UDK 8.41.35 ***DOI 10.52578/2305-9397-2025-4-2-16-23***
IRSTI: 68.41.53

Abishov A.A., Doctor of Veterinary Sciences, **the main author**, https://orcid.org/0000-0002-7646-6275
LLP «SPE DiaVak-ABN», Almaty, 4/1 Bulkusheva Street, A20F3H4, Kazakhstan, adishov-64@mail.ru
Abisheva A.K., Master of Veterinary Sciences, https://orcid.org/0000-0002-6291-1876
LLP «SPE DiaVak-ABN», Almaty, 4/1 Bulkusheva Street, A20F3H4, Kazakhstan, Uak.89@mail.ru
Khairullayeva K.A., https://orcid.org/0009-0006-8925-2969
LLP «SPE DiaVak-ABN», Almaty, 4/1 Bulkusheva Street, A20F3H4, Kazakhstan, Khayrullaeva69@mail.ru
Kalissynov B.S., Head of the Virology Laboratory, https://orcid.org/0000-0002-7928-3589
LLP «SPE DiaVak-ABN», Almaty, 4/1 Bulkusheva Street, A20F3H4, Kazakhstan, b.kalisynovy@mail.ru
Maikhin K.T., Candidate of Veterinary Sciences, https://orcid.org/0000-0003-1744-2860
Almaty branch «National Reference Center for Veterinary», Almaty, 221в Rayymbek Avenue, A20C2D0Ю, Kazakhstan, maikhin67@mail.ru
Samarkhanov S.K, Postgraduate student, https://orcid.org/0000-0001-8166-6784
«Kyrgyz National Agrarian University named after K.I. Scriabin»
Almaty branch «National Reference Center for Veterinary», Almaty, 221в Rayymbek Avenue, A20C2D0Ю, Kazakhstan, serik_sk@mail.ru
Kaiypbay B.B., Doctor of Veterinary Sciences, https://orcid.org/0000-0002-8237-5564
«Kostanay Regional Territorial Inspection of the Veterinary Control and Supervision Committee», Kostanay, 43 Embankment Street, P01B8B7, Kazakhstan, Berikzhan_7@mail.ru
Moldakhanov Ye.S., PhD, https://orcid.org/0000-0002-6168-4376
«Research and Production Center for microbiology and virology», Almaty, Bogenbay batyr Str., 105
NJSC «Kazakh National Agrarian Research University», Abai Avenue 26, A15C8A3, Kazakhstan, ergali86@mail.ru
Syrym N.S., Candidate of Veterinary Sciences, Professor, https://orcid.org/0000-0002-4361-5676
RSE «Research Institute of Biological Safety Problems», Zhambyl region, Kordaysky district, the village of Gvardeysky, 15B. Momyshuly Street H20B1C4, Kazakhstan, nazym-syrym@mail.ru

IMMUNOBIOLOGICAL EVALUATION OF THE VIRAL VACCINE AGAINST EQUINE RHINOPNEUMONITIS

ANNOTATION

During the conducted studies, a dry viral vaccine against equine rhinopneumonitis, **"**RinoVac", was developed and experimentally tested. The vaccine was produced using the equine rhinopneumonitis virus strain **"**AK-2011" on a monolayer culture of TT cells. A comprehensive set of biological tests was carried out, including the evaluation of immunogenicity (CFT, ELISA) and protective activity in laboratory animals, as well as safety assessment in white mice. In laboratory conditions, the procedures for obtaining virus-containing biomass were optimized, and the optimal doses and routes of administration were determined, along with the assessment of immunogenicity – all studies were performed on model rabbits (subcutaneous and intramuscular administration at doses of 0.5, 1.0, and 2.0 cm^3). Additionally, the effect of revaccination on the intensity and durability of the immune response was evaluated.

In vaccinated animals, a significant increase in the level of specific antibodies and the formation of a pronounced protective response compared to the control group were recorded. In rabbits, antibody titers reached 1:300 on day 7, increased to 1:500 on day 14, and remained at 1:400 by day 30; the protective effectiveness was 80% (versus 20% in the control group). Revaccination performed 90 days after the primary immunization led to an additional enhancement of the humoral response: in all dilutions (1:50–1:400), optical density values in ELISA in the revaccinated animals were significantly higher than in the control group (for example, at 1:50 – 1.89 vs. 0.73), indicating the development of a more stable and long-lasting immunity.

Key words: *Equine rhinopneumonitis, strain, herpesvirus, vaccine, prevention.*

Introduction. Equine rhinopneumonitis is a highly contagious viral disease in horses, with clinical manifestations ranging from mild respiratory symptoms to severe outbreaks that may cause multiple abortions [1, 2, 3]. The infection typically presents with catarrhal and necrotic lesions in the respiratory

tract mucosa, fever, conjunctivitis, general malaise, and meningoencephalitis in foals. Additionally, adult horses can also develop neurological signs, reproductive system disorders, and acute respiratory disease in young animals, while mares may experience abortions during the second half of pregnancy [4, 5, 6] . The disease is caused by equine herpesviruses, mainly EHV-1 and EHV-4, which possess double-stranded DNA and a lipid-rich envelope, rendering them sensitive to ether, chloroform, and many detergents. Transmission occurs through both respiratory droplets and direct contact. The virus is capable of establishing latent infections that can reactivate under stress, and it demonstrates efficient replication and cytopathic effects in equine tissue cell cultures [7, 8, 9].

Equine rhinopneumonitis is widely distributed across the Americas, Europe, the CIS countries, including the Republic of Kazakhstan [10,11,12] . Infected animals excrete the virus via the respiratory tract and serve as the primary source and vector of infection. Transmission occurs through aborted fetuses and placental membranes, aerosolized respiratory secretions, as well as via contaminated feed, water, and bedding. Stallions may also serve as carriers during mating. It is suggested that domestic and wild animals are likely able to mechanically spread infected fetal material between farms [13,14,15]. Factors such as immunodeficiency, stress, and the use of immunosuppressive drugs contribute to increased outbreak risks. In pregnant mares, the virus adheres to leukocytes, crosses the placental barrier, replicates within the fetus, and induces necrotic damage to the liver, spleen, and lungs, ultimately resulting in fetal death and abortion [16,17,18].

During an epizootic, equine enterprises incur substantial economic losses, manifested as reduced foal production, loss of valuable breeding stock, and increased expenditures on veterinary and sanitary interventions. In herds containing pregnant mares, equine rhinopneumonitis can lead to abortions. Notably, abortions may occur not only in mares exhibiting the respiratory form of the disease with pronounced clinical signs but also in apparently healthy virus-carrying animals. Infection can cause abortions in up to 90% of pregnant mares, with the process potentially persisting for 8-10 months [19, 20, 21].

Based on these considerations, the objective of our study was to develop a methodology for producing a viral vaccine against equine rhinopneumonitis.

Materials and Methods. The study utilized the equine rhinopneumonitis virus strain “AK-2011”, which was isolated from pathological material obtained from horses during abortion outbreaks at equine breeding farms in the Almaty and Zhambyl regions in 2011.

Pathological material from naturally infected animals, various primary and passaged cell cultures, nutrient media, chemical reagents, as well as whole blood and serum from animals, were used in the experiments.

The susceptibility of cell cultures to equine rhinopneumonitis virus was evaluated by serial passaging in a panel of primary and continuous cell lines, including LK (primary lamb kidney), LT (primary trypsinized lamb testis), PEF (primary porcine embryo fibroblasts), BHK-21 (baby hamster kidney; continuous line), BK-80 (bovine kidney), BT-Tr (primary bovine trachea), and Vero (African green monkey kidney epithelial; continuous line).

Viral biomass was produced using the “AK-2011” strain, which was propagated on a monolayer of TT cells (tracheal epithelial cells) at a titer of 10^5 $TCID_{50}$. The biological activity of the equine rhinopneumonitis vaccine was assessed by virus titration in TT cell test-tube cultures.

The immunogenic potential of the equine rhinopneumonitis vaccine was determined through virus titration in TT cell cultures. Ten-fold serial dilutions (10^{-1}–10^{-7}) of the initial virus-containing suspension in maintenance medium were prepared. Each dilution (1 cm^3) was used to infect four test-tube cell cultures. Biological activity was evaluated based on the onset and severity of cytopathic effects, as well as the accumulated virus titer calculated using the Reed and Muench method. Titration was performed in triplicate.

Vaccine safety was evaluated using 20 clinically healthy white mice (18-20 g) and 5 healthy rabbits (3.0-3.5 kg). Mice were administered the vaccine subcutaneously at a dose of 0.5 cm^3, whereas rabbits received intramuscular injections in the thigh with 3 cm^3 of the original preparation. Animals were observed continuously over a 14-day period.

Preliminary studies to establish the optimal vaccine dose, administration route, and immunogenic potential were performed on laboratory rabbits. Rabbits (10 animals per group) received the vaccine either subcutaneously or intramuscularly at doses of 0.5 cm^3, 1.0 cm^3, or 2.0 cm^3. Serum samples were collected on day 21 after immunization to assess the immune response using complement fixation (CFT) and ELISA assays. At this stage, the necessity and timing of revaccination were also determined.

Results and Discussion. The studies confirmed that the attenuated equine rhinopneumonitis virus strain "AK-2011" possesses strong immunogenic properties, making it a viable candidate for vaccine development. Following comprehensive evaluation of its biological characteristics, the experimental vaccine, provisionally named "RinoVac," was developed and subjected to preliminary testing. Throughout the development process, a fully documented production protocol for the dry cell culture vaccine was established, detailing the preparation of media and solutions, cell culture propagation, harvesting of virus-containing biomass, filling, and lyophilization.

In the course of the study, optimal parameters for the accumulation of viral biomass were determined. TT cell cultures at a density of 150-200 × 10^3 cells/mL, infected with 0.1-0.5 $TCID_{50}$ per cell, formed a confluent monolayer within 40-72 hours, producing a viral suspension with a titer of 5.25-6.0 log $TCID_{50}$/mL. These conditions were identified as ideal for generating standardized virus-containing material suitable for prophylactic application.

The attenuated "AK-2011" strain demonstrated strong immunogenic properties, eliciting a prolonged and stable production of specific antibodies. These findings underscore its promise for the development of a domestic equine rhinopneumonitis vaccine and establish a solid scientific foundation for subsequent scaling of the production process.

The obtained results provide a solid scientific and production basis for the manufacture and implementation of the dry culture-based equine rhinopneumonitis vaccine "RinoVac" in veterinary practice. A key quality parameter of the vaccine is its protective efficacy—the ability to prevent infection and safeguard the organism—which is assessed through comparative testing of vaccinated and unvaccinated animals followed by controlled pathogen challenge.

In this study, the protective activity of the vaccine against a heterologous (cross) strain of the equine rhinopneumonitis virus was evaluated. Experimental infection was performed in both vaccinated and control groups, and subsequent assessments included clinical signs, pathological changes, and antibody titers. Based on these data, the epizootic efficacy of the vaccine was determined.

The equine rhinopneumonitis viral vaccine was administered intramuscularly to rabbits at a dose of 2.0 cm^3. The immune response was monitored on days 7, 14, and 30 post-vaccination. Animals were challenged with a heterologous (cross) strain of the equine rhinopneumonitis virus by intramuscular injection of a virus-containing suspension at 2.0 cm^3.

Evaluation criteria included clinical signs (fever, rhinitis, cough, depression) and pathological changes (evidence of lung and bronchial inflammation). Antibody titers were measured using ELISA, and protective efficacy (%) was calculated based on the ratio of affected to recovered animals.

The results of the vaccine's protective efficacy are presented in Table 1 and Figure 1.

Table 1 – Effect of the viral vaccine on the development of specific antibodies and protective response

Groups	Antibody titer (day 7)	Antibody titer (day 14)	Antibody titer (day 30)	Protective efficacy (%)
Vaccinated	1:300	1:500	1:400	80
Control	0	1:50	1:30	20

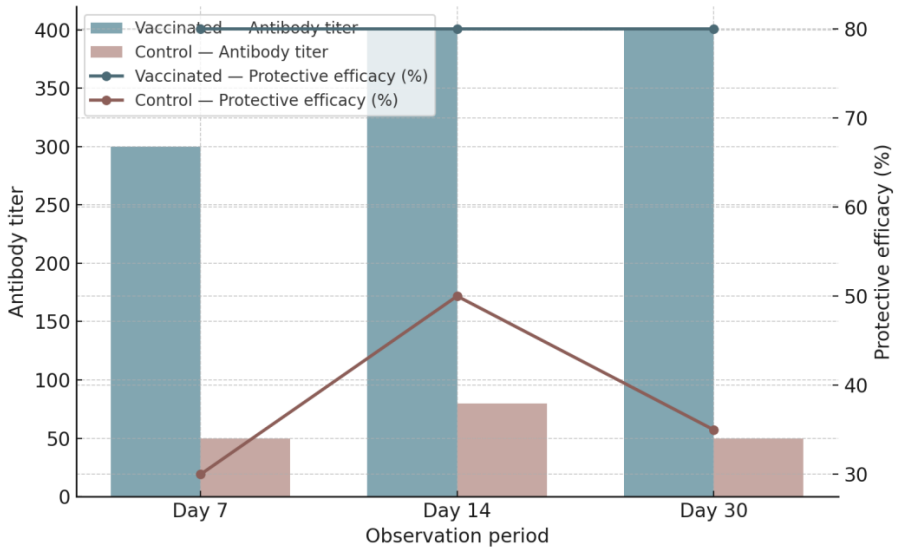

Figure 1 – Dynamics of antibody titers and protective efficacy

Protective efficacy was assessed by measuring antibody titers and protection rates on days 7, 14, and 30 post-vaccination in both the vaccinated and control groups. In the vaccinated cohort, antibody titers increased to 1:300 by day 7, peaked at 1:500 on day 14, and slightly decreased to 1:400 by day 30, reflecting a rapid and robust immune response.

In contrast, the control group displayed markedly lower titers, reaching only 1:50 on day 14 and 1:30 on day 30, with antibodies undetectable at day 7. Protective efficacy was 80% in vaccinated animals versus 20% in controls, highlighting a strong correlation between antibody titer and vaccine-mediated protection.

As shown in the figure, vaccinated animals maintained high antibody levels and consistent protection, while the control group exhibited a weak immune response, indicating inadequate protection.

These findings demonstrate that the vaccine provides strong and durable immunity against the target infectious disease.

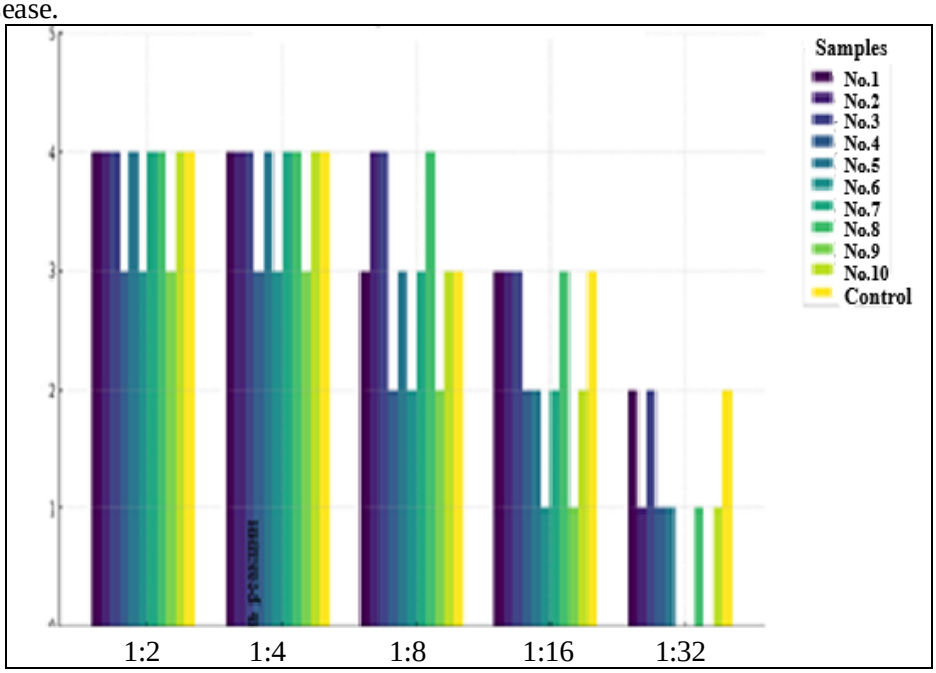

Figure 2 – Serial dilution of blood serum

As shown in Figure 2, increasing the degree of serum dilution (1:8, 1:16, 1:32) resulted in a gradual decrease in reaction intensity, indicating a reduction in antibody concentration.

Sera samples No. 2 and No. 3 exhibited a stable response even at high dilution (1:32) with a reaction intensity of 2 points, suggesting a high antibody content. In contrast, samples No. 6, No. 7, and No. 9 showed a much earlier decline in titer, with no detectable reaction at 1:32 dilution, reflecting a weaker immune response.

The control (reference) serum demonstrated consistent reactivity across all dilution levels, confirming its reliability as a positive control.

It was determined that intramuscular administration of the “RinoVac” viral vaccine at a dose of 2 cm^3 in rabbits caused no adverse effects.

Complement fixation testing (CFT) of sera collected on day 21 after immunization revealed antibody titers ranging between 1:16 and 1:32. The specific antiserum against the equine rhinopneumonitis virus showed a titer of 1:32. The results are presented in Figure 3.

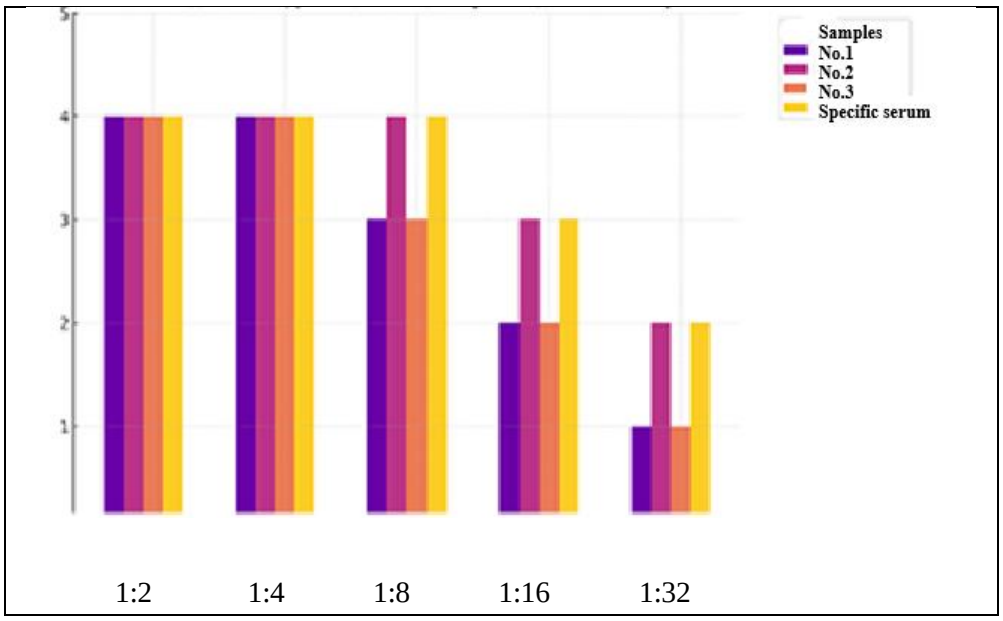

Figure 3 – Evaluation of the immunogenicity of the viral vaccine by the complement fixation test (CFT) on day 21 post-vaccination

As shown in Figure 3, serum sample No. 2 demonstrated the highest reaction intensity even at dilutions from 1:8 to 1:32. In comparison, serum samples No. 1 and No. 3 exhibited a weaker response that gradually diminished as the dilution increased to 1:16–1:32. The specific control serum displayed a stable and high-level reaction across all dilutions, confirming its reliability as a positive control.

These results indicate that the tested viral vaccine induces a pronounced immune response, particularly in animals represented by serum samples No. 1–3, which developed the highest antibody titers.

Subsequently, revaccination (booster immunization) was carried out to enhance the primary immune response and ensure long-term protective immunity, thereby increasing the overall vaccine efficacy. Revaccination was performed 90 days after the initial immunization, with each rabbit receiving 2 cm³ of the vaccine intramuscularly.

The ELISA data collected after revaccination, reflecting the kinetics of antibody formation, are summarized in Figure 4.

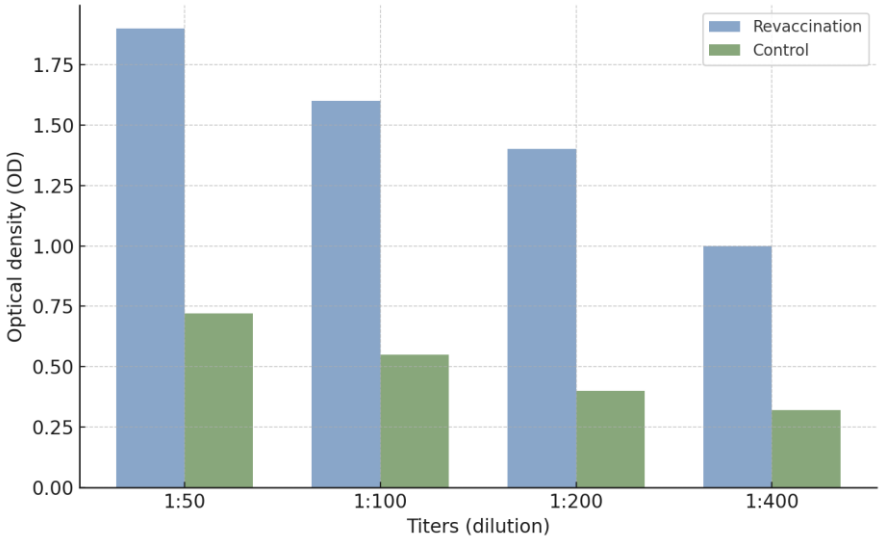

Figure 4 – ELISA results following revaccination

As a result of the conducted experiments, experimental batches of a lyophilized viral vaccine against equine rhinopneumonitis, produced using a continuous cell culture system, were obtained, and its immunobiological characteristics were comprehensively evaluated.

Post-revaccination analysis using ELISA revealed the induction of a robust and sustained immune response in immunized animals. At all serum dilutions (1:50, 1:100, 1:200, and 1:400), the optical density

(OD) readings in the revaccinated group were markedly higher than those in the control group. Specifically, at a 1:50 dilution, the mean OD reached 1.89, compared to 0.73 in controls. At higher dilutions, OD values in vaccinated animals remained within the 1.2–1.8 range, indicating persistent antibody activity, whereas control samples maintained low baseline levels (0.3–0.5).

These data demonstrate the high immunogenic potential of the developed vaccine formulation and confirm that revaccination significantly enhances the humoral immune response, promoting the formation of durable, specific antibodies against the equine rhinopneumonitis virus.

Thus, revaccination carried out 90 days after the primary immunization is a critical factor for maintaining antibody levels at consistently high titers.

Conclusion. As a result of the conducted studies, the equine rhinopneumonitis virus strain "AK-2011" was isolated and adapted for production, demonstrating stable reproductive and antigenic properties. The optimal conditions for its cultivation on the TT cell line were established, enabling the generation of virus-containing biomass with a titer of approximately 10^5 $TCID_{50}$. These findings provided the basis for the development of the cell-culture-based dry viral vaccine against equine rhinopneumonitis, "RinoVac."

A comprehensive evaluation was conducted on experimental batches of the equine rhinopneumonitis vaccine, encompassing assessments of physicochemical quality, sterility, biological activity, safety, and immunogenic potential. The vaccine demonstrated an excellent safety profile, eliciting no adverse reactions in white mice or rabbits and being well tolerated by horses following intramuscular administration at a dose of 2 cm^3.

The immunobiological activity of the vaccine was confirmed through controlled studies in model organisms. In rabbits, a robust and specific humoral immune response was observed within 7–14 days post-vaccination, persisting at high levels up to 30 days. Revaccination at 90 days further enhanced and stabilized antibody titers, indicating effective induction of long-term immunological memory. The vaccine exhibited a protective efficacy of 80%, reflecting substantial prophylactic potential against equine rhinopneumonitis.

In horses under field conditions, administration of the "RinoVac" vaccine led to the development of specific antibodies reaching titers of up to 1:32, in accordance with regulatory requirements for antiviral vaccines of this category. No adverse clinical signs were recorded, confirming both the safety and immunological effectiveness of the vaccine under field conditions.

Thus, the developed equine rhinopneumonitis viral vaccine, "RinoVac":

-exhibits high immunogenicity;

-induces a durable and specific immune response;

-demonstrates strong protective efficacy;

-is safe for animals;

-complies with veterinary-sanitary and biological regulatory standards.

The results obtained support the recommendation of "RinoVac" for use in horse-breeding farms that are affected by or at risk of rhinopneumonitis, for the purpose of prophylaxis, outbreak prevention, and reduction of epizootic losses.

REFERENCES

1 OIE (World Organization for Animal Health). Equine rhinopneumonitis (infection with equid herpesvirus-1 and -4) // OIE Terrestrial Manual. – Paris, 2017. – Гл. 2.5.9.

2 Oladunni F.S., Horohov D.W., Chambers T.M. EHV-1: A Constant Threat to the Horse Industry // Frontiers in Microbiology. – 2019. – Vol. 10. – P. 2668.

3 OIE. Equine rhinopneumonitis (infection with equid herpesvirus-1 and -4) // OIE Terrestrial Manual. – 2017. – P. 894–903.

4 Юров К.П. Этиология, патогенез альфа- и гаммагерпесвирусных инфекций у лошадей // Материалы VI Междунар. ветеринарного конгресса. – 2016. – С. 312–314.

5 Autorino G.L., Corradi V., Frontoso R. и др. Gestione di un focolaio neurologico da Equine herpesvirus 1 (EHV-1) // Workshop Nazionale di Virologia Veterinaria. – Roma, 2014. – P. 15.

6 Negussie H., Gizaw D., Tessema T.S., Nauwynck H.J. Equine Herpesvirus-1 Myeloencephalopathy // Transboundary and Emerging Diseases. – 2017. – Vol. 64. – P. 389–397.

7 Khusro A., Aarti C., Rivas-Caceres R.R., Barbabosa-Pliego A. Equine herpesvirus-1 infection in horses: pathogenicity and prevention // Journal of Equine Veterinary Science. – 2020. – Vol. 87. – P. 102923.

8 Oladunni F.S., Horohov D.W., Chambers T.M. EHV-1: A Constant Threat to the Horse Industry // Frontiers in Microbiology. – 2019. – Vol. 10. – P. 2668. (повтор по требованию)

9 Castro E.R., Arbiza J. Detection and genotyping of equid herpesvirus 1 in Uruguay // Revue Scientifique et Technique (OIE). – 2017. – Vol. 36(3). – P. 799–806.

10 Yildirim Y., Yilmaz V., Kirmizigul A.H. Equine herpesvirus 1 and 4 infections in horses and donkeys // Iranian Journal of Veterinary Research. – 2015. – Vol. 16(4). – P. 341–344.

11 Azab W., Bedair S., Abdelgawad A. и др. Detection of equid herpesviruses in Arabian horses // Veterinary Medicine and Science. – 2019. – Vol. 5(3). – P. 361–371.

12 Bryant N.A., Wilkie G.S., Russell C.A. и др. Genetic diversity of EHV-1 isolated from outbreaks // Transboundary and Emerging Diseases. – 2018. – Vol. 65. – P. 817–832.

13 Koelle D.M., Norberg P., Fitzgibbon M.P. и др. Worldwide circulation of HSV-2 × HSV-1 recombinant strains // Scientific Reports. – 2017. – Vol. 7. – P. 44084.

14 Stasiak K., Dunowska M., Rola J. Prevalence of equid herpesviruses in Polish horses // Virology Journal. – 2018. – Vol. 15. – P. 106.

15 Damiani A.M., de Vries M., Reimers G. и др. EHV-1 abortion outbreak // Veterinary Microbiology. – 2014. – Vol. 172(3–4). – P. 555–562.

16 Dunowska M., Gopakumar G., Perrott M.R. и др. Investigation of EHV-1 in New Zealand // Veterinary Microbiology. – 2015. – Vol. 176(3–4). – P. 219–228.

17 Stasiak K., Dunowska M., Hills S.F., Rola J. Genetic characterization of EHV-1 from abortion cases // Archives of Virology. – 2017. – Vol. 162. – P. 2329–2335.

18 Nugent J., Birch-Machin L., Smith K.C. и др. DNA polymerase mutation associated with neuropathogenic EHV-1 // Journal of Virology. – 2006. – Vol. 80. – P. 4047–4060.

19 Matczuk A.K., Skarbek M., Jackulak N.A., Bażanów B.A. Molecular characterization of EHV-1 in Poland // Virology Journal. – 2018. – Vol. 15. – P. 186.

20 Garvey M., Lyons R., Hector R.D. и др. Molecular characterisation of EHV-1 in Ireland // Pathogens. – 2019. – Vol. 8(1). – P. 7.

21 Bryant N.A., Wilkie G.S., Russell C.A., Compston L., Grafham D., Clissold L., McLay K., Medcalf L., Newton R., Davison A.J., Elton D.M.Genetic diversity of equine herpesvirus 1 isolated from neurological, abortigenic and respiratory disease outbreaks // Transboundary and Emerging Diseases. – 2018. – Vol. 65. – P. 817–832.

ТҮЙІН

Жүргізілген зерттеулер нәтижесінде ринопневмонияға қарсы «RinoVac» құрғақ вирусвакцинасы әзірленіп, эксперименттік тұрғыда апробациядан өткізілді. Вакцина вирусвакцинаның өндіруші штаммы «АК-2011» негізінде ТТ жасуша мәдениетінің моноқабатында алынды. Иммуногенділікті (КБР, ИФТ) және протективтік белсенділікті зерттеу, сондай-ақ ақ тышқандарда қауіпсіздігін бағалауды қамтитын кешенді биологиялық сынақтар жүргізілді. Зертханалық жағдайда вируссыйымдылық биомассасын алу режимдері жетілдірілді, енгізу дозалары мен тәсілдері айқындалды, сондай-ақ иммуногенділік бағаланды – барлық осы зерттеулер модельдік қояндарда (тері астына және бұлшықет ішіне 0,5; 1,0; 2,0 см³ көлемдерінде) жүргізілді. Қосымша, ревакцинацияның иммундық жауаптың қарқындылығы мен тұрақтылығына әсері анықталды.Вакцинацияланған жануарларда бақылау тобымен салыстырғанда арнайы антиденелер титрінің едәуір жоғарылауы және айқын қорғаныс реакциясының қалыптасуы тіркелді. Қояндарда антидене титрі вакцинациядан кейін 7-тәулікте 1:300 құрап, 14-тәулікте 1:500 деңгейіне дейін артты және 30-тәулікте 1:400 деңгейінде сақталды; қорғаныс тиімділігі 80% болды (бақылау тобында – 20%). Бастапқы иммундаудан кейін 90 күн өткен соң жүргізілген ревакцинация гуморальды иммундық жауапты қосымша күшейтті: барлық сұйылтуларда (1:50–1:400) ИФТ әдісі бойынша оптикалық тығыздық көрсеткіштері ревакцинацияланған жануарларда бақылаумен салыстырғанда айтарлықтай жоғары болды (мысалы, 1:50 сұйылтуында – 1,89 қарсы 0,73), бұл қалыптасқан иммунитеттің тұрақты және ұзақ мерзімді екенін көрсетті.

РЕЗЮМЕ

В ходе проведенных исследований разработана и экспериментально апробирована сухая вирусвакцина против ринопневмонии лошадей «RinoVac», полученная на монослойной культуре клеток ТТ с использованием штамма «АК-2011». Проведен комплекс биологических испытаний, включающий оценку иммуногенности (РСК, ИФА) и протективной активности на модельных животных, а также проверку безопасности на белых мышах. В лабораторных условиях отработаны режимы получения вируссодержащей биомассы, определены оптимальные дозы и пути введения, а также оценена иммуногенность – все исследования выполнены на модельных кроликах

(подкожно и внутримышечно, 0,5; 1,0; 2,0 см³). Дополнительно оценено влияние ревакцинации на выраженность и устойчивость иммунного ответа. У вакцинированных животных зафиксировано существенное повышение титра специфических антител и формирование выраженной защитной реакции по сравнению с контролем. У кроликов титр антител достигал 1:300 на 7-е сутки, повышался до 1:500 на
14-е сутки и удерживался на уровне 1:400 к 30-м суткам; показатель защитной эффективности составлял 80% (против 20% в контроле). Ревакцинация, выполненная через 90 дней после первичной иммунизации, приводила к дополнительному усилению гуморального ответа: во всех разведениях (1:50–1:400) значения оптической плотности по ИФА у ревакцинированных животных были заметно выше контрольных (например, при 1:50 – 1,89 против 0,73), что указывает на формирование более устойчивого и длительного иммунитет