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### Received April 20, 1990 to yr cog ogaloe na oni parwi o de si bidi

# eyes alt i le se man a l'attait a pida gua **Abstract** se pils al level a significare

# subside at a desired Rybnikář, A., J. Chumela, V. Vrzal, J. Lysák and J. Petráš: Vaccination of Horses against Trichophytosis. Acta vet. Brno, 60, 1991: 165–169.

Prophylactic potency of the vaccine against equine trichophytosis manufactured by Bioveta, Ivanovice na Hané (Czechoslovakia), was tested in a challenge bioassay on 15 horses. Non-vaccinated controls (6 horses) challenged with a Trichophyton equinum culture by epicutaneous inoculation developed trichophytosis with dissemination of mycotic foci beyond the site of primary infection. Vaccinated horses challenged with the same inoculum doses showed only short-term superficial dermal changes which disappeared spontaneously.

Therapeutic potency of the vaccine was confirmed in an experiment including 8 horses with natural T. equinum-produced trichophytosis and 6 experimentally infected horses. The animals with natural trichophytosis were healed in 4 to 6 weeks and the experimentally infected animals in 3 to 4 weeks after vaccination.

Trichophyton equinum, vaccine, prophylactic and therapeutic potency

From detailed mycological studies conducted in the past 20 years it appears that an overwhelming majority of equine dermatomycoses both in our country and abroad are produced by the dermatophyte Trichophyton equinum. In the USSR, Petrovich and Sarkisov (1981) reported the isolation of T. equinum in 82 % of the cases examined, whereas other species were isolated from mycotic changes in horses less frequently (Trichophyton mentagrophytes in 10 % and Microsporum equinum in 7 % of the samples). Similar results were also reported by Woloszyn et al. (1976), Weiss et al. (1979), Takatori et al. (1981), Connole and Pascoe (1984), Stenwig (1985) and Aho (1988).

In Czechoslovakia, the first case of equine trichophytosis produced by T. equinum was described by Otčenašek et al. (1962). Further cases of this infection in our country (Buchvald 1964, Stros et al. 1978, Vrzal et al. 1985) prompted our present research in which we were guided by successful results of the vaccination of horses against trichophytosis in the USSR (Petrovich and Sarkisov 1981).

In our laboratory we developed a lyophilized immunobiological preparation based on a live *T. equinum* mutant, TE-M-141, of low virulence for horses, guinea-pigs and cattle. The results of our tests for its prophylactic and therapeutic potency are reported in the present study.

#### Materials and Methods

The experimental animals were warm-blooded horses in a good nutritional state aged 8 to 13 months.

In the 1st experiment 9 horses were vaccinated i.m. with prophylactic doses of the vaccine against equine trichophytosis manufactured by Bioveta, Ivanovice na Hané (Czechoslovakia), and revaccinated 14 days later. One month after the revaccination they were challenged, together with a group of non-vaccinated controls of the same age, by epicutaneous inoculation of a suspension of a highly virulent Trichophyton equinum strain onto a  $10 \times 10$  cm clipped and gently scarified area of the left flank at the rate of 80 000 conidia per animal. During the next 37 days the animals were observed for mycotic dermal changes at the site of inoculation of the challenge culture and for possible dissemination of trichophytic changes. At the end of the experiment scale and hair samples were taken from the vaccinated and control animals for examination by culture.

The 2nd experiment was designed to test the therapeutic potency of the vaccine. Eight horses with natural T. equinum-produced trichophytosis and 6 control animals of the 1st experiment (37 days after challenge) showing numerous trichophytic foci (6 to 80 lesions 1 to 15 cm in diameter per animal) were vaccinated twice with the vaccine to assess its therapeutic potency. The periods elapsing between vaccination and recovery of the vaccinated animals were recorded.

# Results

The results of testing the protective potency of the vaccine are presented in Table 1. In the immunized animals the only changes observed after challenge were slight surface lesions of the skin which disappeared spontaneously without any further treatment. The skin at the site of administration of the infectious agent soon became covered with new hair. At the end of the experiment all vaccinated animals were completely negative without any clinical signs of trichophytosis (see Fig. 1 and 2. Plate XVIII at the end of the volume).

The course of experimental infection in non-immunized controls was serious. Confluent trichophytic lesions painful to the touch were seen all over the infected area (Fig. 3, Plate XIX). From the 23rd day after challenge dissemination of mycotic changes was observed in places outside the incolutation site of the culture (Fig. 4 and 5, Plates XIX and XX). Trichophytic foci persisted in the controls up to the 37th post-challenge day (end of the experiment) without tendency to self-healing.

The results of examination by culture (day 37 after challenge) agreed with the clinical findings. The challenge strain of T. equinum was recovered from the controls, whereas recultivation attempts from the vaccinated animals yielded negative results.

Group	Horse No.	Clinical dermal mycotic changes after challenge, days after challenge					
		day 10	day 17	day 23	day 29	day 33	day 37
Non-vaccinated horses	012 013 016	± ±	++ +++	+++++++++++++++++++++++++++++++++++++++	++++	+++++	+++++
Controls	272 290 293		± + ++	+++++++++++++++++++++++++++++++++++++++	$\left \begin{array}{c} + + + + \\ + + + + \\ + + + + \end{array}\right $	+++++	
Vaccinated horses	005 007 011 047 271 288 289 291	+++++++++++++++++++++++++++++++++++++	++ ++ ++ ++ + + +	+ + ± ± + ±			
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Table 1 Tests of prophylactic potency of the vaccine against equine trichophytosis

No clinical dermal mycotic changes.

+ Minute dermal changes.

+ Solitary mycotic foci.
+ + More than 10 mycotic foci.
+ + Merging of the foci into confluent crusts.

++ Presence of confluent crusts, dissemination beyond the inoculation site.

Therapeutic administration of the vaccine to clinically affected horses produced disintegration and shedding of trichophytic crusts within 1 to 2 weeks of revaccination. The skin at these sites soon became covered with new hair. Seven out of 8 horses with natural trichophytosis recovered within 4 weeks of revaccination. One horse with extensive mycotic lesions (Fig. 6, Plate XX) recovered 6 weeks after revaccination.

Four horses with experimental trichophytosis recovered within 3 weeks and the other 2 horses of this group became clinically negative 4 weeks after the therapeutic vaccination.

# Discussion

Until recently the main therapeutic means in the control of equine dermatophytosis were pharmaceuticals administered locally and per os and the only method of prevention was disinfection. Besides being laborious and costly, all these procedures have been unable to induce immunity to a secondary infection of the animals.

Positive results obtained with vaccination against trichophytosis in cattle inspired the hope that immunization may prove successful also in horses. The first potent vaccine against equine dermatophytosis has been produced in the USSR since 1981 (Sarkisov and Kolesnikov 1989). This biological, containing a living strain of *T. equinum*, is known under the trade-mark S-P-1. The prophylactic potency of the vaccine in challenge experiments conducted by Petrovich and Sarkisov (1981) was 95 %. According to these investigators the lowest age of foals for immunization with S-P-1 was 2 to 3 months, active production of immunity occurred in 17 days and was completed by 30 days after immunization; the duration of post-vaccination immunity was reported to be at least 5 years.

The horses in our experiments were challenged 31 days after revaccination. The results demonstrated a high protective potency of the Czechoslovak vaccine: the character of clinical dermal changes found after challenge in the vaccinated horses was markedly different from that observed in the non-vaccinated controls. In the immunized animals only superficial skin lesions developed at the site of epicutaneous inoculation of a highly virulent *T. equinum* strain and disappeared soon afterwards. By the end of the experiment the inoculation site was covered with new healthy hair. The non-vaccinated challenged controls, on the other hand, showed the typical pattern of trichophytosis: in addition to the primary mycotic lesions dozens of foci were produced by dissemination and continued to spread till the end of the experiment.

Of importance was also the therapeutic effect of the vaccine. Horses showing previously numerous mycotic lesions without a tendency to healing recovered after its application, mostly in 3 to 4 weeks.

The vaccine against equine trichophytosis has been manufactured by Bioveta, Ivanovice na Hané, since 1986. Besides its high prophylactic and therapeutic potency it has the merit of containing an attenuated vaccination strain of low virulence. In animals exposed to a heavy physical load, vaccination and particularly revaccination may produce painful swelling at the site of application of the vaccine and a decline in their food intake for one week. Similar post-vaccination reactions were also observed in animals vaccinated with S-P-1. Although these changes disappear spontaneously within a week, we recommend that the vaccination against equine trichophytosis should be carried out when the animals are at relative rest.

The protection of horses against *T. equinum*-produced infection also reduces the risk of infection with this dermatophyte in man. Also the possibility of spread of further causative agents of mycotic diseases of the horse (*Microsporum equinum*, *M. canis*, *Trichophyton mentagrophytes* a. o.) should be considered. In studies on cross-immunization it has become apparent that the most resistant animals were those infected with homologous dermatophyte species, whereas immunization and infection experiments with heterologous cultures did not result in full protection of all experimental animals (Petrovich 1985). Further studies along this line are therefore warranted.

Experience to date has shown that specific vaccines are the most convenient means of protection against equine trichophytosis in terms of efficacy and economy. The control of this disease requires a systematic approach under due observation of all epizootiological recommendations.

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# Vakcinace koní proti trichofytóze

V čelenžním biologickém pokusu na 15 koních byla ověřována profylaktická účinnost vakcíny proti trichofytóze koní (výrobce Bioveta, Ivanovice na Hané, ČSFR). Kontrolní nevakcinovaná zvířata onemocněla po epikutánní inokulaci čelenžní kultury *Trichophyton equinum* trichofytózou s rozsevem mykotických ložisek i mimo místo primární infekce. Stejné dávky čelenžního inokula vyvolaly u vakcinovaných koní jen krátkodobé povrchové kožní změny, které samovolně vymizely.

Dobrá terapeutická účinnost vakcíny byla potvrzena v pokusu s osmi přirozeně infikovanými (původce *T. equinum*) a šesti uměle nakaženými koňmi. U zvířat s přirozenou trichofytózou došlo k vyléčení po 4-6 týdnech a u zvířat experimentálně infikovaných po 3-4 týdnech od vakcinace.

## Вакцинация лошадей против трихофитоза

В проводимом на 15 лошадях биологическом эксперименте проверяли профилактическое действие вакцины против трихофитоза лошадей (выпускает Биовета Ивановице-на-Ханой, Чехословакия). Контрольные, не принимавшие вакцину животные (6 лошадей) заболели после эпикутанной инокуляции культуры *Trichophyton equinum* трихофитозом с рассеиванием микотических очагов также за пределы первичной инфекции. Одинаковые дозы инокулята вызвали у вакцинированных лошадей лишь кратковременные поверхностные изменения на коже, самопроизвольно несколько позже исчезнувшие.

Хорошее терапевтическое воздействие вакцины подтвердил также опыт восьми естественно нифицированных (возбудитель *T. equinum*) и шести искусственно зараженных лошадей. Заболевшие естественным трихофитозом лошади излечились через 4 – 6 недель, животные экспериментально инфицированные – через 3 – 4 недели со дня вакцинации.

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Plates XVIII. to XX.: Rybnikář A. et. al.: Vaccination of horses...pp. 165-169.

Fig. 1. Vaccinated horse No. 011 - day 37 after challenge.



Fig. 2. Vaccinated horse No. 291 - day 37 after challenge.

Plate XIX.



Fig. 3. Non-vaccinated control horse No. 290 - day 23 after challenge.



Fig. 4. Non-vaccinated control horse No. 293 - day 23 after challenge.

Plate XX.



Fig. 5. Non-vaccinated control horse No. 272 - day 37 after challenge.



Fig. 6. A horse with natural trichophytosis before therapeutic vaccination.