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EXPERIMENTAL DERMATOPHYTIC INFECTION OF GUINEA-PIGS AND CALVES

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Abstract

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Typical mycotic lesions occurred in all infected guinea-pigs after application of ca 10^3 viable units of *Microsporum canis* onto 1 cm^2 of shaved and scarified skin. However, similar lesions on shaved but non-scarified skin were only observed with inocula of the same culture containing 10^5 units/cm^2 skin, i.e. 100 times higher doses. Minimum infective doses of *Trichophyton equinum* and *T. verrucosum* necessary to produce experimental clinical skin trichophytosis in guinea-pigs amounted ca 100 units/cm^2 of shaved scarified skin (1 animal showed signs of the disease after application of 10 units/cm^2 of *T. verrucosum*). Susceptibility of calves to the infective agent *T. verrucosum* was lower than that of guinea-pigs. Trichophytosis occurred only after inoculation of 10^4 units/cm^2 *T. verrucosum* in shaved and scarified skin, i.e. 100 times higher dose than that used with guinea-pigs.

M. canis, T. equinum, T. verrucosum, inoculation doses.

Experimental infection of animals has been an important component of efficacy testing of biolcgicals in veterinary medicine. The results of these tests may be influenced by a number of factors, e.g. the mode of experimental infection and the amount of inoculum. These factors also play an important role in challenge experiments with dermatophytic cultures. Experimental inoculation of pathogenic skin fungi is usually carried out by rubbing suspensions or pastes of the fungal culture into the shaved and often scarified skin of animals (Cox and Moore 1968; English et al. 1979; Veronese et al. 1981). Some workers inoculated the infective agent into non-scarified skin (Lepper 1972; Poulain et al. 1980 and others). Various doses of infective material have been inoculated in such studies. However, no optimum inoculation doses for the individual dermatophyte cultures have been recommended. Therefore the present work was aimed at completion of these data.

Materials and Methods

In the experiment, lyophilized samples of 3 dermatophytes were employed: *Microsporum canis* Bodin, 1902, *Trichophyton equinum* (Matruchot et Dassonville) Gedoelst, 1902, *Trichophyton verrucosum* Bodin, 1902. The lyophilized samples were diluted in saline up to the selected concentrations based on previously determined density of viable microorganisms. Their density was determined both microscopically and using the plate dilution method. The culture was inoculated by rubbing 0.2 cm^3 of its suspension into the shaved and scarified skin of the right side of the bodies of 3—4 guinea-pigs using a rubber stopper. The size of the inoculation area was about 10 cm^2 in all animals. The same animals were then inoculated with *M. canis* into the shaved non-scarified skin on the left side of the body. The number of inoculated organisms was verified by seeding of the respective diluted culture onto Sabouraud glucose-peptone agar. The experimental animals were examined from day 7 until day 12 after inoculation. Mycotic changes at the inoculation site were evaluated macroscopically. In an exploratory study also skin scrapings were seeded on Sabouraud's agar and the isolated cultures were identified.

Similar experimental design was also employed with calves which were inoculated with various

doses of *T. verrucosum*. Two cm^3 of suspension of *T. verrucosum* microconidia was rubbed into shaved scarified skin of the left side the body, on an area of approximately 100 cm^2. Mycotic lesions were evaluated both macroscopically and by cultivation.

Results

The local mycotic skin lesions in guinea-pigs after inoculation of *M. canis* are indicated in Table 1. Typical lesions developed after inoculation of a minimum of 1000 elements/1 cm^2 of scarified skin. Inoculation of smaller doses caused typical mycotic changes only in one animal infected with 100 units *M. canis*/1 cm^2 of scarified skin. No changes in the rest of the animals were found. Inoculation of into non-scarified skin led to solitary mycotic lesions only at a dose as high as 10^5 units/cm^2 whereas the same dose inoculated into scarified skin resulted in a confluent mycotic crust on the whole scarified skin surface. The dose of 10^5 units/cm^2 caused confluent mycotic lesions also with *T. equinum* (Table 2) and *T. verrucosum* (Table 3). Minimum concentration leading to trichophytic lesions was ca 100 units/cm^2 with both cultures (in one guinea-pig a mycotic lesion developed after only 10 units/cm^2 of *T. verrucosum*).

In calves inoculated with *T. verrucosum* (Table 4) clinical trichophytosis occurred at a dose of ca 10^4 units/cm^2 forming up to 10 typical circular crusts 1 to 2 cm in dia-

Table 1
Experimental epicutaneous inoculations of guinea-pigs with various doses of Microsporum canis

Inoculation dose (number of viable M. canis units/cm^3 skin)	Exp. animal No.	Skin lesions at the inoculation site					
		Scarified skin			Non-scarified skin		
		Day 7	Day 14	Day 17	Day 7	Day 14	Day 17
10	563	—	—	—	—	—	—
	564	—	—	—	—	—	—
	566	—	—	—	—	—	—
100	567	—	—	—	—	—	—
	568	±	×	× ×	—	—	—
	569	—	—	—	—	—	—
1.000	570	×	×	×	—	—	—
	575	×	×	×	—	—	—
	577	×	× ×	× ×	—	—	—
10.000	581	×	× ×	× ×	—	—	—
	583	—	× ×	× ×	—	—	—
	584	× ×	× × ×	× ×	±	—	—
100.000	585	× × ×	× × ×	× × ×	×	× ×	×
	586	× × ×	× × ×	× × ×	×	× ×	×
	589	× ×	× × ×	× × ×	×	× ×	×

— no macroscopic changes
± suspect small skin changes
× solitary mycotic lesions
× × more than 10 mycotic lesions
× × × confluent mycotic crusts

Table 2

Experimental epicutaneous inoculations of guinea-pigs with various doses of Trichophyton equinum

Inoculation dose (number of viable Trichophyton equinum units/cm² skin)	Exp. animal No.	Skin lesions at the inoculation site - days after infection		
		Day 12	Day 14	Day 17
10	502	—	—	—
	504	—	—	—
	505	—	—	—
100	506	—	×	×
	518	±	×	×
	539	±	×	×
1.000	338	×	×	×
	521	—	×	×
	563	±	×	×
10.000	526	× × ×	× × ×	× × ×
	561	× ×	× × ×	× × ×
	579	× × ×	× × ×	× × ×
100.000	507	× × × ×	× × × ×	× × × ×
	575	× × × ×	× × × ×	× × × ×
	600	× × × ×	× × × ×	× × × ×

— no macroscopic changes
± suspect small skin changes
× solitary mycotic lesions
× × more than 10 mycotic lesions
× × × confluent mycotic crusts
× × × × one confluent crust covering the entire inoculation site

Table 3

Experimental epicutaneous inoculations of guinea-pigs with various doses of Trichophyton verrucosum

Inoculation dose (number of viable Trichophyton verrucosum units/cm² skin	Exp. animal No.	Skin lesions at the inoculation site - days after infection		
		Day 12	Day 14	Day 17
10	566	—	—	—
	567	—	×	×
	571	—	—	—
	574	—	—	—
100	568	±	×	×
	573	—	×	×
	590	—	×	×
	593	—	—	—
1.000	595	×	× ×	× ×
	596	±	×	× ×
	597	×	× ×	× × ×
	598	×	× ×	× ×
10.000	508	× ×	× × ×	× × ×
	511	±	×	× ×
	516	× ×	× × ×	× × ×
	541	× ×	× × ×	× × ×
100.000	070	× × × ×	× × × ×	× × × ×
	556	× × × ×	× × × ×	× × × ×
	557	× × × ×	× × × ×	× × × ×
	569	× × × ×	× × × ×	× × × ×

For footnotes see Table 2.

Table 4
Experimental epicutaneous inoculations of calves with various doses of Trichophyton verrucosum

Inoculation dose (number of viable Trichophyton verrucosum units/cm² skin)	Exp. calf No.	Skin lesions at the inoculation site - days after infection			
		Day 14	Day 19	Day 23	Day 34
10	34938	—	—	—	—
100	34937	—	—	—	—
1.000	90	—	—	±	±
10.000	34936	±	××	××	××
100.000	92	×	×××	××××	××××

For footnotes see Table 2.

meter. Inoculation of ca 10^5 units/cm² resulted in formation of confluent trichophytic lesions on the inoculated skin surface similar to those found in guinea-pigs. The crusts were prominent up to 1 cm above the skin surface, they broke in skin folds and after they had fallen off, haemorrhagic bottom was exposed.

The inoculated cultures were re-isolated from the cutaneous lesions in all guinea-pigs and calves with these lesions.

Discussion

Attempted experimental inoculation infections of guinea-pigs with 6 strains of *T. verrucosum* isolated from cattle, horses and sheep failed (Cox and Moore 1968). However, inoculations of rabbits and calves with the same doses of these strains resulted in formation of typical trichophytic lesions. The minimum infective dose for rabbits was ca 10^3 units/cm².

Our results are at variance with data of the above-mentioned authors. Whereas successful experimental infection with *T. verrucosum* in guinea-pigs was achieved with as little as 100 viable units/cm² skin, in calves no lesions occurred unless a 100 times higher infective dosis of the same strain (i.e. 10^4 units/cm² skin) was used. Also virulence of various strains of the same dermatophyte species may differ considerably for one species of experimental animals, and appropriate inoculation doses should be selected. Determination of minimum infective doses for the individual strains of dermatophytes at standard modes of application is essential for successful experimental infection. For cattle, a minimum infective dose of *T. verrucosum* 10^3 viable units has been recommended (Lepper 1972). In our experiment, the disease in calves occurred only after application of 10^4 viable units/cm². Hence the virulence of our *T. verrucosum* strain was lower than that reported by the above-mentioned author.

The minimum infective doses of *T. equinum* and *T. verrucosum* for guinea-pigs were practically identical (about 100 units/cm²). However, with *M. canis* the minimum infective dose for guinea-pigs was 1000 units/cm² (in 1 of 3 animals it was only 100 units/cm²). Similar infective doses of *M. canis* have been reported by English et al. (1979). However, these data are only valid for inoculation into shaved and scarified skin. Our results show that the same *M. canis* culture inoculated into shaved but not scarified skin caused the disease only at doses 100 times higher. These results indicate that impaired skin integrity as achieved by scarification is a factor enhancing successful experimental dermatophytic infection.

It is noteworthy that a certain error is involved in the determination of minimum infective doses as after inoculation of the culture the animals often rub and scratch the inoculated skin so that a portion of the inoculum may be removed. In spite of this disadvantage we consider the method employed in our trials closer to natural conditions than e.g. protection of the inoculum by tapes (Jones et al. 1974) or by a polyethylene chamber (Weigl 1976) even if these methods enable application of precise doses of inoculums with no losses.

In practice, our results may be used for estimation of optimum challenge doses in testing the efficacy of antimycotic vaccines. In our earlier experiments a breakdown of immunity occurred after application of extremely high challenge inoculum. Thus biased efficiency tests of otherwise high-quality preparations were obtained. Therefore, for challenge tests we recommend doses 10 to 100 ID_{50}, i.e. 10^3 to 10^4 fungus elements/cm^2 in guinea-pigs and 10^5 to 10^6 fungus elements/cm^2 skin in calves when using *M. canis*, *T. verrucosum* and *T. equinum* strains.

Experimentální dermatofytická infekce morčat a telat

Po aplikaci asi 1000 životaschopných jednotek *Microsporum canis* na 1 cm^2 oholené skarifikované pokožky došlo k vytvoření typických mykotických lézí u všech pokusných morčat. K vyvolání klinické lokální dermatomykózy při vtírání inokula téže kultury do oholené neskarifikované pokožky morčat bylo třeba použít přibližně 100× vyšších dávek (asi 100.000 jednotek/cm^2). Minimální infekční dávky kultur *Trichophyton equinum* a *T. verrucosum*, nutné k vyvolání experimentální klinické kožní trichofytózy morčat, byly asi 100 jednotek/cm^2 oholené skarifikované pokožky (1 morče onemocnělo již po aplikaci 10 jednotek *T. verrucosum*/cm^2 pokožky). Vnímavost telat k infekčnímu agens *T. verrucosum* byla oproti morčatům nižší. K onemocnění telat trichofytózou došlo teprve po inokulaci řádově 10^4 jednotek *T. verrucosum*/cm^2 oholené skarifikované pokožky, což představuje 100× vyšší dávky než u morčat.

Экспериментальная дерматофитическая инфекция морских свинок и телят

После ввода около 1 000 жизнеспособных единиц *Microsporum canis* на 1 $см^2$ сбреенной скарифицированной кожи у всех подопытных животных произошло характерное микотическое повреждение. Для того, чтобы вызвать клинический местный дерматомикоз при втирании инокула той же культуры в сбреенную нескарифицированную кожу морских свинок, нужно было использовать дозу приблизительно в сто раз больше (около 100 000 единиц/$см^2$). Минимальная инфекционная доза культур *Trichophyton equinum* и *T. verrucosum*, необходимая для того, чтобы вызвать экспериментальный клинический кожный трихофитоз у морских свинок, достигала около 100 единиц/$см^2$ сбреенной скарифицированной кожи (одна морская свинка заболела уже после введения 10 единиц *T. verrucosum* /$см^2$ кожи). Восприимчивость телят к инфекционному веществу *T. verrucosum* по сравнению с морскими свинками была ниже. Заболевание телят трихофитозом имело место только после инокуляции порядка 10^4 единиц *T. verrucosum*/$см^2$ сбреенной скарифицированной кожи, что представляет собою дозу в сто раз больше чем у морских свинок.

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