogenicity of the vaccine was tested on 3 camels, which were injected subcutaneously with 3.0 cm^3 in the groin area.

The camels were monitored for 14 days. After blood sampling from vaccinated animals, blood sera were examined for the presence of antibodies to camelpox virus. The work was carried out on the basis of "Antigen" LLP.

Compliance with biosafety regulations. To comply with the rules of biosafety when working with camelpox virus, the work was carried out in isolation rooms equipped with all means of protection and excluding the possibility of carrying the infection. Air exchange in them is carried out through supply and exhaust ventilation with purification of exhaust air through special HEPA filters.

Results and their discussion. In the development of inactivated culture vaccine against camelpox, the deposited strain of camelpox virus M-0001, isolated in one of the farms of Mangistau region in 2020, was used. For inactivation, 0.05% final concentration of inactivant ß-propiolactone was used. To stimulate the immune response was used adjuvant Montanidae Gel01 in 1% concentration. The technology of this experimental series of inactivated camelpox vaccine consisted of 5 steps: inactivation of camelpox virus; testing for residual virulence was performed on lambs kidney cell culture.

When testing the avirulence of the suspension on susceptible animals, five guinea pigs were injected with inactivated material in a volume of 1 cm^3 subcutaneously. The animals were monitored for 14 days. Harmlessness test was conducted on 2 camels (1 of them control), 5 rabbits and 5 guinea pigs. The vaccine was administered intramuscularly to camels in the thigh region in the initial dilution of 6 cm^3 (Figure 1). Rabbits and guinea pigs were administered the vaccine intramuscularly at a dose of 1 cm^3 each. The observation period was 10 days. Determination of vaccine immunogenicity was tested on 3 camels, which were injected subcutaneously with 3.0 cm^3 of vaccine in the inguinal region. The camels were monitored for 14 days. After blood collection from vaccinated animals, blood serum was tested for the presence of antibodies to camelpox virus. The whole technological process of vaccine production consisted of three stages outlined in the normative and technical documentation developed and agreed by the Veterinary Committee of the Committee of Science of the Ministry of Agriculture of the Republic of Kazakhstan.

The results of the literature analysis allowed us to choose formaldehyde and β-propiolactone as the most promising and effective chemical compounds for inactivation of M-0001 camelpox strain. All works were carried out at the production base of DiaVak-ABN LLP.

Experimental-laboratory series of the vaccine was tested on naturally susceptible animals in order to study harmlessness and immunogenicity.

As a base for approbation of the culture inactivated vaccine against camelpox from the strain "M-0001" under production conditions, we used the vivarium of "Antigen" LLP, which is located at the address: c/o "Ұmtyl", Karasai district, Almaty oblast Karasay district of Almaty region. The veterinarian of the vivarium of "Antigen" LLP carried out clinical examination of the experimental camels with body temperature measurement. Blood serum samples were taken from camels to determine the presence or absence of antibodies to the causative agent of camel pox by serological reaction.

a

b

c

d

Figure 1 – Determination of harmlessness and immunogenicity in animals (a,b,c,d)

The animals were followed by daily clinical examination with measurement of body temperature and with subsequent data logging for 10 days (table 1).

Table 1 – Temperature list of animals after immunization

№	Individual animal number	Animal body temperature		Note
		morning	evening	
1	2	3	4	5
04.04.2023 г				

1	2	3	4	5
1	KZ B5 112550115 (harmlessness)	38,0	38,5	Clinical condition of the animals is satisfactory, body temperature is normal (at 35.0-38.6°) C. The animals are of average fatness, without any abnormalities.
2	KZ B5 112550113 (control)	36,9	37,2	
05.04.2023				
1	KZ B5 112550115 (harmlessness)	39,1	39,3	The animal has an increase in body temperature, no changes were found at the site of vaccine administration. The general condition of the animal is satisfactory.
2	KZ B5 112550113 (control)	36,7	37,6	Clinical condition of the animals is satisfactory, body temperature is within normal limits.
06.04.2023				
1	KZ B5 112550115 (harmlessness)	39,3	39,5	The animal has an increase in body temperature, no changes were found at the site of vaccine administration., The general condition of the animal is satisfactory.
2	KZ B5 112550113 (control)	37,0	37,5	Clinical condition of the animals is satisfactory, body temperature is within normal limits.
07.04.2023				
1	KZ B5 112550115 (harmlessness)	39,7	39,8	The animal has an increase in body temperature, no changes were found at the site of vaccine administration. The general condition of the animal is satisfactory.
2	KZ B5 112550113 (control)	37,0	37,4	Clinical condition of the animals is satisfactory, body temperature is within normal limits.
08.04.2023				
1	KZ B5 112550115 (harmlessness)	39,2	39,5	The animal has an increase in body temperature, no changes are found at the site of vaccine administration. Appearance of nodular-pustular rash on the skin and mucous membranes of experimental animals is not observed. The general condition of the animal is satisfactory.
2	KZ B5 112550113 (control)	37,2	37,5	Clinical condition of the animals is satisfactory, body temperature is within normal limits.
09.04.2023				
1	KZ B5 112550115 (harmlessness)	38,7	38,9	The animal has an increase in body temperature, no changes are found at the site of vaccine administration. Appearance of nodular-pustular rash on the skin and mucous membranes of experimental animals is not observed. The general condition of the animal is satisfactory.
2	KZ B5 112550113 (control)	37,1	37,3	Clinical condition of the animals is satisfactory, body temperature is within normal limits.
10.04.2023				
1	2	3	4	5
1	KZ B5 112550115 (harmlessness)	38,2	38,4	Clinical condition of the animals is satisfactory, body temperature is within normal limits.
2	KZ B5 112550113 (control)	37,0	37,2	Clinical condition of the animals is satisfactory, body temperature is within normal limits.
11.04.2023				

1	2	3	4	5
1	KZ B5 112550115 (harmlessness)	37,9	38,0	Clinical condition of the animals is satisfactory, body temperature is within normal limits.
2	KZ B5 112550113 (control)	36,8	37,1	Clinical condition of the animals is satisfactory, body temperature is within normal limits.
	12.04.2023			
1	KZ B5 112550115 (harmlessness)	37,5	37,7	Clinical condition of animals is satisfactory, body temperature is within normal limits. Appetite is normal.
2	KZ B5 112550113 (control)	37,0	37,2	Clinical condition of the animals is satisfactory, body temperature is within normal limits.
	13.04.2023			
1	KZ B5 112550115 (harmlessness)	37,3	37,6	Clinical condition of animals is satisfactory, body temperature is within normal limits.
2	KZ B5 112550113 (control)	36,1	36,5	Clinical condition of the animals is satisfactory, body temperature is within normal limits.
	14.04.2023			
1	KZ B5 112550115 (harmlessness)	37,2	37,5	On the 10th day in experimental animals, which was used for testing the determination of harmlessness of "Inactivated culture vaccine against camelpox from strain "M-0001" clinical condition of animals is satisfactory, body temperature is within normal limits. Appetite is normal.
2	KZ B5 112550113 (control)	37,3	37,9	Clinical condition of the animals is satisfactory, body temperature is within normal limits.

The data in table 1, indicate that the vaccine is considered harmless, as the vaccinated camels remained clinically healthy for 10 days after vaccination. Camels are allowed to have body temperature increase up to 40.5℃ for 1- 4 days. Administration of inactivated camelpox culture vaccine to guinea pigs and rabbits at a dose of 1 cc did not cause death of laboratory animals during 10 days of observation. Also, all control animals used in the experiment during the entire observation period remained clinically healthy.

The control animal was in contact with the vaccinated camel during the entire observation period. During the observation period, the control animal remained clinically healthy, which indicates the biological safety of the veterinary drug.

Immunogenicity of the vaccine was tested on 3 camels, which were injected subcutaneously in a dose of 3.0 cm^3 in the groin area. The animals were subjected to daily clinical examination with body temperature measurement for 14 days. Subsequently, blood sera were sampled from experimental animals on 7, 14, 21 days for serologic study for antibodies.

Table 2 – Virus neutralizing antibody levels in camel blood sera at 0, 7, 14 and 21 days after vaccination

№	Name of material	Animal identification numbers	Serum dilution				availability VNA
			1:2	1:4	1:8	1:16	
1	2	3	4	5	6	7	8
0 day							
1	Blood serum	KZB5 112550114	++++	++++	++++	++++	0,0
2		KZB5 112550116	++++	++++	++++	++++	0,0
3		KZB5 112550117	++++	++++	++++	++++	0,0
7 day							

1	2	3	4	5	6	7	8
1	Blood serum	KZB5 112550114	- - - -	++++	++++	++++	1:2
2		KZB5 112550116	- - - -	- - - -	++++	++++	1:4
3		KZB5 112550117	- - - -	++++	++++	++++	1:2
№ n/n	Name of material	Animal identification numbers	Serum dilution				availability VNA
			1:2	1:4	1:8	1:16	
14 day							
№ n/n	Name of material	Animal identification numbers	Serum dilution				availability VNA
			1:2	1:4	1:8	1:16	
1	Blood serum	KZB5 112550114	- - - -	- - - -	- - - -	++++	1:8
2		KZB5 112550116	- - - -	- - - -	- - - -	++++	1:8
3		KZB5 112550117	- - - -	- - - -	++++	++++	1:4
21 day							
№ n/n	Name of material	Animal identification numbers x	Serum dilution				availability VNA
			1:2	1:4	1:8	1:16	
1	Blood serum	KZB5 112550114	- - - -	- - - -	- - - -	++++	1:8
2		KZB5 112550116	- - - -	- - - -	- - - -	++++	1:8
3		KZB5 112550117	- - - -	- - - -	- - - -	++++	1:8
Note: "-" - absence of cytopathogenic effect; "+" - presence of cytopathogenic effect.							

As shown in table 2, the presence of antibodies in sera was determined by performing neutralization reaction (NR) with a constant dose of virus and different dilutions of serum. To set up the reaction, the tested sera were inactivated at 56℃ for 30 minutes and twofold dilutions were made, ranging from 1:2 to 1:16, with 0.2 ml of each dilution. For the dilution, DMEM maintenance nutrient medium for lamb kidney cell culture was used, on which the neutralization reaction was performed. An equal volume of 0.2 ml of virus at the selected dose (200 TCD50) was then added to each serum dilution. The virus mixture with serum dilutions was incubated at (37 ±0.5) ℃ for 1 h. Then the mixture from each well was added to 4 wells of the plate with lambs kidney cell culture in a volume of 0.1 ml, having previously drained the supporting medium from them. At the same time, a control of cell culture was placed in a separate plate (4 wells with a change of nutrient medium and 4 wells without change), as well as a subtitration of the working dose of virus was made in parallel.

Infected plates with cell culture were incubated under CO^2 conditions at (37 ±0.5) ℃ for 10 days depending on the time of cytopathic action (CPA). The nutrient medium in the plates was changed every 2 days.

The results of the study of immunogenic properties of the inactivated culture vaccine against camelpox from the strain "M-0001" produced by NAO "Kazakh National Agrarian Research University" on 7, 14 and 21 days, the vaccinated animals formed virus neutralizing antibodies to camelpox virus in titers of 1: 2-1:8.

Blood serum samples were collected from vaccinated animals after 6 months of the current year to study the duration of immunity against camelpox virus.

Conclusion. In developing the parameters of technological control of the vaccine (harmlessness, immunogenicity) on laboratory, target animal species by the neutralization reaction (NR) method, the developed culture inactivated vaccine was injected intramuscularly into the inguinal region of the thigh in the initial dilution of 6 cm^3 1 one camel, 5 rabbits and 5 guinea pigs intramuscularly in a dose of 1 cm^3 each. The vaccine is harmless as the vaccinated animals remained alive and clinically healthy during 10 days of observation.

Immunogenicity of the vaccine was tested on 3 camels, which were injected subcutaneously at a dose of 3.0 cm^3 in the inguinal region. Blood serum samples were collected from experimental animals on 7, 14, 21 days for serologic examination for antibodies. Inactivated culture vaccine against camelpox

from the strain "M-0001" produced by NAO "Kazakh National Agrarian Research University" on 7, 14 and 21 days in vaccinated animals formed virus neutralizing antibodies to camelpox virus in titers of 1:2-1:8.

After studying the immunobiological properties and confirming the optimal schemes of camel immunization, 1 experimental series of culture inactivated vaccine against camel pox from strain M-0001 was produced according to the developed technology.

Acknowledgments: This work was supported by the Ministry of Agriculture of the Republic of Kazakhstan: for №04/8-21-29 "Program-targeted financing of scientific research and activities" 2021-2023.

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ТҮЙІН

Түйе шешегіне қарсы бейбелсендірілген дамылсыз дамитын торша өсіндісінде өсірілген вакцинаны әзірлеу кезінде Маңғыстау облысы Бейнеу ауданының шаруа қожалығының түйелерінен 2020 жылы оқшауланып, бөлініп алған М-0001 түйе шешек вирусының депозиттелген штаммы пайдаланылды. Иммундық жауапты ынталандыру үшін 1% концентрацияда montanide GEL 01 адъюванты қолданылды.

Иммунопрофилактика кезіндегі мал иммунитетінің шынайы жай-күйі жоспарлы иммунологиялық (серологиялық) мониторинг және инфекцияның пайда болған ошағын жою нәтижелері бойынша айқындалатын болады. "М-0001" штаммынан түйе шешегіне қарсы инактивтендірілген торша өсіндісінде өсірілген вакцинаны сынақтан өткізу мақсатында өндірістік жағдайларда ЖШС "Антиген" өндірістік ғылыми кешенінің виварийін база ретінде пайдаланылды. Вакцинаның иммуногенділігін зерттеу мақсатында вакцина түйелердің шап аймағының тері астына 3,0 см^3 дозада енгізіп, 3 бас 6 айлық жас түйелерде сыналды. Зерттеуге шешек ауруынан қолайлы аймақтан алынған түйелерге жүргізілді. Вакцинацияланған жануарларда "М-0001" штаммынан түйе шешегіне қарсы инактивтендірілген дамылсыз дамитын торша өсіндісінде өсірілген вакцинамен 0 тәулікке шешекке қарсы антиденелердің титрі теріс нәтиже көрсетті, 7 тәулікке 1:2 – 1:4, 14 тәулікке 1:4-1:8, 21 тәулікке антиденелердің титрі 1:8 құрады.

РЕЗЮМЕ

При разработке инактивированной культуральной вакцины против оспы верблюдов был использован депонированный штамм вируса оспы верблюдов М-0001, выделенный в одном из КХ Мангистауской области Бейнеуовском районе в 2020 г. Для инактивации использовали 0,05 % конечных концентрации инактивант ß-пропиолактон. Для стимулирования иммунного ответа был использован адъювант Montanidae Gel01 в 1% концентрации.

Истинное состояние иммунитета поголовья при иммунопрофилактике будет определяться по результатам планового иммунологического (серологического) мониторинга и ликвидации возникшего очага инфекции. С целью апробации культуральной инактивированной противооспенной вакцины из штамма «М-0001» против оспы верблюдов в производственных условиях в качестве базы был использован виварий ТОО НПП «Антиген». Иммуногенность вакцины испытали на 3-х верблюдах, которым вводили вакцину подкожно в дозе 3,0 см^3 в паховую область. У вакцинированных животных инактивированной культуральной вакциной против оспы верблюдов из штамма «М-0001» на 0 сутки титр антител против оспы показал отрицательный результат, на 7 сутки составлял 1:2 – 1:4, на 14 сутки составлял 1:4-1:8, на 21 сутки титр антител составлял 1:8.

UDC 582.62.876.777.4 ***DOI 10.52578/2305-9397-2024-2-1-278-286***
IRSTI: 68.41.35. 0403301

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