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Evaluation of manufacturing specification of antifungal vaccines

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Abstract

In this article are presented the algorithm of manufacture vaccines against dermatomycoses carnivores based on original research stages manufacture of vaccines. Shown biotechnological aspects of selection isolates the study of immunological properties of strains of dermatophytes, ways to optimize cultivation conditions and methods of working hours of raw material.

The basic stages of biotechnology development inactivated vaccines against dermatomycoses dogs and cats, for example vaccines "Funhikanifel" inactivated associated against dermatomycoses dogs and cats. The authors have developed technological regulation manufacture of vaccines against dermatomycoses, allowing for high immunogenic properties and the harmlessness for immunization of dogs, cats and laboratory animals. In the article focuses on the selection of isolates of dermatophytes on morphological features that will continue to be crucial in the production of highly effective vaccine.

Experimentally, the possibility of a vaccine using domestic raw materials, which has priority from the standpoint of national biosafety.

Key words: dermatomycosis of dogs and cats, *Trichopyton mentagrophytes* and *Microsporum canis,* vaccine, immune response.

Introduction

Dermatomycoses of pet have a high level of infectious pathology that activates as search the methods of obtaining vaccines and search approaches to the development universal vaccine production technologies against dermatomycoses [Nedosekov 2013].

Vaccination of dogs and cats against dermatomycoses (trichofitia, microsporia) allows you to control the incidence rate of these animals [Stetsyura 2005]. Recently, production of biologics against dermatomycoses animals car-

ried out in many countries by different technologies [Nedosekov 2013; Panin 2004].

However for all vaccines must be a technological regulation and evaluation of the finished product. It was necessary to conduct the analysis and synthesis of existing technologies and develop own regulation and considering this show experimentally biotechnological aspect that need to focus in the manufacture of vaccines against dermatomycoses dogs and cats [Nedosekov 2013; Martyniuk 2005].

The purpose our investigation were analysis of biotechnological aspects of the development of specific prevention dermatomycoses dogs and cats.

Develop. Studies carried out by stages of producing vaccine against dermatomycoses dogs and cats:

1) The selection and study of biological properties of selected isolates of dermatophytes for create vaccine;
2) Development of regulations producing vaccine;
3) Conducting pre-clinical and clinical trials developed vaccine.
4) Evaluation of the effectiveness of the developed drug on vectors animals.

Biotechnological aspects of the development of vaccines against dermatomycoses dogs and cats were considered by the example of vaccines "Funhikanifel" – an inactivated associated vaccine against dermatomycoses (trichophitia, microsporia) dogs and cats (TU 24.4-31112822-004:2005).

Isolation of dermatophytes were performed on Sabouraud agar, worth-agar (during 10–14 days).

The criterion for the selection of promising strains of dermatophytes was homogenic cultures without signs of dissociation, with the release of viable, stable and spore-forming plants that have immunogenicity (for vaccine strains) and virulence (for control strains).

As results our investigation we have been proposed regulation of production vaccine that included the following steps:

1) The selection and study of immunological properties of *T. mentagrophytes* and *M. canis*

This phase of work is the primary and fundamental, because it determines the effectiveness of the drug to be developed [Skrypnyk 2004].

After planting of selected samples from the animals it was estimed that strains of *T. mentagrophytes* were characterized by rapid growth in the 3rd day of cultivation, powdery texture, from light cream to beige of colors, sometimes white colonies. Microscopic examination of cultures as vaccine and epizootic strains observed several features that have been described previously [Nedosekov 2013].

Important biological properties of epizootic strains of dermatophytes is the ability to induce disease, so to estimation the pathogenicity of dermatophytes,

laboratory animals were infected by epizootic strains of *T. mentagrophytes* [Stetsyura 2005; Martyniuk 2005].

According to the study of biological properties of dermatophytes for these studies were selected strains *T. mentagrophytes* (Co, Th, 15, TM-48 K) and *M. canis* (№ № 11, 22, 39, 50, 65 K).

Selection of vaccine strains of T. mentagrophytes and M. canis proved that strains of Trichophyton mentagrophytes-15 and Microspore canis-22 had stable morphological and cultural characteristics, intense accumulation microconidiy and were technological. Using the method of selection of fast growing monocolony managed to increase the formation microconidia.

As a result, strains of Trichophyton mentagrophytes-15 and Microspore canis-22 were identified as being suitable for the manufacture of vaccines, followed by the study of their immunogenicity.

The selection control strains of dermatophytes. Immunogenicity of vaccines to control a prerequisite is the selection and study of the control strains. To increase the virulence of strains of dermatophytes were conducted passaging fungi in vivo (dogs and cats) with the following reisolation. As a result, strains were selected high virulent strain T. mentagrophytes TM-48 K and M. canis № 65 K (2.5 x 10^6 microconidia).

Due to the necessity of using inactivated components we performed the o*ptimization condition of inactivation of dermatophytes* (t- 58 0 C – 3 days).

Determined, test samples for the presence of live vaccine agents found that in all cases the growth of dermatophytes and any other microflora was observed. This indicated that the prepared samples were inactivated vaccine.

Testing the safety of the vaccine samples showed that after intramuscular application (3.0 cm^3 rabbits and 1.0 cm^3 guinea pigs), during 10–14 days post–vaccination complications in the area of administration was not observed. Thus, vaccine is full inactivated and safety.

2) Development of regulations making vaccine against dermatomycoses dogs and cats. Biotechnological production of vaccine regulation "Funhikanifel" consists of 8 stages, but there are some critical control points that are crucial:

A) Determination of safety and immunizing dose of vaccine. After the selection of vaccine strains of dermatophytes strains were determined immunizing dose of *T. mentagrophytes-15* and *M. canis-22* (ratio 1:2) in rabbits and guinea pigs by application a dose of vaccine samples 1.0 cm^3 containing microconidia 3.0–24.0 x $10^6/cm^3$, twice, intramuscularly. Protective properties of the vaccine samples were determined by cutaneous infection virulent strains of *T. mentagrophytes TM-48 K* and *M. 65 K canis.*

Determined that immunizing dose (6.0–24.0 × $10^6/cm^3$ microconidia) provides protective activity of vaccines for laboratory animals.

Test experimental vaccine series (13.0–24.0 x 10^6/cm^3 microconidia) of susceptible animals, showed that the vaccine dose of 0.5 and 1.0 cm^3 (13.4–16.8 x 10^6/cm^3 microconidia) did not result in dogs and cats post–vaccination complications and local reactions at the site of administration. At higher dosages observed swelling, pain and abscesses.

Two–time injection of vaccine formed protection dogs and cats from virulent cultures of challenge *T. mentagrophytes TM-48 K, M. 65 K canis.* Unvaccinated animals (control) sick with typical clinical signs trichofitia and microsporia.

It is established that the application of the vaccine in a dose of 0.5 and 1.0 cm^3 (13.4–16.8 x 10^6/cm^3) dogs and cats were recorded safety and potency of vaccine.

B) Determination of the duration and intensity of immunity in the vaccine "Funhikanifel". Criterions of immunity in the presence of grafted animal vaccines areagainst dermatomycoses resistance of animals to cutaneous infection.

To determine the duration of immunity in the vaccine "Funhikanifel" performed infection immunized rabbits, guinea pigs, dogs and cats strains of T. mentagrophytesTM-48 and Microsporum canis K 65 K, on 30 days, 6 and 12 months after vaccination. Subsequently, the disease was observed animals, while all control animals during infection by challenge strains were ill with sing of dermatomycoses. Immunity in dogs and cats vaccinated the vaccine "Funhikanifel" lasts at least 12 months.

Studies of antibody level showed that titers in vaccinated animals vaccine "Funhikanifel" compared with subtitles experimentally infected with challenge strains, to both antigens were accurate ($p < 0.05$).

3) Conducting pre–clinical and clinical trials of vaccine "Funhikanifel".

The results of clinical trials of vaccine "Funhikanifel" dermatomycoses for dogs and cats found that after the first vaccination recovered 27.0% (n = 17) dogs and 34.1% (n = 44) cats sick from dermatomycoses, 7–14 days after the second vaccination – 84.1% (n = 53) and 93.0% (n = 120).

After double vaccination recovered 96.8% of the animals, only 3.2% (n = 6) animals sick from microspores, used vaccine three times. Note that the recovery period was dependent on the presence of both primary and secondary dermatological diseases (infectious, parasitic, non-contagious) and natural resistance of the body. During the observation period in animals immunized for medical reasons, relapses dermatomycoses not registered as a prophylactic – a disease not been reported.

4) Evaluation of the effectiveness of the developed product on vector animals.

The final stage of the research is an independent assessment of the drug, which is the commission interagency vaccine trials on dogs and cats. Deter-

mined that the vaccine meets the parameters and requirements documentation, and vaccine is safety and immunogenic and susceptible to laboratory animals. The vaccine was registered in Ukraine № 1392-04-0197-05, implemented in the production and practice of veterinary medicine.

Conclusions

Shown the basic aspects of biotechnology development inactivated vaccines against dermatomycoses dogs and cats, for example vaccines "Funhikanifel" inactivated associated to dermatomycoses (trichofitia, microsporia) dogs and cats. Developed technological regulation manufacture of vaccines against dermatomycoses dogs and cats, including the selection and study of immunological properties of *T. mentagrophytes* and *M. sanis*, clinical vaccine trials, evaluating the effectiveness of the developed drug on susceptible animals. It is possible to provide high immunogenic properties and the safety for immunization of dogs, cats and laboratory animals.

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