CROSS-IMMUNITY IN CALVES AFTER VACCINATION AGAINST TRICHOPHYTOSIS

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Abstract

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Calves vaccinated against *Trichophyton verrucosum* were resistant to experimental infection with *T. verrucosum* strain and *T. mentagrophytes* strain. Immunization with a vaccine containing *T. mentagrophytes* produced reliable immunity against the homologous strain but failed to confer a satisfactory degree of protection against the heterologous dermatophyte *T. verrucosum*. Four out of 12 calves of this group reacted to challenge by development of mycotic changes persisting throughout the observation period. All non-vaccinated controls inoculated with one or the other of the two challenge cultures showed a similar clinical picture of trichophytosis.

Trichophyton mentagrophytes, Trichophyton verrucosum, immunization, challenge

Immune cross-reactions in dermatophytes have been studied both in vitro and in vivo with frequently conflicting results. The differences have been explained particularly by different preparation and composition of antigens and by the use of qualitatively and quantitatively different methods (Kaaman and Wasserman 1981). In in-vivo experiments the immune response was studied in animals and humans sensibilized, infected or immunized with dermatophytic antigens.

Foals injected i. m. with living *Trichophyton equinum* antigen developed immunity not only to the homologous species but also to *Trichophyton mentagrophytes* (Petrovich and Sarkisov 1981). However, from the observations reported by Petrovich (1985) it appears that horses that recovered from trichophytosis lacked cross-immunity to subsequent infection with the causative agent of microsporidia and vice versa. Woloszyn (1987) reported that cattle infected with *T. verrucosum* cross-reacted with trichophytin prepared from *T. mentagrophytes*. Wawrzkiewicz and Rzechowski (1983) who vaccinated guinea-pigs with living and inactivated vaccine against trichophytosis found immune cross-reactions between *T. verrucosum* and *T. mentagrophytes* strains. Petrovich et al. (1972) and Rasulev and Turdiev (1975), on the other hand, found no cross-immunity between *T. verrucosum* and *T. mentagrophytes* in calves.

The present study was designed to assess the development of post-vaccination immunity against homologous and heterologous dermatophyte species in 69 calves on the basis of challenge experiments.

Materials and Methods

The experimental animals were calves of the Bohemian Pied breed from herds without a history of trichophytosis, aged 4 to 6 weeks at the beginning of the experiments.

The following two vaccines were used:

(a) a commercial vaccine against bovine trichophytosis containing living *T. verrucosum* strain TV-M-310 attenuated by ultra-violet radiation (manufacturer: Bioveta, Ivanovice na Hané, Czechoslovakia) and

(b) an experimental living vaccine prepared from microconidia of a *T. mentagrophytes* strain.

Each vaccine was injected i. m. into the gluteal muscle in two doses of 2.5 ml. Each vaccination dose contained approximately 15 million CFU. The interval between vaccination and revaccination was 12 days. One month after revaccination the vaccinated calves and non-vaccinated controls were challenged by inoculation of a suspension of the challenge culture into a clipped and gently scarified 10 × 10 cm area of the right flank. In the first experiment the calves were challenged

with a virulent *T. verrucosum* strain (isolated from bovine skin lesions) at the rate of approximately 10 million conidia per animal. In the second experiment the calves were challenged with a *T. mentagrophytes* culture (cultured from a mycotic focus of a guinea-pig) at the rate of approximately 1 million conidia per animal. The size of the challenge doses was chosen on the basis of the results of preliminary experiments.

The animals were observed for the presence of clinical skin lesions at the site of inoculation of the challenge cultures for 32 days after challenge. At the end of the experiments crust and scale specimens were collected from clinically positive calves and examined by culture on Sabouraud's agar with actidion and chloramphenicol. For microscopic examination the specimens were stained with Blankophore and examined in a fluorescence microscope.

Results

Results are presented in Tables 1 and 2. It can be seen that in vaccinated groups challenged with the homologous dermatophyte species the prophylactic efficacy of both vaccines proved very good. Clinical skin lesions at the site of inoculation of the challenge culture were either absent or were only negligible and of short duration. Animals vaccinated with the vaccine containing the *T. verrucosum* strain proved highly resistant also to experimental infection with *T. mentagrophytes* strain. In calves challenged with *T. verrucosum* the efficacy of the vaccine containing *T. mentagrophytes* was obvious, though it remained below the level recorded in the foregoing groups.

All non-vaccinated controls developed typical trichophytosis after challenge. The clinical picture of skin changes was the same irrespective of the species of the challenge culture *(T. mentagrophytes* or *T. verrucosum)*. Nodules observed at the inoculation site changed into alopecia of confluent crusts painful to the touch. In some calves prominent confluent lesions covered the whole challenge area. Examination by culture as well as microscopic examination of clinically affected calves yielded positive results.

Discussion

Vaccination of cattle against bovine trichophytosis is the most effective way of controlling this infection (Sarkisov and Kolesnikov 1989). Its adoption in the veterinary practice in Czechoslovakia proved very successful: after 8 years of the vaccination programme covering all newborn calves bovine trichophytosis was brought under control (Polák 1984). The very good prophylactic efficacy of the Czechoslovak vaccine containing a living *T. verrucosum* strain was also confirmed in challenge experiments on calves (Rybnikář et al. 1986, 1989) in which immunized animals proved resistant to experimental infection with the homologous species and, unlike non-vaccinated controls, did not develop trichophytosis. A similar result was recorded in the present study. Whereas non-vaccinated controls challenged with *T. verrucosum* culture developed extensive alopecia and painful deep crusts, vaccinated animals were practically without clinical mycotic changes after inoculation of the same challenge dose. Where in some of these animals such changes occurred, they were only of short duration and superficial in character. Analogous results were obtained in testing the *T. mentagrophytes*-containing vaccine for protection against the homologous species.

The calves vaccinated against *T. verrucosum* were resistant also against infection with *T. mentagrophytes*. The protective cross-efficacy was almost the same as with the homologous species. On the other hand, the experimental vaccine con-

Table 1
Post-vaccination immunity against trichophytosis in calves challenged with T. mentagrophytes

Strain used for vaccination	Strain used for challenge	Calf No.	Skin mycotic changes after challenge, days after challenge				
			14	18	22	27	32
Trichophyton mentagrophytes	*Trichophyton mentagrophytes*	459	±	−	−	−	−
		700	−	−	−	−	−
		755	−	−	−	−	−
		757	−	−	−	−	−
		758	−	−	−	−	−
		821	±	±	−	−	−
		822	+	±	−	−	−
		828	±	±	−	−	−
		835	−	−	−	−	−
		882	−	−	−	−	−
		883	−	−	−	−	−
		884	−	−	−	−	−
		984	±	−	−	−	−
		992	±	−	−	−	−
Trichophyton verrucosum	*Trichophyton mentagrophytes*	457	+	−	−	−	−
		458	+	+	−	−	−
		699	−	−	−	−	−
		819	−	−	−	−	−
		823	+	+	±	−	−
		832	±	±	±	±	−
		885	+	±	−	−	−
		886	−	—	—	—	—
		901	±	±	−	−	−
		903	−	−	−	−	−
		905	−	−	−	−	−
Non-vaccinated controls	*Trichophyton mentagrophytes*	779	±	++	+++	+++	+++
		785	++	+++	+++	++	++
		848	++	+++	+++	+++	+++
		849	+	+++	+++	+++	++
		850	++	+++	+++	+++	+++
		851	±	+	+	+	++
		852	++	+++	+++	+++	++
		878	+++	+++	+++	+++	+++
		879	++	++	++	++	+
		900	++	+++	+++	+++	+++

taining *T. mentagrophytes* culture failed to confer satisfactory protection against challenge with the heterologous *T. verrucosum* strain: skin mycotic changes persisting throughout the observation period were seen in 4 out of 12 calves of this group. In the remaining 8 calves the efficacy of the vaccine was good; all of them were clinically negative at the end of the experiment.

The questions of immune cross-reactions are closely related to the antigenic properties and antigenic structure of dermatophytes. The presence in dermatophytes of species-specific antigens sharing several antigenic determinants became apparent from the experiments reported by Christiansen and Svejgaard (1976) and Philpot (1978). Some further studies, however, confirmed major antigenic differences between dermatophytes (Svejgaard et al. 1976; Walters et al. 1976). In their in-vivo and in-vitro experiments Kaaman and Wasserman (1981) found that sensibilized guinea-pigs responded with a delayed-type skin reaction to purified antigens of all three dermatophyte species under study. The strongest reactions, however, were those to homologous antigen. These investigators suggested the presence of a major group-specific antigen as well as the existence of species-specific antigens in dermatophytes.

According to Wawrzkiewicz and Wawrzkiewicz (1989) the capacity of

Table 2
Post-vaccination immunity against trichophytosis in calves challenged with T. verrucosum

Strain used for vaccination	Strain used for challenge	Calf No.	Skin mycotic changes after challenge, days after challenge				
			14	18	22	27	32
Trichophyton verrucosum	*Trichophyton verrucosum*	452	−	−	−	−	−
		453	±	±	−	−	−
		454	−	−	−	−	−
		455	−	−	−	−	−
		805	±	−	−	−	−
		810	+	±	±	−	−
		811	−	−	−	−	−
		819	−	−	−	−	−
		869	±	±	−	−	−
		870	−	−	−	−	−
Trichophytoton mentagrophytes	*Trichophyton verrucosum*	003	++	++	+	−	−
		205	++	++	+	+	+
		206	±	+	−	−	−
		207	++	++	++	++	++
		208	+	+	+	+	+
		244	++	++	+	+	+
		358	±	±	−	−	−
		538	+	+	±	±	−
		693	−	−	−	−	−
		859	+	±	−	−	−
		860	+	+	±	−	−
		861	++	++	+	−	−
		862	+	+	+	−	−
		893	+	+	±	±	−
Non-vaccinated controls	*Trichophyton verrucosum*	542	+	++	+++	+++	+++
		543	+	++	+++	+++	+++
		549	++	+++	+++	+++	+++
		697	+	+++	+++	+++	+++
		752	++	+++	+++	+++	+++
		766	−	+	++	++	++
		767	−	+	++	++	++
		883	±	++	++	++	++
		887	+	++	++	++	++
		891	+	++	+++	+++	+++

Explanatory notes to Tables 1 and 2
− without clinical skin mycotic changes;
± minute skin changes;
+ solitary mycotic foci;
++ more than 10 mycotic foci tending to merge;
+++ merging of the foci into confluent crusts.

a strain to induce hypersensitivity is not associated with its immunogenicity and no direct relation exists between immunity and allergic state.

The experiments carried out by Golovina et al. (1989) suggest the possibility of major differences in antigenic structure between strains of one and the same species. Vaccination of sheep with vaccine LTF-130 containing *T. verrucosum* did not confer immunity against experimental infection with *T. verrucosum* var. *autotrophicum*. Only vaccine prepared from the homologous strain proved efficacious. In this connection it is of interest to note that vaccine LTF-130 was found to confer a high degree of protection to cattle (Sarkisov and Kolesnikov 1989; Gudding et al. 1991). Apparently differences existed in antigenic structure between the vaccination strain and the challenge strain of *T. verrucosum*.

From the experiments reported by Wawrzkiewicz and Rzechowski (1983) it appears that solid immune cross-reactions between *T. mentagrophytes* and *T.*

verrucosum can be induced. The degree of resistance may vary from case to case, depending both on the strain used for production of the vaccine and on the strain used for infection. These conclusions are in keeping with the results of the present study which confirmed the possibility of inducing post-vaccination cross-immunity against heterologous dermatophyte species and also differences in its level in dependence upon the vaccination strain and the challenge strain. From the results reported here it can be concluded that *T. mentagrophytes* and *T. verrucosum* strains used in our study were similar in antigenic structure. A higher degree of cross-protection against *T. mentagrophytes* infection in a vaccine containing *T. verrucosum*, compared with the cross-reactivity of a vaccine containing *T. mentagrophytes*, was reported also by Wawrzkiewicz and Wawrzkiewicz (1989). In our view these results can be accounted for by a high degree of specificity of *T. verrucosum* for cattle and by a higher immunogenic potential of *T. verrucosum*-containing vaccines used in the afore-mentioned experiments.

Křížová imunita u telat po vakcinaci proti trichofytóze

Telata vakcinovaná proti *Trichophyton verrucosum* byla resistentní k umělé infekci, vyvolané kmenem *T. verrucosum* a *T. mentagrophytes*. Po imunizaci vakcínou, obsahující *T. mentagrophytes*, došlo ke vzniku spolehlivé imunity k homologním druhu. Chráněnost zvířat proti experimentálnímu nakažení heterologním dermatofytem *T. verrucosum* nebyla však dostačující. Z dvanácti telat této skupiny se u čtyř kusů objevily po čelenži mykotické změny, které přetrvávaly po celou dobu pozorování. Všechna kontrolní nevakcinovaná telata onemocněla po inokulaci obou čeležních kultur trichofytózou s podobným klinickým průběhem.

Перекрестный иммунитет у телят после вакцинации против трихофитоза

Вакцинированные против *Trichophyton verrucosum* телята отличались резистентностью к искусственной инфекции, вызванной штаммами *T. verrucosum* и *T. mentagrophytes*. После иммунизации вакциной, содержащей *T. mentagrophytes*, наступил надежный иммунитет к гомологическому виду. Защищенность животных перед экспериментальным заражением гетерологическим дерматофитом *T. verrucosum* не была однако достаточной. Из двенадцати телят группы появились после ввода микотические изменения, длившиеся в течение всего периода наблюдения. Все контрольные не вакцинированные животные заболели после инокуляции обеих культур трихофитозом с аналогичным клиническим протеканием.

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