

Immunoprophylaxis of Dermatophytosis in Animals

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Abstract Dermatophytosis is a relatively common disease in many countries occurring endemically both in companion and food animals. Fungi belonging to the genera *Trichophyton* and *Microsporum* are most often isolated from clinical cases. Measures to control and prevent dermatophytosis include sanitation, hygienic measures and treatment. In some countries, successful control and eradication have been achieved by mass vaccination of cattle and fur-bearing animals. Vaccines containing live attenuated cells of the fungus stimulate a cell-mediated immune response conferring long-lasting protection against subsequent challenge by the homologous fungus. A delayed type hypersensitivity (DTH) skin test using appropriate dermatophyte antigens is suitable to assess the response. Inactivated dermatophyte vaccines are available for use in cattle, horse, dog, and cat in some countries. However, the scientific literature is scarce making it difficult to conclude on efficacy and appropriate use. Current vaccines are all first generation vaccines. Attempts have been made to prepare subunit vaccines based on new knowledge about

virulence factors like the keratinases, so far with limited success. Candidate antigens must be able to stimulate a strong T helper 1 cell response and future research should focus on identification of major T-cell epitopes that specifically elicit a DTH reaction. Dermatophytosis is a zoonotic disease. In Norway and a few other countries, systematic vaccination against cattle ringworm has almost eliminated the disease, and ringworm in man caused by *T. verrucosum* is almost nonexistent. A similar benefit could be expected if a safe and efficacious vaccine was available for *Microsporum canis* infection in cats and dogs.

Keywords Dermatophytosis · Immunoprophylaxis in animals · Ringworm · Vaccination

Introduction

Dermatophytosis or ringworm is a fungal infection of the hair and of the superficial keratinized cell layers of the skin occurring in animals and man. Several species of genus *Microsporum* or genus *Trichophyton* belonging to the groups of zoophilic or geophilic dermatophytes can cause clinical infections in animals. There is a certain degree of host specificity, but some of the dermatophyte species may infect a variety of hosts, including man [1, 2]. Upon introduction to a herd, kennel or cattery, the disease spreads easily from one animal to another by direct

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transmission or indirectly via environmental contamination or fomites. Measures to prevent and control dermatophytosis have included sanitation, hygienic measures, prophylactic treatment, and vaccines have been available against ringworm in some animal species [3, 4]. In Norway and a few other countries, ringworm in cattle and fur-bearing animals is a notifiable disease. To limit spread, bio-security measures are imposed on affected herds, e.g., restrictions on sale of animals, access to common grasslands, and participation in shows.

In the endemic situation, ringworm is a disease of the young animal. Naturally occurring ringworm is seldom recurrent suggesting an effective and long-lasting immunity, and observations from experimental studies confirm that animals express increased resistance to subsequent challenge by the homologous fungus [5–7]. Successful resolution of lesions correlates with development of a cell-mediated immune response as demonstrated by a delayed type hypersensitivity (DTH) skin reaction [8, 9]. As pointed out by Smith and Griffin [10], this knowledge must form the basis for vaccine development against ringworm.

In the 1960s, Kielstein and coworkers [11] used inactivated preparations of *Trichophyton verrucosum* for vaccination of cattle, but with limited success. Pioneering work was carried out by researchers at the All-Union Institute of Experimental Veterinary Science (VIEV), Moscow, in the late 1960s and 1970s, reviewed by Tretiakov et al. [12] and Sarkisov [13]. A large number of field isolates of dermatophytes from different animal species belonging to the genus *Trichophyton* was characterized and sub-cultured in the process of attenuation. Strains producing abundant numbers of microconidia were selected for vaccine production [13]. This procedure of attenuation was used for *T. verrucosum* (VIEV culture collection strain 130), *T. equinum* (VIEV culture collection strain 2251/70), and *T. mentagrophytes* (VIEV culture collection strain 1024) [14–16] and vaccine formulations were developed for cattle, horse, and farmed fox, respectively. Efficacy and safety were demonstrated in experimental studies and field trials [13]. During the 1970s, the Russian vaccine against cattle ringworm was introduced in Bulgaria [17], Hungary [18], former German Democratic Republic [19], former Yugoslavia [20], and in Norway as the first country in Western Europe [21]. In former Czechoslovakia, a *T. verrucosum* vaccine

containing live cells of the strain CCM 8165 (A Rybnikar, personal communication) was available from 1974 and manufactured by a national company [22].

Several attempts have been made to develop a vaccine against *Microsporium canis* infection in dogs and cats; however, a well documented, safe and efficacious vaccine is still lacking [3].

In this article, we review immunoprophylaxis against dermatophyte infections in companion animals and in fur-bearing and food animals.

Host-Fungus Relationships of Importance for Vaccination and Protection

Host Immune Response

The immunopathogenesis of dermatophyte infections is reviewed in a previous chapter in this issue of Mycopathologia, therefore, only some aspects of the host immune response important for protection against disease will be discussed here. Both innate and adaptive immune mechanisms are involved in the response to a dermatophyte infection. Furthermore, it has been demonstrated that antigens of *Microsporium canis* and several species belonging to the genus *Trichophyton* elicit both humoral and cell-mediated immune responses [23–26]. Dermatophytosis seems to be another example of a disease illustrating the dichotomy of the immune system and outcome of the infection. Predominant activation of T helper type 2 (Th2) cells and corresponding cytokine profile leads to antibody formation and is associated with a chronic disease [10, 26, 27]. Activation of Th1 cells stimulates a cell-mediated response characterized by the cytokines interferon- γ (IFN- γ), interleukin 12 (IL-12), and IL-2 leading to recovery [10, 26, 27]. Passive transfer of anti-dermatophyte antibodies does not protect against challenge with the homologous fungus in a mouse model [28], whereas transfer of lymphoid cells from infected donors confers protection to susceptible recipients. A positive DTH reaction confirms that a cell-mediated immune response has been established. Conversely, an immediate hypersensitivity skin test reaction indicates a humoral response. IFN- γ plays an important role in the effector phase of the DTH reaction [29]. In patients with dermatophytosis, in vitro release of IFN- γ by peripheral blood mononuclear cells has

been demonstrated [30], and in those with a chronic course of infection a decreased IFN- γ production was detected upon stimulation with trichophytin [31]. Experimental inoculation of calves with a virulent strain of *T. verrucosum* resulted in IFN- γ production in cultures of whole blood cells sampled 4 weeks post infection and stimulated by trichophytin [32]. Furthermore, vaccination of calves with a live attenuated *T. verrucosum* vaccine (Bovilis[®] Ringvac, Intervet, the Netherlands), revealed production of IFN- γ indicating stimulation of a cell-mediated immune response [32]. Vaccinated calves were protected upon challenge. In conclusion, an efficient dermatophyte vaccine must stimulate appropriate lymphocyte subsets which prime the animal for a subsequent cell-mediated immune response leading to clearance of the fungus and recovery from infection [10]. A DTH skin test using appropriate dermatophyte antigen is suitable to assess the type of immune response.

Dermatophyte Antigens

Initial studies by Sarkisov and co-workers [13] demonstrated that vaccines containing hyphal elements of dermatophytes did not confer protection upon challenge, whereas vaccines of conidia from the same dermatophytes did. Hussin and Smith [7] examined immunogenicity and protection of different vaccine preparations from *T. mentagrophytes* in a guinea pig model. The live spore vaccine was the most effective and the authors suggested that antigens responsible for a protective immune response are likely to be exposed at time of spore germination and early hyphal growth. The cytoplasmic extract vaccine had no beneficial effect on the course of infection, whereas the cell wall antigen vaccine prepared from inactivated hyphae cells produced an intermediate type of protection [7]. The efficacy of an *M. canis* cell wall vaccine was examined in kittens both by direct challenge and contact exposure with infected animals [33, 34]. However, all animals developed ringworm lesions. Calves and guinea pigs were vaccinated with preparations from the ribosomal fraction of *T. verrucosum* and *M. canis*, respectively [35, 36]. Signs of ringworm developed after challenge in both trials, but the duration of the course of infection was significantly reduced compared to mock-vaccinated animals. The antigens used in these experimental vaccines were not well characterized.

Typical for dermatophytes is the production of keratinases considered as virulence factors playing a role in the pathogenesis of infection [37–39]. There are two families of keratinolytic enzymes, the subtilase family, which are serine proteases, and the zinc metalloprotease family. In a guinea pig model, humoral and cell-mediated immune responses to a recombinant metalloprotease from *M. canis* have been described [40]. However, a subunit vaccine based on this enzyme [41] or a subtilase [42] failed to protect the animals against development of skin lesions. Interestingly, Woodfolk and co-workers [26, 43] demonstrated that a subtilase recombinant enzyme derived from *T. rubrum*, Tri r 2, had the ability to stimulate immediate and delayed-type hypersensitivity skin test reactions in different test persons. Furthermore, the peptide P5 was an immunodominant epitope of the Tri r 2 specifically associated with the DTH. In the search for protective immunogens, peptides specifically stimulating strong memory T-cell responses are candidate vaccine antigens. Raska and co-workers [44] examined the potential protective effects in calves and guinea pigs of two vaccine formulations based on heat shock proteins (hsp) from *T. mentagrophytes* and compared with commercial inactivated and live *T. verrucosum* vaccines. The animals were challenged by epicutaneous inoculation of a virulent strain of *T. mentagrophytes*. The DNA plasmid vaccine designated pVAX1-hsp60-TM814K seemed to confer some protection especially in the guinea pig model. The recombinant Freund adjuvanted protein vaccine designated r-hsp60-TM664 gave highly variable results. For both vaccine products, it is not possible to draw conclusions because of the small number of animals in each group and of different vaccine compositions with respect to antigen amount and adjuvant content. Therefore, current dermatophyte vaccines are first generation vaccines with inactivated or attenuated live fungal cells.

Vaccination Against Dermatophytosis in Cattle

In cattle, dermatophytosis is most often caused by *T. verrucosum*; occasionally *T. mentagrophytes*, *T. equinum*, and *M. canis* are isolated from ringworm lesions [1, 4]. There are four commercialized vaccine products against cattle ringworm caused by

T. verrucosum (Table 1); three are monovalent, containing live cells of *T. verrucosum* [45, 46], whereas the inactivated vaccine contains the three dermatophyte species *T. verrucosum*, *T. mentagrophytes*, and *T. sarkisovii* (now synonymous with *T. mentagrophytes* on the basis of recent molecular studies) [47]. The most common procedure for evaluation of vaccine efficacy and safety and characterization of the immune response involves the target animal species; moreover, these parameters are studied in a homologous system where animals are challenged with a virulent strain belonging to the same dermatophyte species as in the vaccine. A few experiments have used heterologous challenge strains demonstrating some degree of cross reactions [48, 49].

Inactivated Dermatophyte Vaccines

Vaccines for cattle containing formalin-inactivated cells of strains of *T. verrucosum* have been examined for immunogenicity and protective ability in experimental and field trials [50, 51]. Vaccination of calves aged 5–8 days stimulated a cellular immune response assessed by skin test and in a leukocyte migration inhibition test. In an experimental trial, some protection was demonstrated after epicutaneous challenge of calves with a virulent strain of *T. verrucosum* [50]. However, in a field trial, the design included vaccination of already infected animals and was considered suboptimal for the evaluation of prophylactic efficacy of the vaccine preparation [51]. Limited information is available regarding efficacy and duration of immunity of the inactivated trivalent vaccine Insol[®] Trichophyton (Boehringer Ingelheim Vetmedica GmbH, Germany), but annual revaccination is recommended [47]. In a blinded challenge study, comparison was made between this vaccine and a monovalent live attenuated vaccine (Bovilis[®] Ringvac, Intervet, the Netherlands) [52]. None of the calves vaccinated with the live vaccine developed ringworm signs, whereas all calves vaccinated with the inactivated vaccine had severe ringworm lesions comparable to the control group (Table 2). The difference between live and inactivated *T. verrucosum* vaccine preparations in stimulating protective immunity has been confirmed in guinea pigs and calves [53].

Dermatophyte Vaccines Containing Live Fungal Cells

The vaccine strains *T. verrucosum* LTF-130 (Bovilis[®] Ringvac, Intervet, the Netherlands) [45] and CCM 8165 (Trichoben, Bioveta Inc., Czech Republic) [46] are both characterized by an abundant production of microconidia (Fig. 1) and remaining virulence properties. Interestingly, the Czech research group has further attenuated the CCM 8165 vaccine strain by ultraviolet light-induced mutagenesis [54] producing an avirulent strain designated TV-M-310 (Trichoben AV) [46]. Efficacy has been demonstrated for these vaccines in experimental trials with commingling exposure (i.e., a study in which the test animals are challenged by exposure to an actively infected animal mixed in with the group) and epicutaneous inoculation of a virulent strain of *T. verrucosum* in the target animal [55–57]. Protective immunity in animals vaccinated with the two first mentioned products has been compared and found to be equally satisfactory [56]. In vaccinated calves, epicutaneous inoculation of the virulent strain of *T. verrucosum* elicits a mild inflammatory reaction appearing a couple of days post challenge and resolving within 4–6 days (Table 2) [52]. This reaction is a visible evidence of the anamnestic immune response involving rapid mobilization of effector cells leading to elimination of the fungus.

Field trials have been conducted in areas with high prevalence of bovine ringworm and all have demonstrated significant reduction of cases of disease and concluded that vaccination should be part of a ringworm control program [58–60]. For the purpose of obtaining experience with the Russian ringworm vaccine under Norwegian conditions, a field trial was conducted in an area of dairy production with a herd prevalence rate of ringworm of 70% [59]. For 6 years, approximately 95% of all herds in the area participated in the program, which included vaccination of non-infected animals of all ages, followed by vaccination of all calves born and purchased animals. The prevalence rate of ringworm-infected herds was reduced to 0% 8 years after inception of the program.

The vaccination schedule consists of intramuscular injection of two doses with an interval of 10–14 days. One dose or doubling the dose given once does not confer satisfactory protection [61]. The significance of the fungal antigen amount to trigger a protective

Table 1 Summary of dermatophyte vaccines commercially available in different countries in Europe (October 2007). See text for further detail

Manufacturer	Vaccine trade name*	Dermatophyte species	Status of organisms	Target animals	Indication for use		References
					Prophylactic	Treatment	
Boehringer Ingelheim Vetmedica	Insol [®] Trichophyton	<i>T. verrucosum</i> , <i>T. mentagrophytes</i> , <i>T. sarkisovii</i>	Inactivated	Cattle	X	X	[85]
	Insol [®] Dermatophyton	<i>T. verrucosum</i> , <i>T. mentagrophytes</i> , <i>T. sarkisovii</i> , <i>T. equinum</i> , <i>M. canis</i> , <i>M. canis</i> var <i>distortum</i> , <i>M. canis</i> var <i>obesum</i>	Inactivated	Dog, cat, horse	X	X	[86]
Bioveta	Biocan M Plus	<i>M. canis</i>	Inactivated	Dog	X	X	[83]
	Biofel M Plus	<i>M. canis</i>	Inactivated	Cat	X	X	[83]
	Trichoben	<i>T. verrucosum</i>	Live attenuated	Cattle	X	X	[46]
	Trichoben AV	<i>T. verrucosum</i>	Live avirulent**	Cattle	X	X	[46]
	Trichoequen	<i>T. equinum</i>	Live attenuated	Horse	X	X	[83]
	Trichopelen	<i>T. mentagrophytes</i>	Live attenuated	Rabbit and fur-bearing animals	X	X	[96]
Intervet	Bovilis [®] Ringvac	<i>T. verrucosum</i>	Live attenuated	Cattle	X	X	[45]

* Other names may appear depending on country where the products are marketed

** Ultraviolet radiation of *T. verrucosum* vaccine strain

Table 2 Ringworm lesions in calves vaccinated with Bovilis® Ringvac (Intervet, the Netherlands) (group R), Insol® Trichophyton (Boehringer Ingelheim Vetmedica GmbH, Germany) (group I) and non-vaccinated controls (group C) after inoculation of a virulent strain of *Trichophyton verrucosum*

Vaccine group	Calf no.	Clinical score ^a , weeks after inoculation								
		1	2	3	4	5	6	7	9	
R	31	–	–	–	–	–	–	–	–	–
	34	(+)	(+)	(+)	(+)	–	–	–	–	–
	36	(+)	(+)	–	–	–	–	–	–	–
	38	(+)	(+)	(+)	–	–	–	–	–	–
	40	(+)	(+)	(+)	(+)	–	–	–	–	–
I	33	–	–	+	+	++	++	+	–	–
	35	(+)	(+)	++	++	++	++	(+)	–	–
	37	–	(+)	+	++	++(+)	++	+(+)	+	–
	39	(+)	(+)	+	++	++	+	–	–	–
	41		(+)	+	+(+)	++	+(+)	(+)	–	–
C	42	(+)	(+)	+(+)	++	+++	++	+	–	–
	43	(+)	(+)	+	++	++	+	+	–	–
	44	(+)	(+)	+	++	++(+)	+(+)	–	–	–
	46	–	(+)	+	+(+)	++	++	(+)	–	–

Data adapted from study referenced herein [52]

^a Clinical score: –, no visible signs; (+), small spots about 1 mm in diameter, with scaling; and + to +++ increasing degrees of ringworm lesions, from mild to severe



Fig. 1 Scanning electron micrograph of the vaccine strain of *Trichophyton verrucosum* LTF 130 (Bovilis® Ringvac, Intervet, the Netherlands) producing abundant numbers of microconidia on wort agar (Lund and Bratberg, unpublished)

immune response has been studied [62] and was further demonstrated in a field trial in calves in specialized beef production [63]. Under these conditions, the vaccine dose had to be doubled of that recommended, to produce a statistically significant preventive effect. Newborn calves can be vaccinated

[64–66] and there is no interference with maternal antibodies from previously vaccinated dams [66, 67]. Onset of immunity has been studied in calves and when challenged at different time points after the last vaccination, a gradual increase in protection was observed [56]. In calves challenged 14–25 days after the last vaccination, up to 10% developed mild signs of ringworm, and when challenged at 28 days, only one of 108 calves presented mild signs of infection at the inoculation site. All animals challenged 35 days after the last vaccination were fully protected.

Field experience indicates that duration of immunity is long lasting following vaccination with live dermatophyte vaccines and no booster vaccination is recommended [12, 68]. Rybnikar and co-workers [56] demonstrated full protection of all animals challenged 1 year after vaccination. The authors commented on Russian experiments where a few cattle were challenged by epicutaneous inoculation of a virulent strain of *T. verrucosum* 1 year after vaccination and others after 3 years. Signs of ringworm were observed in one of three and one of five animals, respectively. No reason for this outcome was presented, but the inoculated dose of the fungus may have been very high and in a sense overwhelmed the vaccine-induced

immunity. Moreover, epicutaneous inoculation of the fungus onto depilated and sometimes slightly scarified skin is rather different from natural exposure. Vaccine failure has been reported [57, 58], and likely explanations may be occurrence of other dermatophyte species than *T. verrucosum*, notably *M. canis* and *T. equinum*, incorrect vaccination procedure, high level of natural challenge exposure, or vaccination during the incubation period.

The vaccine strains used in live vaccines are attenuated, but have some remaining virulence properties [69]. Following intramuscular injection, a lesion may develop, 1–2 cm in diameter with some scaling or moderate hair loss and crust formation (Fig. 2) [45, 46, 70]. Regression of the lesion is seen within 2–4 weeks. Histological examination of the skin lesion reveals presence of fungal hyphae in hair follicles accompanied by an inflammatory reaction with influx of neutrophils and CD4+ T cells (Fig. 3). The development of the lesion confirms that the vaccine strain has retained certain virulence properties likely to be crucial for the stimulation of an appropriate protective immune response. Culture of specimens from the skin surface yields the vaccine strain (69, AM Bratberg, personal communication). In commingling trials, examination of transmission of the vaccine strain from calves presenting lesions at the injection site to non-vaccinated calves, revealed no transfer (AM Bratberg, personal communication). Direct inoculation of skin scrapings from the lesion onto skin of non-vaccinated calves did not produce

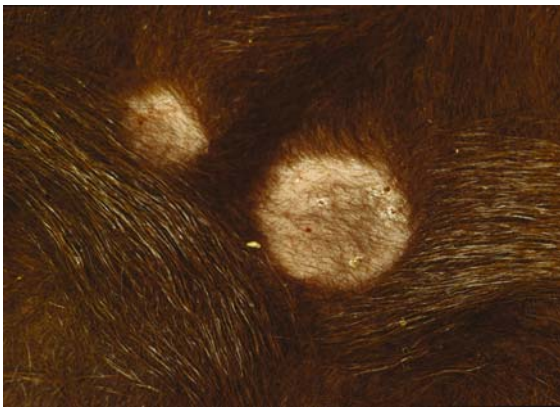


Fig. 2 Injection site skin lesion after intramuscular vaccination in calf of Bovilis® Ringvac (Intervet, the Netherlands) 2 weeks after vaccination (Lund and Bratberg, unpublished)

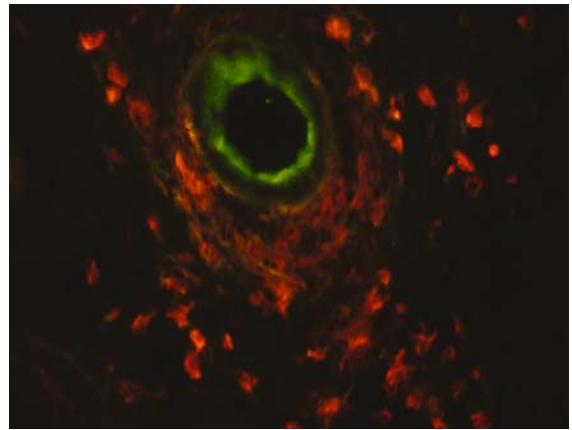


Fig. 3 Immunohistochemical staining of cryosection of biopsy sampled from the injection site in calf 12 days after vaccination with Bovilis® Ringvac (Intervet, the Netherlands) ($\times 400$). *T. verrucosum* stained green with FITC-labeled goat anti-rabbit antibodies (primary antibody: rabbit polyclonal *T. verrucosum* antibodies, National Veterinary Institute, Oslo, Norway). CD4 + T-cells stained red with phycoerythrin-labeled goat anti-mouse antibodies (primary antibody: IL-A12, ILRI, Nairobi, Kenya) (Lund and Bratberg, unpublished)

any skin reaction, most likely because of low number of fungal cells in the inoculum material (AM Bratberg, personal communication). Regarding safety of humans using the vaccine or being exposed by contact with vaccinated animals, the authors are not aware of any published reports of suspected disease in humans associated with the vaccines containing attenuated strains of *T. verrucosum*. However, the manufacturers underscore that the vaccine should not be used by immunosuppressed individuals [45] and the need for gloves when vaccinating [46].

A Russian-attenuated *Trichophyton* vaccine, Vermet, was recently described in a preliminary report as efficacious in multiple species, including cattle, goats, camels, rabbits, and foxes [71]. No additional information or studies on this product have come forth.

Vaccination in Control and Eradication Programs

In the former Soviet Union, bovine ringworm was common and considered present in all farms in the 1960s [13]. A nationwide campaign was initiated in 1969, and approximately 250 million cattle were vaccinated in the period 1970–1980 [12]. Systematic mass vaccination was promising and in 1975 less than

10% of the farms were infected [72]. The prevalence decreased further to less than 1% by 1984 [12]. When vaccination was introduced in the herds, both adult and young stocks were vaccinated to establish herd immunity. Thereafter, only calves were vaccinated starting at 4 weeks of age, and the authorities recommended continuing vaccination in herds for 7–10 years. Thus, the former Soviet Union was the first country to employ vaccination against bovine ringworm in a program to eliminate the disease. Successful vaccination campaigns have been reported from other European countries [73–76].

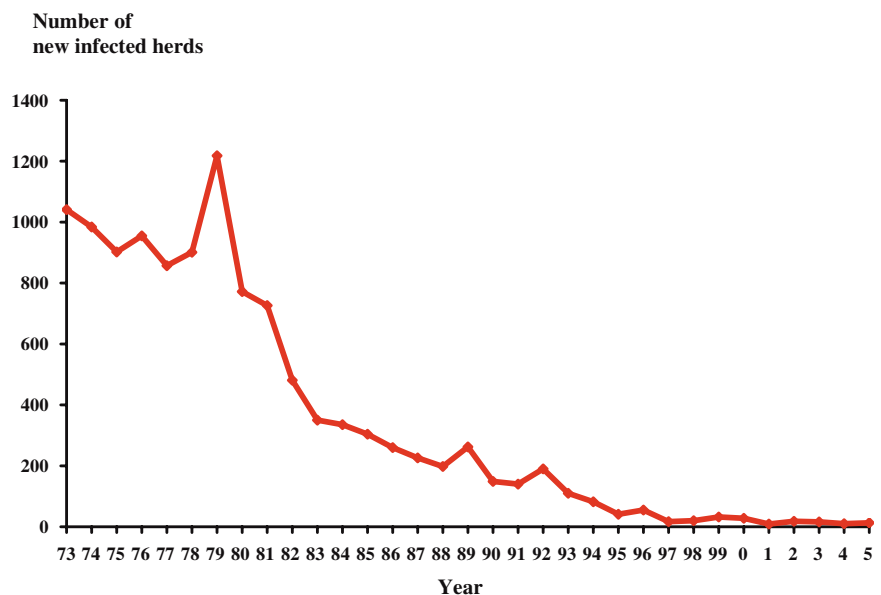
In Norway, prevalence data of herds with clinical ringworm are based on monthly reports submitted by the local veterinarians to the animal health authority. Herds are recorded once when the first case appears, and this reporting system of new infected herds has provided information about the ringworm situation since 1973 (Fig. 4) [68]. The number of new infected herds reported annually has decreased from approximately 1000 (annual incidence rate new infected herds 1.8%) before 1979 to less than 10 in 2006 (annual incidence rate 0.040%). Currently, a concerted action has been undertaken in order to focus the control measures on those herds still having problems with ringworm to eliminate the disease from these herds.

The control strategy in herds with a positive ringworm diagnosis largely follows the Russian

recommendations. All non-infected cows and calves are vaccinated. Infected animals, or those with a history of ringworm, have presumably developed protective immunity and are not vaccinated. Vaccination of cows during the last 2 months of pregnancy is postponed until after calving. This program is followed by vaccination of all calves born from day one and purchased animals with no vaccination status. Duration of immunity is long lasting and revaccination is not recommended. Development of clinical lesions is prevented as well as transmission of dermatophytes to other animals and contamination of the environment. The spores may survive for several years, therefore vaccination should continue for a period of 3–5 years once started. In a program for eradication of ringworm from herds, cleaning and disinfection is recommended as additional measures and contributing to reduction in the level of contamination. Improved hygiene shortens the time needed to achieve the goal of animals without signs of ringworm and the long-term goal of eradication of ringworm from the herd.

In Sweden, a different approach was employed concerning ringworm control, in a program aiming at improving hide quality [76]. Farmers organized in the “Faultless hide scheme” agreed to vaccinate the cattle and also focus on other measures to reduce hide damage. A beneficial effect on both ringworm prevalence and quality of the hides was observed in

Fig. 4 Number of new ringworm-infected cattle herds reported per year to the Animal Health Authority in Norway. Vaccination against bovine dermatophytosis started in 1979. Data adapted from study referenced herein [68]



the herds participating in the program, whereas the disease situation remained almost unchanged for those outside the scheme.

Vaccination Against Dermatophytosis in Companion Animals (Dogs, Cats and Horses)

Historical Perspective and Development Efforts

The dramatic effectiveness of vaccination programs for food and fur-bearing animals in many countries stimulated the search for an effective (and ideally, prophylactic) vaccine in companion animals. Historically, the biologics industry in Eastern Europe was active in development of vaccines for such use in horses, including the early “SP1” (Sarkisov-Petrovich-1) equine trichophytosis vaccine [15, 77]. These vaccines were never distributed widely to other countries, and faded from use as geopolitical events evolved. Notably, initial efforts often disclosed substantial host species differences in the ability of a vaccine product to protect against infection. For example, a commercial cattle vaccine based on *T. verrucosum* was reported to protect calves against *M. canis* infection, but to lack effectiveness in dogs [78]. Apparently, to stimulate long lasting and appropriate cell-mediated immunity, inactivated vaccines intended for companion animals required addition of an adjuvant. An adjuvanted vaccine formulation containing two inactivated strains of *T. equinum* was studied in horses [79]. The relative protection was 75% for epicutaneous challenge and 87% for challenge by commingling studies.

Only recently were efforts made to address vaccination of cats. Preliminary studies in a guinea pig model using live and inactivated vaccine formulations of two different strains of *M. canis* demonstrated best protection following challenge in animals vaccinated with the inactivated vaccine [80]. The vaccines were prepared according to a special protocol and stimulated DTH response; however, there was no mention of adjuvant content. The authors proposed follow-up studies in cats, but so far nothing has been published. Two experimental vaccine preparations have been studied in this host. A killed preparation of *M. canis* cell wall components, injected into 8–9-week-old kittens biweekly for five doses, induced both anti-dermatophyte antibody titers

and cell-mediated immunity (as measured by antigen-stimulated lymphocyte blastogenesis), but these immunologic responses were not as strong as those seen in natural infection. The vaccination procedure did not confer resistance to challenge infection either via direct spore application [33] or via commingling with an infected cat [34].

Subsequently, an experimental combined live-inactivated dermatophyte vaccine, consisting of attenuated live *Trichophyton equinum* plus killed components from two *Microsporum* strains, was tested in cats under laboratory conditions. The product was administered to kittens using several different protocols for up to 3 times at 2-week intervals. This vaccine induced a strong antibody response, but only slight and variable cell-mediated immunity. Six out of 10 cats vaccinated developed apparent, though mild, local infection with the vaccine strain at the injection site. The conferred response was not protective against direct challenge infection with *M. canis*, and did not provide a more rapid cure of the infection once established, but slightly reduced severity of the initial stages of the challenge infection [81]. In this same study (Fig. 5), the effectiveness of a commercially available killed vaccine product was evaluated in parallel with the

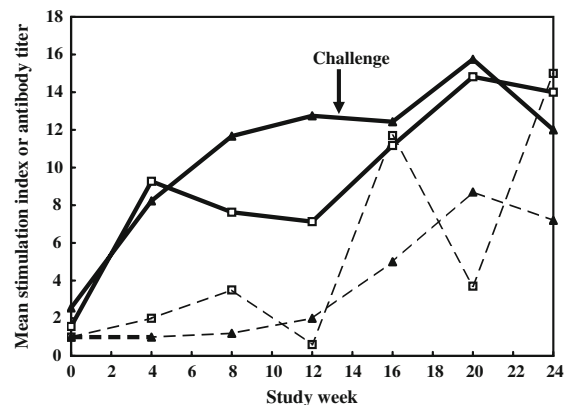


Fig. 5 Immunologic response in cats induced by an experimental dermatophytosis vaccine (□) and a commercial feline vaccine (▲). Note that both vaccines induced strong antibody titers (–) by Week 12, but only slight and variable cell-mediated responses (– –). Neither vaccine was protective against challenge infection at week 13 (arrow) via commingling with an infected cat. This study highlights the relative lack of importance of inducing a humoral immune response in feline dermatophytosis vaccines intended for prophylaxis. Data adapted from study referenced herein [80]

experimental vaccine, and was also found to lack prophylactic activity (see below).

A commercial vaccine product (Fel-O-Vax MC-K, Fort Dodge Laboratories) consisting of killed *M. canis* components in adjuvant was licensed in the United States for feline use in 1994. The major indication for this product was to reduce severity of, or hasten resolution of, the clinical signs of dermatophytosis in cats, rather than for use as a prophylactic vaccine. When tested in an experimental infection model in cats, the commercial vaccine did not prevent establishment of challenge infection, although the initial severity of the infection was slightly reduced. Moreover, the vaccine did not hasten resolution of the challenge infection as compared with unvaccinated controls [81]. Anecdotal reports of field experience with the vaccine were generally not positive, with the main perception being that though it appeared to reduce clinical signs in some cats, it did not prevent infection nor eliminate the fungus from an active infection. The product was withdrawn from the market by the manufacturer in 2003 and is no longer available.

A commercially developed inactivated *M. canis* preparation in aluminum hydroxide adjuvant (MICANFIN, Bioveta Inc.; later called Biocan M) was tested for prophylactic efficacy in 13 cats [82]. Following two subcutaneous or intramuscular injections 14 days apart, cats were challenged by direct topical spore application. Vaccinated cats developed only minute skin changes at the challenge site, consisting of scaling and papules, with the skin returning to normal at 28 days postchallenge or less, and negative fungal cultures at that time. In contrast, unvaccinated controls developed much more severe lesions; at day 28, lesions were still present and all animals were culture positive. Thus, there was some indication that this preparation had prophylactic properties. However, the product has now been replaced on the market by a preparation that does not contain an adjuvant, hence may or may not have the same properties (see below).

Currently Available Products and Recommended Uses

Dermatophyte vaccines for use in companion animals contain either attenuated or inactivated whole fungal

cells of different species. The Czech company Bioveta Inc. (Ivanovice na Hane, Czech Republic) has developed monovalent, inactivated, unadjuvanted *M. canis* vaccines for use in the cat (Biofel M *Plus*) and the dog (Biocan M *Plus*; a different formulation than Biocan M), and an attenuated live *T. equinum* vaccine intended for horses (Trichoequen) [83] (Table 1). Currently, Bioveta vaccines have market authorization in some countries mostly in Eastern Europe, but recently Biocan M has become available in Germany under the trade name RIVAC[®] Mikroderm[®] (Riemser Arzneimittel AG). It is difficult to formulate an informed opinion on the efficacy of these products, due to lack of available information in the scientific literature.

Currently, there are no published studies on the use of Biofel M *Plus* or Biocan M *Plus* for cats and dogs. Information about these products is only available from the manufacturer, claiming protection when used prophylactically and a shorter disease course when used in animals presenting signs of infection [83]. The manufacturer states that the feline vaccine can be administered to kittens at 8 weeks of age, and that immunity persists for 1 year. Puppies can be vaccinated from the age of 8 weeks, and they need two doses 10–21 days apart. Immunity is established 1 month after the last injection, and annual revaccination is recommended.

Bioveta's equine vaccine (Trichoequen) is based on the live-attenuated mutant strain *T. equinum* TE-M-141 [83, 84]. Foals can be vaccinated as early as 4 months of age. Two doses at 10–16 day intervals are required. Upon epicutaneous challenge, vaccinated horses show only short-term superficial skin changes which disappear spontaneously, whereas unvaccinated controls develop more severe and disseminated disease. Some therapeutic efficacy has also been demonstrated [84]. Duration of immunity is 1 year, therefore requiring annual revaccination.

Another dermatophyte vaccine (Insol[®] Dermatophyton, Boehringer Ingelheim) is available in several continental European countries and in the United Kingdom, but not in the Americas or Asia [85] (Table 1). It is a polyvalent, unadjuvanted, inactivated vaccine containing fungal species *T. verrucosum*, *T. mentagrophytes*, *T. sarkisovii*, *T. equinum*, *M. canis*, *M. canis* var. *distortum*, *M. canis* var. *obesum*, and *M. gypseum*. According to the

manufacturer's literature, it is produced using a special manufacturing process that enables the inactivated microconidia to stimulate a cell-mediated immune response [85]. For treatment of active infections in horses over 5 months of age, the manufacturer's recommended protocol is to administer two doses by deep intramuscular injection, 14 days apart. If no visible improvement occurs within 2 weeks after the second injection, a third injection may be given. For prophylactic use, two injections are given IM, 14 days apart, with repeat of the series every 9 months. Limited published investigations on the efficacy of Insol[®] Dermatophyton make it difficult to draw firm conclusions as to its most appropriate use. The only studies published are in horses, where the product was evaluated for therapeutic efficacy and not as a prophylactic [86, 87]. The latter studies are open, uncontrolled field studies in which horses with active infections were treated with the vaccine, and recovery monitored. It was claimed that up to 95% of horses were successfully treated, but it is unknown how many of these successful outcomes could have represented spontaneous recovery (an expected result in the majority of normal horses). Insol[®] Dermatophyton has also been recommended, at least in some countries, for use in dogs (over 6 weeks of age) and cats (over 10 weeks of age) as well, and even in rabbits [88]. Again, two doses, 2 weeks apart have been recommended [89]. In all species, local reactions in the form of swellings and/or pain may occur in up to 3% of vaccinated animals, and more generalized systemic malaise can occur in up to 1%. It must be emphasized that published studies on the efficacy of Insol[®] Dermatophyton as a prophylactic vaccine in any species are not available, nor have studies of any kind been published on its use in dogs or cats. Moreover, personal experiences and opinion expressed at a roundtable event at the Fifth World Congress of Veterinary Dermatology suggest limited efficacy [90]. Due to the lack of published information on this product, it is difficult to make specific recommendations for its use.

It appears that the goal of a widely available, truly prophylactic vaccine against dermatophytosis in dogs, cats, or horses has not been attained, but success in other species in several countries, increasing knowledge regarding the immune response to infections, and increasing globalization of manufacturing, marketing, and regulatory efforts hold promise that this goal will be eventually achieved.

Vaccination Against Dermatophytosis in Fur-Bearing Animals and Rabbits

In fur-bearing animals (including silver fox, blue fox, chinchilla, and mink) and rabbits, dermatophytosis is most often caused by *T. mentagrophytes* and *M. canis* [91–94]. Two vaccines have been used to prevent the disease in these animal species, both containing live attenuated cells of *T. mentagrophytes* [95, 96]. The Russian vaccine Mentavak, developed by researchers at the VIEV, Moscow [13] was used extensively in former Soviet Union in farmed foxes and rabbits [97]. A shift in the predominant dermatophyte species isolated from these animals was observed; *T. mentagrophytes* being most prevalent prior to the vaccination campaign and *M. canis* being isolated from most cases of ringworm after the campaign. The Russian vaccine was used during an outbreak of ringworm caused by *T. mentagrophytes* in two Swedish fox farms [98]. Adults and cubs were vaccinated by two injections and no new cases appeared after the second vaccination was completed. All cubs born the next year were vaccinated as *T. mentagrophytes* had been isolated from a few adult foxes and in environmental samples. No signs of ringworm were observed the following year and therefore vaccination was suspended during the next birth season. Samples collected from the environment and from animals were all negative. The authors concluded that vaccination and hygienic measures contributed to successful eradication of the disease by creating herd immunity and reducing level of environmental contamination. However, mock-vaccinated control animals were not included in this field trial and therefore the authors mentioned the need for further studies.

The vaccine Trichopelen (Bioveta, Ivanovice na Hane, Czech Republic) contains an attenuated strain of *T. mentagrophytes* and is indicated for use in chinchilla, rabbit, and fur-bearing animals [96] (Table 1). The vaccination schedule includes two doses by intramuscular injection with an interval of 8–12 days. Immunity is established one month after the last dose with an expected duration of minimum one year. In an experimental study in rabbits, animals vaccinated by the subcutaneous route at 11–14 days of age and those vaccinated intramuscularly at 4 months of age were all protected against epicutaneous challenge of a virulent strain of

T. mentagrophytes [99]. The vaccine strain survived in the muscle for 9–10 days and hair loss and some scaling was observed at the injection site confirming some residual virulence. In an outbreak of ringworm in a large breeding colony of rabbits, all female breeding animals were vaccinated with Trichopelen protecting approximately 90% of the animals [100, 101].

Kostro [102] isolated different strains of *T. mentagrophytes* var. *granulosum* from arctic fox and examined immunological properties in guinea pig and fox models. Two of six strains stimulated strong cell-mediated immunity. Guinea pigs immunized with these two strains were well protected against challenge with a virulent strain of *T. mentagrophytes*.

Efficacy and safety of an experimental vaccine against *M. canis* infection were evaluated in silver fox and blue fox [103, 104]. The polyvalent vaccine, containing live attenuated *T. equinum* and inactivated cells of two strains of *M. canis*, was given twice intramuscularly to four- and six-week old cubs. Five weeks post last vaccination, the animals were challenged by epicutaneous inoculation of a virulent strain of *M. canis*. Vaccinated animals presented a transient reaction with superficial scaling 7–14 days post inoculation, whereas the controls developed typical signs of ringworm. None of the animals showed general adverse reactions. However, at the injection site, hair loss and scaling appeared 2–4 weeks post vaccination. In a field trial, 32% of the animals examined presented similar local reactions lasting for 2–6 weeks. The live vaccine component was regularly cultured from crust material at the injection site.

Impact of Vaccination

Zoonotic Situation

Dermatophytosis in animals is an important zoonotic disease transmitted to man most often by direct contact with an infected animal or indirectly from the environment contaminated by fomites from the animal. Thus, dermatophytes belonging to the zoophilic group are the most prevalent when considering transmission from animals to man [2] including *M. canis*, *T. verrucosum*, *T. equinum*, and others. Control and prevention of the disease in the animal

reservoir is the most important measure to reduce occurrence in man. Ringworm transmitted from food and fur-bearing animals is an occupational disease of farmers and their households, veterinarians and people working in abattoirs and tanneries [105–107]. Exposure from companion animals, concerns owners of these animals and often include children [108]. Attention has also been given to the group of immunocompromised persons regarding risk of disseminated disease after exposure to dermatophytes [109–111].

The systematic vaccination campaign against cattle ringworm in the former Soviet Union has obviously contributed to a significant decrease in dermatophytosis in man caused by *T. verrucosum* [13]. In Norway, cattle ringworm is confined to less than 10 farms after almost 30 years of vaccination and in parallel, number of cases in the human population has decreased [112]. Today private and public diagnostic laboratories rarely isolate *T. verrucosum* indicating that ringworm transmitted from cattle to man is almost nonexistent. The former German Democratic Republic experienced a similar reduction in number of human cases of ringworm caused by *T. verrucosum* in the 1980s, and this was seen as a consequence of extensive use of vaccination in the cattle population [113]. Successful campaigns to eliminate the dermatophyte from the reservoir population clearly demonstrate the beneficial effect on the zoonotic situation. *M. canis* infections in companion animals are on the increase in some countries, and there are several reports on transmission to man [108, 114]. If an efficacious vaccine had been available, the experience from vaccination against cattle ringworm is likely to be applicable also in these animals.

Pelt and Hide Quality

In farms keeping fur-bearing animals, dermatophytosis is among the conditions reducing pelt quality and having serious economic consequences [115, 116]. When there is an increased risk of infection, vaccination may be a measure to avoid an outbreak. However, the importance to maintain good level of biosecurity and hygiene must be underscored. Vaccination has been used successfully in the former Soviet Union and some other Eastern European

countries to prevent the disease caused by *T. ment-agrophytes* [13].

In cattle, the hide is the most valuable by-product of the carcass. The economic significance of ringworm is related to the downgrading of hides as apparently healed skin lesions reappear after the tanning process (Fig. 6) [117]. The British Leather Confederation estimated losses in the UK leather trade industry from poor quality cattle hides to about £35 million per year of which ringworm accounted for 5% [116, 118]. In the international hide market, price setting is based on quality assessment by buyers and the level reflects prices obtained previous years. This system does not allow price differentiation between hides with and without ringworm scars. Moreover, prevalence of hides presenting ringworm lesions or damage by other causes is not recorded. A vaccination program was offered to farmers by the Swedish leather industry as one of several measures to improve hide quality [76]. Vaccination against bovine ringworm has contributed to improved quality of hides in Norway and Sweden and better prices on the European market [119, O Nafstad, personal communication]. This aspect is underscored in cost-benefit considerations of vaccination against cattle ringworm [120]. Obviously, other parameters also contribute as the zoonotic character of the disease, and in Norway, the costs related to restrictions

imposed on ringworm positive herds add to the bill and justify vaccination.

Concluding Remarks

Obviously, use of live attenuated vaccines against trichophytosis in cattle and fur-bearing animals has contributed to the control or eradication of ringworm in these species, positively impacting overall herd health and hide and pelt quality, and greatly diminishing zoonotic transmission and concurrent infection in farm personnel. Despite these resounding successes, however, one must exercise caution in translating these positive results to other clinical situations. For example, in addition to prophylactic use, manufacturers also often recommend therapeutic use, and claim effects on the duration of the course of infection. In the literature, convincing data on this procedure are lacking. Importantly, very few efficacy studies (prophylactic or therapeutic) have been conducted and published in companion animals, even where there is a commercial product on the market for such use. When such efficacy studies exist, they may consist of a very small number of animals, or may be uncontrolled (and therefore difficult to interpret), or they may have been done using a preparation different from the one that eventually appeared on the market. Indeed, the evidence for efficacy of commercially available companion animal vaccines is extremely limited at this point.

Dermatophyte vaccines with the most proven success are those which contain live attenuated fungal strains. However, vaccines containing live components with residual virulence are certainly not an alternative in companion animals. Close human contact increases the risk of transfer of the vaccine strain in those cases where a lesion develops at the injection site, and may be of increased concern in households with immunosuppressed humans. There is definitely a need for well documented, safe and efficacious vaccines against dermatophytosis in companion animals. Prospects for vaccine development should be based on insight into the immune mechanisms providing protection and immunodominant dermatophyte antigens stimulating a strong DTH reaction. Some areas for future research are listed in Box 1.

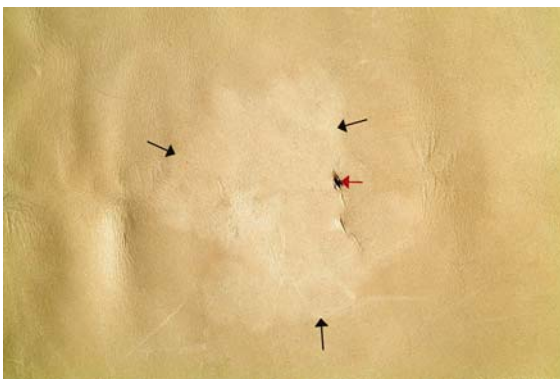


Fig. 6 Tanned cattle hide from a calf experimentally inoculated epicutaneously with a virulent strain of *Trichophyton verrucosum* producing a typical ringworm lesion 5 weeks after challenge. The black arrows point to the juncture between the paler, diseased hide and the normal-colored hide. The red arrow points to a defect following biopsy sampling. (Lund and Bratberg, unpublished)

Box 1 Future research directions

Several important questions remain regarding host–dermatophyte interactions, vaccine formulations and use, and how to stimulate protective immune response.

- Langerhans cells and dermal dendritic cells and interaction with dermatophytes. Study the maturation process, antigen uptake, antigen presentation, expression of co-stimulatory molecules, and production of cytokines. Develop a model for the study using dermatophytes and/or products thereof being a pathogen confined to epidermis.
- Targeting dendritic cells in skin with dermatophyte vaccines. Is it possible, or even desirable, to deliver antigens specifically to dendritic cells?
- Topical application of dermatophyte vaccine formulations—is it possible? Study immunogenicity of antigens applied onto skin and induction of Th1 and Th2 responses.
- Role of Toll-like receptors and link between innate and adaptive immunity in dermatophyte infections; TLR signaling pathways and triggering of adaptive immune response after immunization with dermatophyte vaccine formulations.
- Identification of immunodominant dermatophyte antigens and ability to stimulate Th1 type responses.
- Generation of memory T cells in dermatophyte infections and following immunization with dermatophyte vaccine formulations.
- Duration of immunity following vaccination against dermatophytosis.
- Public health significance, or lack thereof, in attenuated live-spore vaccines, notably in immunosuppressed humans.
- Development of safe and efficacious vaccines against *Microsporum canis* infection in cats and dogs; to include vaccination schedule, vaccination strategies for prevention and in outbreak situation and epidemiological follow-up to assess impact on zoonotic disease.

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