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DEVELOPMENT OF TECHNOLOGICAL PRODUCTION REGULATIONS OF VACCINES AGAINST DERMATOMYCOSES DOGS AND CATS

Shown the algorithm of production vaccines against dermatomycoses carnivores based on 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 «Fungicanifel» 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 and cultural- morphological features that will impact to effectiveness vaccine. Experimentally, the possibility of a vaccine using domestic raw materials, which has priority from the standpoint of national biosafety.

Recently, the question is highly relevant prevention, diagnosis and treatment of mycoses is the focus of WHO and FAO. Dermatomycoses animals found in different countries, and search the methods of obtaining high immunity for vaccines against these infections are rather topical issue [1].

Development of A.H. Sarkisov et al. (1967) first live vaccine against of cattle trichophitia allow to eliminate trichophitia animals in many regions. Dermatomycoses different species of animals have a high level of infection of animals that activates as search the methods of obtaining vaccines and search approaches to the development universal vaccine production technologies against dermatomycoses [4].

The rationale for relevance development of these drugs is that vaccines as a means of causal treatment dermatomycoses animals contributes to the rapid elimination causative agent from the body of animals and formation of stress immunity as a prophylactic purpose – to prevent the occurrence of infection and environmental contamination fungi [3]. Prophylactic vaccination of dogs and cats against dermatomycoses (trichophitia, microsporia) allows you to control the incidence rate of these animals.

Production of biologics against dermatomycoses animals carried out in many countries by different technologies [1, 3].

However for all vaccines must be a technological regulation and evaluation of the finished product. Therefore, on this basis, it was necessary to conduct the analysis and synthesis of existing technologies and develop own scheme and considering this show experimentally biotechnological aspect that need to focus in the manufacture of vaccines against dermatomycoses dogs and cats.

The **purpose** of the study was analysis of biotechnological aspects of the development of specific prevention dermatomycoses dogs and cats.

MATERIALS AND METHODS

Studies carried out by stages:

1) study, the selection and study of immunological properties of selected isolates of dermatophytes for making vaccines against dermatomycoses dogs and cats;

2) development of regulations making vaccine against dermatomycoses dogs and cats;

3) conducting pre-clinical and clinical trials developed vaccine against dermatomycoses dogs and cats.

4) evaluation of the effectiveness of the developed drug on susceptible animals.

Biotechnological aspects of the development of vaccines against dermatomycoses dogs and cats were considered by the example of vaccines «Fungicanifel» – an inactivated associated against dermatomycoses (tryhofity, microspore) dogs and cats (ТУ 24.4-31112822-004:2005).

Isolation of dermatophytes were performed pathological material and cultured on agar Sabouraud broth, worth-agar (either 27 ± 1°C for 10–14 days). Monocolony strains of dermatophytes obtained by serial dilutions of suspensions of fungi and replanting touch.

Identification of crop pathogens tryhofity and microspores was carried out morphologically-cultural characteristics, the results of microscopic examination of cultures and by determinants of fungi [5, 7].

The criterion for the selection of promising strains of dermatophytes was to obtain homogeneous cultures with no 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 the vaccine strains were selected *Trichophyton (T.) mentagrophytes 15* and *Microsporum (M.) canis 22* (isolated from patients trichophitia microspores and cats, respectively), control – *Trichophyton mentagrophytes TM-48 K* and *65 K Microspore canis* (isolated from patients trichophitia and microspores dog and cat, respectively).

Definition of immunogenic activity of the vaccine conducted in the laboratory (rabbits, guinea pigs) and pets (dogs, cats) animals by methods approved State Scientific Control Institute of Biotechnology and strains of microorganisms.

The intensity of immunity in laboratory and susceptible animals in the application of the vaccine was determined after 30 days, 6 and 12 months after vaccination.

With medical treatment and preventive purposes vaccine was injected 63 dogs and 129 cats of all ages, breeds and for prevention – 7 and 18, respectively [5].

RESULTS

We have been proposed scheme 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. Due to this special treatment is given morpho-culture properties of the pathogen. After planting of selected samples from the animals it was found that strains of *T. mentagrophytes* were characterized by rapid growth in the 2-3rd day of cultivation, powdery texture, sometimes velvety, from light cream to beige, yellow, sometimes white colonies with red-brown reverse. Microscopic examination of cultures as vaccine and epizootic strains observed several features that have been described previously [5].

Important biological properties of epizootic strains of dermatophytes is the ability to specify a distinct disease, so to assess the pathogen city of dermatophytes laboratory animals infected by coetaneous epizootic strains of *T. mentagrophytes* [2, 5].

According to the study of morphological, cultural and 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 15 strains of *Trichophyton mentagrophytes* and *Microspore canis* 22 had stable morphological and cultural characteristics, intense accumulation microconidii and were technological. Using the method of selection of fast growing and sporonosy mono colony managed to increase the formation microconidii. As a result, 15 strains of *Trichophyton mentagrophytes* 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 through the body of dogs and cats with the following allocation of net culture. As a result, strains were selected very virulent *T. mentagrophytes TM-48 K* and *M. canis № 65 K* of the infecting dose – $2,5\times10^6$ microconidii.

Due to the necessity of using inactivated components we performed the following:

Optimization method of inactivation of dermatophytes. Dermatomifites used to inactivate universal termomethod (at a temperature of 58 °C – 3 days).

As a result, 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 harmlessness of the vaccine samples showed that after intramuscular administration (3,0 cm^3 rabbits and 1,0 cm^3 guinea pigs) vaccine in the thigh area, within 10–14 days post-vaccination complications in the area of administration was not observed.

Thus, at this stage investigation not only chosen if inactivation, but also show harmful to laboratory animals.

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

a) determination of harmlessness and immunizing dose of vaccine. After the selection of vaccine strains of dermatophytes strains were determined immunizing dose of *T. 15 mentagrophytes* and *M. canis 22* (ratio 1:2) in rabbits and guinea pigs by administering a dose of vaccine samples 1,0 cm^3 containing microconidii $3,0–24,0\times10^6/cm^3$, twice, intramuscularly in the thigh area. Protective properties of the vaccine samples were determined by cutaneous infection virulent strains of *T. mentagrophytes TM-48 K* and *M. 65 K canis.*

Recognized that immunizing dose ($6,0–24,0\times10^6$ microconidii/cm^3) provides protective activity of vaccines for laboratory animals.

Test experimental vaccine series ($13,0–24,0\times10^6$ microconidii/cm^3) of susceptible animals (dogs, cats), showed that the vaccine dose of 0,5 and 1,0 cm^3 ($13,4–16,8\times10^6$ microconidii) 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 vaccine shaped stressful immunity and protection dogs and cats from virulent cultures of challenge *T. mentagrophytes TM–48 K, M. 65 K canis.* Checklists (unvaccinated) animals sick with typical clinical signs trihofitii and microspores.

It is established that the application of the vaccine in a dose of 0,5 and 1,0 cm^3 of the number of microconidii $13,4–16,8\times10^6/cm^3$ dogs and cats were recorded harmless and protective activity of vaccines;

b) determination of the duration and intensity of immunity in the vaccine «Fungicanifel». 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 «Fungicanifel» performed infection immunized rabbits, guinea pigs, dogs and cats strains of T. *TM-48 mentagrophytes* and *Microsporum canis K 65 K*, 30 days, 6 and 12 months after vaccination.

Subsequently, the disease was observed animals, while all control animals during infection by strains *Trichophyton mentagrophytes TM-48 K* and *65 K Microsporum canis* ill with tryhofity and microspores, the subsequent release retrokultur. Immunity in dogs and cats vaccinated the vaccine «Fungicanifel» lasts at least 12 months.

Studies of humeral immunity showed that antibody titers in vaccinated animals vaccine «Fungicanifel» compared with subtitles experimentally infected with strains of *T. 15 mentagrophytes* and *M. canis 22* to both antigens were suspicion (P<0,05).

3. Conducting pre-clinical and clinical trials of vaccine «Fungicanifel».

The results of clinical trials of vaccine «Fungicanifel» dermatomycoses for dogs and cats found that after the first vaccination recovered 27,0 % (n = 17) dogs and 34,1 % (n = 44) cats suffering from ringworm, 7–14 days after the second vaccination – 84,1 % (n = 53) and 93,0 % (n = 120).

After double vaccinotherapy recovered 96,8 % of the animals, only 3,2 % (n = 6) animals suffering 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. Found that the drug meets the parameters and requirements documentation, and vaccine is harmless and immunogenic and susceptible to laboratory animals. The vaccine was registered in Ukraine № 1392-04-0197-05 on November 23, 2005, implemented in the production and practice of veterinary medicine («Alteks» JSC NVAP «Novohaleschynska biofactory»).

CONCLUSIONS

Shown the basic aspects of biotechnology development inactivated vaccines against dermatomycoses dogs and cats, for example vaccines «Fungicanifel» inactivated associated to dermatomycoses (trichophitia, microsporia) dogs and cats. The authors have developed technological regulation manufacture of vaccines against dermatomycoses dogs and cats, including the selection and study of immunological properties of *T. mentagrophytes* and *M. canis*, clinical vaccine trials, evaluating the effectiveness of the developed drug on susceptible animals. It is possible to provide high immunogenic properties and the harmlessness for immunization of dogs, cats and laboratory animals.

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Розроблення технологічного регламенту виготовлення вакцин проти дерматомікозів собак і котів. *В.В. Недосєков, О.Г. Мартинюк, Л.Г. Стецюра, В.В. Сливко*

Представлено алгоритм виготовлення вакцин проти дерматомікозів м'ясоїдних на основі дослідження етапів їх створення. Показані біотехнологічні аспекти підбору ізолятів, вивчення імунобіологічних властивостей штамів дерматофітів, шляхи оптимізації умов культивування і способів напрацювання сировини.

Проаналізовано основні етапи біотехнології розроблення інактивованих вакцин проти дерматомікозів собак і котів на прикладі вакцини «Фунгіканіфел». Експериментально показана принципова можливість отримання вакцин з використанням вітчизняної сировини, що має пріоритетне значення з позиції національної біобезпеки.

Разработка технологического регламента изготовления вакцин против дерматомикозов собак и кошек. *В.В. Недосеков, А.Г. Мартынюк, Л.Г. Стецюра, В.В. Сливко*

Представлен алгоритм изготовления вакцин против дерматомикозов плотоядных на основе исследования этапов их создания. Показаны биотехнологические аспекты подбора изолятов, изучение иммунобиологических свойств штаммов дерматофитов, пути оптимизации условий культивирования и способов наработки сырья.

Проанализированы основные этапы биотехнологии разработки инактивированных вакцин против дерматомикозов собак и кошек на примере вакцины «Фунгиканифел». Экспериментально показана принципиальная возможность получения вакцин с использованием отечественного сырья, что имеет приоритетное значение с позиции национальной биобезопасности. ○