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Experimental Assessment Of An Inactivated Vaccine Against *Histoplasma Capsulatum Var. Farciminosum* In Horses

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Abstract **-** Epizootic lymphangitis is a chronic infectious disease of equids, caused by the dimorphic fungus *Histoplasma capsulatum var. farciminosum*, and characterized by inflammation of the lymphatic vessels and formation of purulent lesions. In Kazakhstan, the disease remains endemic due to geographic proximity and active trade with neighboring countries. The objective of this study was to evaluate the safety, immunogenicity, and stability of an experimental inactivated vaccine developed using *H. capsulatum var.. farciminosum* strain "8ZH". Two fungal strains ("8ZH" and control "T") were cultivated, and the biological properties, virulence, and antigenic activity were assessed. The optimal inactivation conditions using 0.05% beta-propiolactone and the most effective adjuvant formulation (10% Montanide Gel 01) were determined. The resulting formulation induced detectable antibodies by day 14 post-vaccination in foals, with immunity lasting at least 12 months. Stability was confirmed under recommended storage conditions. This work represents a significant step toward developing a preventive measure against epizootic lymphangitis in horses in Central Asia.
Keywords: Epizootic lymphangitis, *H.capsulatum var. farciminosum*, horses, inactivated vaccine, beta-propiolactone, Montanide Gel 01

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1. Introduction

Epizootic lymphangitis is a chronic infectious disease of equids that affects the lymphatic vessels of the skin and subcutaneous tissue, leading to the formation of purulent foci and ulcers [1], [2]. The causative agent is the dimorphic fungus *H.capsulatum var. farciminosum* [3], [4], and infected animals excrete large numbers of fungal cells in ulcer exudates, contaminating the environment [5], [6]. Transmission occurs via direct contact or contaminated grooming tools and harnesses [7].

In Kazakhstan, epizootic lymphangitis is reported almost annually. The country's geographic location and trade connections with Russia, China, and other Central Asian states facilitate disease spread [8]. Therefore, development of a vaccine against *H.capsulatum var. farciminosum* is essential for disease control. Demand for such a vaccine is high within Kazakhstan, and its production is a key strategy for epizootic lymphangitis prevention.

The aim of this study was to assess the immunogenicity and stability of an experimental inactivated vaccine developed using *H.capsulatum var. farciminosum* strain "8ZH" in horses.

2. Materials and Methods

2.1. Strains and Culture Conditions

Two fungal strains were used: *H.capsulatum var. farciminosum* "8ZH" for vaccine development and strain "T" as a control. Each strain was cultivated in liquid culture to produce 1.5 liters of fungal suspension. Biological and virulence properties were evaluated.

2.2. Inactivation and Formulation

Inactivation was performed using 0.05% beta-propiolactone for 10 hours at 20–25 °C. Various adjuvants were tested, and 10% Montanide Gel 01 (Seppic, France) was selected based on superior immunostimulatory performance compared to 15% and 20% formulations.

2.3. Vaccine Composition and Immunization Protocol

The final vaccine formulation consisted of 360 mL of fungal suspension and 40 mL of 10% Montanide Gel 01. Experimental vaccination was carried out on foals. Immunogenicity was monitored using complement fixation testing (CFT), and antibody titers were assessed at different time points.

3. Results

Among the two formulations, the "8ZH" strain-based vaccine showed the highest antigenic activity and was selected for further evaluation. Successful cultivation was achieved in both suspension culture and flask systems.

Pathogenicity testing of strain "T" was conducted in foals to determine infectious dose and route of administration. Resulting positive sera were used as a standard for serological evaluation of vaccine responses.

By day 14 post-immunization with the selected formulation (10% Montanide Gel 01), specific antibodies were detected. The protective immune response persisted for at least 12 months.

Stability testing confirmed that the vaccine maintained efficacy under recommended storage conditions, showing no loss of antigenic or immunogenic properties.

4. Conclusion

An inactivated vaccine based on *H.capsulatum var. farciminosum* strain "8ZH" was successfully developed. The optimal inactivation protocol and adjuvant combination provided a strong and long-lasting immune response in foals. This vaccine holds promise as a preventive tool for controlling epizootic lymphangitis in Kazakhstan and neighboring regions.

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