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TESTING THE PROTECTIVE EFFICACY OF MONOVALENT AND BIVALENT ANTIMYCOTIC VACCINES

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Abstract

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Protective efficacy of bivalent vaccines composed of two *Trichophyton verrucosum* strains, *T. verrucosum* + *T. mentagrophytes* strains and *T. verrucosum* + *Microsporum canis* strains was approximately equal to, or even lower than, that of the monovalent vaccine containing *T. verrucosum.*

Vaccination, Trichophyton verrucosum, Trichophyton mentagrophytes, Microsporum canis, challenge.

Antimycotic vaccines used in veterinary practice in the USSR (TF-130, LTF-130, Mentavak, Kamelvak), Czechoslovakia (vaccine against trichophytosis of cattle, vaccine against trichophytosis of horses, vaccine against trichophytosis of furred animals) and in Poland (Trichovac, Bovitrichovac) have one feature in common: each of them contains only one vaccination strain. We thought it of interest to find whether the potency of a monovalent vaccine could be increased by addition of further dermatophyte strains.

The experiments were carried out with the following vaccines:

(a) commercial freeze-dried vaccine against bovine trichophytosis (manufactured by Bioveta, Ivanovice na Hané) the active ingredient of which is *T. verrucosum* strain CCM 8165;
(b) experimental monovalent vaccines containing *T. verrucosum*, *T. mentagrophytes* and *M. canis* strains; and
(c) bivalent vaccines consisting of the afore-mentioned strains.

Materials and Methods

The experimental animals were one-month old calves of the Bohemian pied breed from a herd without a history of trichophytosis. They were vaccinated with freeze-dried living monovalent or bivalent vaccine containing sporulating cultures grown on agar nutrient media. *Trichophyton verrucosum* CCM 8165 and CCM 8167 strains were originally isolated from cattle, *Trichophyton mentagrophytes* strain 1069 from a guinea-pig and *Microsporum canis* strain 6602 from a dog. The vaccines were injected i.m. into the gluteal muscle on two occasions 12 days apart. Twenty-eight days after revaccination the vaccinated calves and non-vaccinated controls were challenged with a sporulating culture of *T. verrucosum* isolated from a bovine deep mycotic focus. The challenge culture was inoculated epicutaneously at the rate of 10 million conidia per animal into a 10×10 cm clipped and gently scarified area of the right flank. The animals were then observed for the presence of clinical dermal changes at the site of inoculation of the challenge culture for the following 32 days. Positive clinical findings were examined by culture and with a microscope (Rybnikář 1992).

Results

Calves vaccinated with *T. verrucosum* strains CCM 8165 and CCM 8167 showed good protection against infection with the homologous species (Table 1). After being challenged, the animals vaccinated with monovalent *T. verrucosum* vaccines showed either no clinical signs of trichophytosis or developed only slight dermal changes of short duration. At the end of the experiment all these animals were clinically negative. In the groups of calves given the vaccine composed of the two *T. verrucosum* strains, trichophytosis persisted throughout the observation period in 1 out of 7 animals. Mycotic changes were less pronounced in a calf vaccinated with higher doses of the mycotic antigen.

All calves immunized with the bivalent *T. verrucosum* + *T. mentagrophytes* vaccine (Table 2) showed variously extensive mycotic changes that could be observed up to 17 to 26 days after challenge. At the end of the experiment all these animals were clinically negative. In 4 out of 9 animals vaccinated with

Table 1

Tests of the protective efficacy of vaccines prepared from T. verrucosum CCM 8165 and T. verrucosum CCM 8167 strains

Vaccination strain Vaccination dose (CFU per calf)	Calf. No.	Dermal mycotic changes after challenge with T. verrucosum strain, days after challenge					
		12	15	19	23	28	32
	007	±	±	−	−	−	−
T. verrucosum	012	+	+	±	−	−	−
	020	+	+	+	+	−	−
CCM 8165	026	±	±	−	−	−	−
	472	−	−	−	−	−	−
(2 × 10 million)	473	−	−	−	−	−	−
	775	−	−	−	−	−	−
	008	+	±	−	−	−	−
T. verrucosum	015	±	+	+	−	−	−
	017	+	+	±	−	−	−
CCM 8167	021	±	+	−	−	−	−
	022	+	+	+	−	−	−
(2 × 10 million)	776	±	±	−	−	−	−
	782	−	−	−	−	−	−
T. verrucosum	006	+	+	±	−	−	−
CCM 8165	013	+	±	−	−	−	−
(2 × 5 million)	682	+	+	+	−	−	−
+	685	+	++	++	++	++	++
T. verrucosum	779	±	±	−	−	−	−
CCM 8167	780	±	+	+	−	−	−
(2 × 5 million)	781	±	±	−	−	−	−
T. verrucosum	009	−	±	−	−	−	−
CCM 8165	014	−	−	−	−	−	−
(2 × 10 million)	016	−	−	−	−	−	−
+	019	−	−	−	−	−	−
T. verrucosum	683	+	+	+	+	±	±
CCM 8167	684	±	±	−	−	−	−
(2 × 10 million)	778	−	−	−	−	−	−
	024	−	++	+++	+++	+++	+++
	025	−	±	++	++	+++	+++
Non-vaccinated	029	−	−	+	++	++	++
	030	−	±	++	+++	+++	+++
Controls	687	±	++	+++	+++	+++	+++
	783	−	+	+++	+++	+++	+++
	784	−	±	++	++	++	++

T. mentagrophytes vaccine, trichophytic foci persisted throughout the observation period.

Calves vaccinated with *M. canis* vaccine (Table 3) developed, after challenge, trichophytosis of the same extent as non-vaccinated controls. At the site of inoculation of the challenge culture, individual or confluent mycotic foci painful to the touch were recorded. In most cases even the deep layers of the skin were affected. The potency of the bivalent *T. verrucosum* + *M. canis* vaccine proved very good, being comparable to that of the monovalent *T. verrucosum* vaccine.

The results of examination by culture and of microscopic examination were in keeping with the clinical findings. In clinically affected animals the challenge *T. verrucosum* strain was demonstrated upon laboratory examination.

Discussion

Experiments with vaccination of animals with living antigens containing two dermatophyte strains have been described by Petrovich et al. (1972). In his experiments all three calves immunized with a vaccine containing *T. verrucosum*

Table 2

Tets of protective efficacy of vaccines prepared from T. verrucosum CCM 8165 and T. mentagrophytes No. 1069 strains

Vaccination strain Vaccination dose (CFU per calf)	Calf No.	Dermal mycotic changes after challenge with T. verrucosum strain, days after challenge					
		10	15	17	21	26	32
T. verrucosum CCM 8165 (2×10 million)	242	±	±	±	−	−	−
	243	−	−	−	−	−	−
	245	−	−	−	−	−	−
	248	±	+	+	−	−	−
	671	+	±	−	−	−	−
	674	−	−	−	−	−	−
	675	+	++	++	+	−	−
	855	±	+	−	−	−	−
	856	−	−	−	−	−	−
T. mentagrophytes No. 1069 (2×6 million)	205	±	++	++	+	+	+
	206	±	±	+	−	−	−
	207	±	++	++	++	++	++
	208	−	+	+	+	+	+
	244	++	++	++	+	+	+
	859	+	+	±	−	−	−
	860	±	+	+	±	−	−
	861	++	++	++	+	−	−
	862	±	+	+	+	−	−
T. verrucosum CCM 8165 (2×10 million) + T. mentagrophytes No. 1069 (2×6 million)	215	+	++	++	±	−	−
	219	+	++	++	+	+	−
	250	+	+	+	−	−	−
	676	++	++	++	+	−	−
	678	+	+	+	−	−	−
	688	++	++	+	+	−	−
	864	±	+	+	−	−	−
	865	+	++	++	−	−	−
	897	±	+	++	+	±	−
Non-vaccinated Controls	539	±	++	+++	+++	+++	+++
	540	−	+	++	+++	+++	+++
	697	±	+	+++	+++	+++	+++
	752	±	++	+++	+++	+++	+++
	880	±	+	++	+++	+++	+++
	885	±	++	++	++	++	++

Table 3

Tests of protective efficacy of vaccines prepared from T. verrucosum CCM 8165 and M. canis No. 6602 strains

Vaccination strain Vaccination dose (CFU per calf)	Calf No.	Dermal mycotic changes after challenge with T. verrucosum strain, days after challenge 11	15	20	25	28	32
	221	+	+	−	−	−	−
T. verrucosum	683	−	−	−	−	−	−
	686	−	−	−	−	−	−
	687	−	−	−	−	−	−
CCM 8165	689	±	−	−	−	−	−
	690	±	±	±	−	−	−
	691	−	−	−	−	−	−
(2 × 10 million)	895	+	±	±	±	−	−
	896	±	±	−	−	−	−
	228	−	±	++	++	++	++
M. canis	229	−	+	++	++	++	++
	692	−	±	+	++	++	++
	694	−	±	+++	+++	+++	+++
No. 6602	869	−	−	++	+++	+++	+++
	870	−	+	++	++	+	+
	872	−	−	++	++	+	+
(2 × 10 million)	874	−	±	++	+++	+++	+++
	891	−	±	+	+++	+++	+++
T. verrucosum	225	−	−	−	−	−	−
CCM 8165	226	+	±	−	−	−	−
(2 × 10 million)	227	−	−	−	−	−	−
+	535	±	±	−	−	−	−
M. canis	536	±	−	−	−	−	−
No. 6602	537	−	−	−	−	−	−
(2 × 10 million)	868	±	−	−	−	−	−
	879	−	−	−	−	−	−
	542	−	+	+++	+++	+++	+++
	543	±	+	+++	+++	+++	+++
Non-vaccinated	766	−	−	++	++	++	++
	767	−	−	+	++	++	++
	883	−	±	++	++	++	++
Controls	887	±	+	++	++	++	++
	891	−	+	+++	+++	+++	+++
	893	+	++	++	++	+	+

Explanation to Tables 1 to 3
CFU = colony-forming units.
− No dermal mycotic changes.
± Minute dermal changes (scales, papillae).
+ Solitary mycotic foci.
++ Mycotic foci covering more than a 1/4 of the inoculated area.
+++ Mycotic foci covering more than half of the inoculated area.

and *T. mentagrophytes* strains developed trichophytosis after challenge with the *T. verrucosum* strain. The use of the monovalent *T. verrucosum* vaccine conferred full protection against experimental infection with the homologous strain. Calves vaccinated with *T. mentagrophytes* vaccine were not resistant to infection with *T. verrucosum* culture. Although these results suggested certain relationships, they cannot be taken as conclusive evidence considering the small numbers of experimental calves (2 to 3 animals) in each group.

Wawrzkiewicz et al. (1991), on the other hand, reported that both monovalent *(T. verrucosum)* and bivalent *(T. verrucosum + T. mentagrophytes)* vaccine conferred a reliable degree of protection against experimental infection with virulent strains of the two dermatophytes.

In our challenge experiments on 102 calves the bivalent vaccines were based on the vaccination *T. verrucosum* strain CCM 8165. Its protective efficacy against

experimental infection with *T. verrucosum* proved very good. The vaccine consisting of two *T. verrucosum* strains was as efficacious as the monovalent *T. verrucosum* vaccine in 6 out of 7 animals. One calf each of the two groups vaccinated with these bivalent vaccines reacted to challenge with mycotic changes that persisted throughout the observation period.

The protection of calves vaccinated with the *T. mentagrophytes* strain against infection with the heterologous *T. verrucosum* strain was lower; 4 out of 9 calves exhibited trichophytic foci up to the end of the experiment. This is in keeping with the results of our previous study (Rybnikář 1992) on postvaccination cross-immunity. The bivalent vaccine consisting of *T. verrucosum* + *T. mentagrophytes* strains conferred a satisfactory degree of protection. However, mycotic changes in response to challenge were observed in all calves of this group and were more conspicuous and persisted for a longer period of time than those seen in calves vaccinated with the commercial monovalent *T. verrucosum* vaccine.

The monovalent vaccine containing *M. canis* proved entirely inefficacious. In this case no cross-immunity against the challenge *T. verrucosum* strain was observed. The protective effect of the *T. verrucosum* + *M. canis* vaccine, on the other hand, was considerable, being comparable to that conferred by *T. verrucosum* vaccine.

The results of our experiments suggest the involvement, to a certain extent, of phenomena described as antigen competition. These phenomena come into play when several antigens are applied simultaneously or closely one after another (Nouza and John 1987). These antigens sometimes potentiate one another; more frequently, however, the response to several antigens is below that one might expect if they were administered separately. Negative effects on the development of postvaccination immunity against trichophytosis in consequence of concurrent administratoin of vaccine TF-130 and vaccine against mouth-and-foot disease have been reported by Ruchljada et al. (1973). In our experiments the protective efficacy of bivalent vaccines was approximately equal to, or even relatively lower than, that of one member of the pair of the monovalent vaccines. Although the differences were not great, it seems possible to conclude that negative effects on the protective efficacy came into operation particularly in the groups vaccinated with strains of presumably similar antigenic structure *(T. verrucosum + T. verrucosum, T. verrucosum + T. mentagrophytes)*. The efficacy of bivalent vaccines containing strains of more differing antigenic structure *(T. verrucosum + M. canis)* was not reduced as against that of the monovalent vaccine.

The results reported here are of value particularly by showing that the use of two dermatohyte strains in an attempt to extend the antigenic structure of a vaccine may not increase its protective efficacy as against the monovalent vaccine and may even result in an opposite effect.

Ověřování protektivní účinnosti monovalentních a bivalentních antimykotických vakcín

Protektivní účinnost bivalentních vakcín, složených ze dvou kmenů *Trichophyton verrucosum*, z kmenů *T. verrucosum* + *T. mentagrophytes* a z kmenů *T. verrucosum* + *Microsporum canis*, byla přibližně stejná nebo i nižší než u monovalentní vakcíny, obsahující *T. verrucosum*.

Проверка защитного действия одновалетных и двухвалентных антимикотических вакцин

Защитное воздействие двухвалентных вакцин, состоящих из двух штаммов Trichophyton verrucosum и из штаммов *T. verrucosum* + *T. mentagrophytes* было ниже в сопоставлении с одновалентной вакциной, содержащей *T. verrucosum*. После иммунизации телят вакциной, подготовленной из культур *T. verrucosum* + *Microsporum canis*, пониженного профилактического эффекта по сравнению с группой, вакцинированной штаммом *T. verrucosum*, не наблюдали.

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