

ASPECTS REGARDING IMMUNE PROPHYLAXY IN ENZOOTIC BOVINE RINGWORM

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ABSTRACT - The aim of this study was the isolation and identification of *Trichophyton* strains and species on various selective culture media (Sabouraud dextrose agar and Dermatophyte Test Medium - DTM); morphological and cultural characterization of *Trichophyton* isolated strains; calves immune response consecutive to the administration of the Trichoben vaccine. The experiment was carried out during October 2008 – February 2009 on a group of 20 animals: 15 animals with typical ringworm lesions and five healthy control calves. For the isolation and the morpho-cultural characterization of *Trichophyton*, samples consisting of crust, squames and hairs were collected and examined by using enlightening solutions and were inseminated on special culture medium for dermatophytes. Ringworm prevention and control were a difficult problem. Various therapeutic schedules gave inconstant results; thus, we took into account the implementation of systemic vaccination programs. We have used the Trichoben vaccine prepared with a non-virulent strain of *Trichophyton verrucosum* (min. 2.5×10^6 CFU), which was

lyophilized. Preventively, the dose was of 2.5 ml in three week –three month old calves, while for older than three month calves, it was of 5 ml, repeated 10-14 days later for both age categories. For the therapeutic purpose, doses were double. Good results were observed since the first administration, while after 28 days, healed placards and lesion diminution, crust thinning and detaching were noticed. Vaccination efficiency for therapeutic purpose was of approximately 95%.

Key words: ringworm, calves, vaccination, prophylaxy, Trichoben

Rezumat- Aspecte imunoprofilactice în tricofitia enzootică bovină. În acest experiment s-au urmărit: izolarea și identificarea de tulpini, respectiv de specii de *Trichophyton* pe diferite medii selective de cultură (Sabouraud dextroză, DTM); caracterizarea morfologică și culturală a tulpinilor de *Trichophyton* izolate din focare; stabilirea capacității imunizante a vaccinului Trichoben, prin administrare la tineret bovin în condiții de fermă și

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urmărirea evoluției clinice a placardelor tricoftice după administrarea vaccinului. Experimentul a fost realizat în perioada octombrie 2008-februarie 2009, pe un lot format din 20 de animale: 15 au prezentat leziuni tipice de tricoftiție, iar un număr de cinci au fost martori clinic sănătoși. Pentru izolarea și caracterizarea morfo-culturală a dermatofizilor s-au recoltat probe de cruste, scuame și fire de păr; probele au fost apoi examinate direct, utilizând soluții clarificatoare și au fost însămânțate pe medii speciale pentru dermatofizi. Prevenirea și combaterea tricoftiției în ferme este o problemă dificilă; diferitele formule terapeutice aplicate au dat rezultate inconstante; astfel, s-a luat în considerare implementarea unor programe sistematice de vaccinare. S-a utilizat vaccinul Trichoben, preparat cu o tulpină avirulentă de *Trichophyton verrucosum* (min. $2,5 \times 10^6$ CFU), liofilizat, administrat pe cale i.m.. În scop preventiv, la vițeii în vârstă de trei săptămâni - trei luni, vaccinul s-a inoculat în doză de 2,5 ml, iar la cei mai mari de trei luni, în doză de 5 ml, cu rapel la 10-14 zile, pentru ambele categorii de vârstă. Terapeutic, doza a fost dublată. Rezultate satisfăcătoare s-au observat încă după prima administrare, iar la 28 zile de la vaccinare s-a observat o tendință de vindecare centripetă a placardelor, cu micșorarea în dimensiune a leziunilor, subțierea și desprinderea crustelor. Eficiența vaccinării în scop terapeutic a fost de aproximativ 95%.

Cuvinte cheie: tricoftiție, viței, vaccinare, profilaxie, Trichoben

INTRODUCTION

Ringworm is a dermatophytosis, a contagious disease with enzootic transmission or sporadic evolution, spread in all the continents in the animals bred under improper hygiene conditions and starving, at which

some stress factors added (Haab *et al.*, 1994). *T. verrucosum* (*T. album*, *T. ochraceum*, *T. discoides*) is the main cause of bovine ringworm in 99.1% of cases and is extremely resistant, surviving 6-8 years in the environment (Coman and Mares, 2000).

Bovines are very receptive to the ringworm infection, while after the disease treatment, they do not have anymore a solid immunity (Sarkisov, 1987). Therefore, the specialists' care was to stimulate the active immunization of bovines, using inactivated vaccines at the beginning, followed by living vaccines (Mitroiu, 1976; Wawrzkiwicz and Wawrzkiwicz, 1992).

The immunological investigations during the last years have brought important contributions to the knowledge of immunogenicity in *Trichophyton* strains, more vaccines being produced for prevention and cure (Lund *et al.*, 2001; Rybnikar *et al.*, 1991). Vaccines proved to be immunogenic, able to induce a satisfactory immune response (Kocik, 1982).

The goal of the investigations were isolating and identifying *Trichophyton* strains, respectively, species on different culture media (Sabouraud dextrose agar and Dermatophyte Test Medium - DTM); morphological and cultural characterization of *Trichophyton* strains isolated from different objects of a natural focus; establishing the immunization capacity of the Trichoben vaccine by its

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administration in calves at farms and following the clinical evolution of ringworm crust after the vaccine was administered.

MATERIALS AND METHODS

Modern therapy involves a diagnosis in the laboratory for this dermatophytosis. Therefore, we have used more methods of paraclinical examination:

Microscopic examination of pathological samples (crust, squame and hair sampling from all the animals with clinical ringworm lesions);

Smear examination after staining (Giemsa Method and Toluidine Blue);

Sample cultures on specific mediums (Sabouraud dextrose agar and Dermatophyte Test Medium - DTM).

Clinical examination was carried out in a cattle farm, where, in the last years, cases of enzootic ringworm were found with a variable incidence depending on season and management conditions, for verifying the preventive and therapeutic effect of the Trichoben vaccine: a group of 15 calves with ringworm placards, localized in the periorcular, periauricular and in the neck area and a group of five clinically healthy calves.

Characteristics: the vaccine was made of a *Trichophyton verrucosum* strain, containing minimum 2.5×10^6 structural elements, spread in a lyophilised medium.

Doses for the prophylactic administration: two doses of 2.5 ml diluted vaccine in week –three month old calves, administered every 10-14 days and two doses of 5 ml in more than three month old calves, administered every 10-14 days. All the animals living together were vaccinated. 1-2 month old and

newly bought animals were vaccinated in the same way, because *Trichophyton verrucosum* is extremely resistant, remaining in the environment for 6-8 years.

The doses for the therapeutic administration were doubled, compared to those used for the prophylactic vaccination. Highly affected animals by ringworm and cachectic animals may be vaccinated for a third time, after 2-4 weeks since the previous administration.

RESULTS AND DISCUSSION

At the examination with immersion lens of Giemsa-stained smears, we have noticed thin hyphae with abundant and scattered microconidia and rare, round-shaped, segmented macroconidia (*Figure 1*).

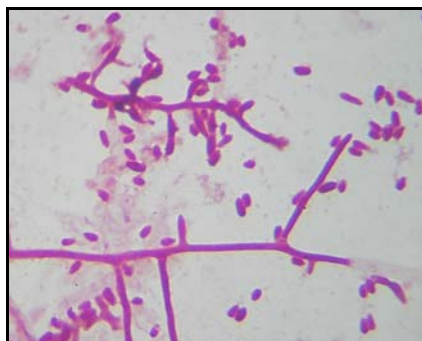


Figure 1 - *Trichopyton* hyphae and spores, isolated from lesions, Giemsa-stained

Bacteriological examination. On all culture mediums (Sabouraud dextrose agar and Dermatophyte Test Medium - DTM), pure cultures of colonies developed. 10 days after the insemination, the colonies had a 10 mm diameter with irregular edges and fleecy whitish surface (*Figures 2, 3*).



Figure 2 - Trichophyton colonies Sabouraud dextrose agar medium, front



Figure 3 - Trichophyton colonies Sabouraud dextrose agar medium, back

For being administered, the lyophilised vaccine was diluted in a diluent found in each vial. It was stirred for a better homogenization. For the prophylactic action, in 3 week-3 month old calves, two vaccine doses of 2.5 ml were administered, while in adult bovines, two doses of 5 ml were administered. The interval between vaccine administrations was of 10-14 days.

At the same category of animals, the doses for the therapeutic action were doubled (5 ml). In five animals highly affected by ringworm, the third vaccine dose was administered 2-4 weeks after the previous vaccination.

After the first inoculation of the vaccine, animals did not show any immediate post-vaccination reactions that show irritation or hypersensitivity reaction.

In animals with ringworm, after almost two weeks since the first vaccination, a ringworm placard with the diameter of 3-4 cm was noticed at the inoculation place, decreasing in 5-6 days (*Figures 5, 8*).

In the animals without ringworm lesions, no reaction was found at the inoculation place. A number of 2-3 placards, located periocularly and in the skin fold, were found only in two animals.

At the examination carried out every 14 days, no local reactions were found at the place of vaccine administration in any animal category. 28 days after vaccination, we have noticed the centripetal regression of lesions; then, they healed without any other treatment (*Figures 6, 9*). The management of vaccinated animals did not have any influence on the animal response to the vaccination.

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Figure 4 – Multiple face and periorbital lesions – Maturation-crust formation stage



Figure 7 – Lesions at the maturation stage with bacterial overinfection



Figure 5 – Lesions at the stage of crust formation, two weeks after the first vaccine administration



Figure 8 - Lesions at the stage of crust formation, two weeks after the administration of the first dose of vaccine



Figure 6 - Lesions at the stage of regression, 14 days after the administration of the second vaccine dose.



Figure 9 - Lesions at the stage of regression, 14 days after the administration of the second vaccine dose

CONCLUSIONS

Vaccination was done on a homogeneous group (calves and young cattle) clinically healthy but with ringworm lesions, using the Trichoben vaccine. Preventively, the administered dose was of 2.5 ml in calves aged between 3 weeks and 3 months and of 5 ml in calves aged over 3 months. For cure, a double dose was used.

In the vaccinated animals for curative effect, the beginning of immunization was demonstrated after two weeks since the end of recommended vaccination scheme; the lesions of vaccinated animals were less severe compared to non-vaccinated ones. The immunization period was not determined, but this experiment shows that by respecting the recommended scheme, cattle are being protected until re-vaccination. The vaccination efficiency was of 95%.

The preventive vaccination had a reduced efficiency. We have found in two animals 3-4 ringworm lesions located periorcularly and in the skin fold. However, lesions regressed and healed without any other treatment.

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