ASPECTS REGARDING IMMUNE PROPHYLAXY IN ENZOOTIC BOVINE RINGWORM

Daniela CĂLINA*, Gh. RĂPUNTEAN, N. FIȚ, G. NADĂȘ, Ramona OLARIU

University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca

Received October 2, 2009

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 x 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 tricofiția 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
Daniela CĂLINA ET AL.

urmărirea evoluției clinice a placardelor tricofitice 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 tricofiție, iar un număr de cinci au fost martori clinic sănătoși. Pentru izolarea și caracterizarea morfo-culturală a dermatofiților s-au recoltat probe de cruste, scuame și fire de păr; probele au fost apoi examine direct, utilizând soluții clarificatoare și au fost însămânțate pe medii speciale pentru dermatofiti. Prevenirea și combaterea tricofiției în ferme este o problemă dificilă; diferitele formule terapeutice aplicate au dat rezultate inconstante; astfel, s-a luat în considerare implementarea unor programe sistemice de vaccinare. S-a utilizat vaccinul Trichoben, preparat cu o tulipă avirulentă de Trichophyton verrucosum (min. 2,5 x 10⁶ CFU), liofilizat, administrat pe cale i.m.. În scop preventiv, la vitelii 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 mișcarea în dimensiune a leziunilor, subțierea și desprinderea crustelor. Eficiența vaccinării în scop terapeutic a fost de aproximativ 95%.

Cuvinte cheie: tricofiție, 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; Wawrzkiewicz and Wawrzkiewicz, 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
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 periocular, 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 $2.5 \times 10^6$ 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*).
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 hypersensivity 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.
Figure 4 – Multiple face and periorbital lesions – Maturation-crust formation stage

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

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

Figure 7 – Lesions at the maturation stage with bacterial overinfection

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

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 periocularly and in the skin fold. However, lesions regressed and healed without any other treatment.

REFERENCES

Kocik T., 1982 - Evaluation of the immunogenic properties of live and killed vaccines against tricophytosis of guinea pigs and calves, Pol Arch Weter, 23(3), 95-107
Mitroiu P., 1976 - Micoze şi micotoxicoze la animale (Mycoses and mycotoxicoses in animals), Edit. Ceres, Bucureşti
Rybnikar A., Vrzal V., Chumela J., 1991- The minimal effective dose of vaccine against trichophytosis in heifers, Bioveta, Ivanovice na Hane, Vet Med 36,(10), 593-597