



# Evaluation of New Compounds Efficacy on Dermatophytosis Treatment in Cattle and Buffalo



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## Abstract

Dermatophytosis is superficial fungal infection of the hairs and outer layers of the skin caused by specialized group of fungi named dermatophytes. Dermatophytosis is worldwide zoonotic disease responsible for high economical losses in farm animals due to skin damages especially in the winter. Several compounds had been used for dermatophytosis treatment such as a chlorhexidine, salicylic acid, iodine-containing shampoos and tinctures, griseofulvin, thiabendazole and ethylenediamine dihydriodide; some of these treatments have been reported to be ineffective or even toxic to the host. Therefore, in this study we evaluate the efficacy of clove oil and local prepared culture filtrate vaccine on treatment of ringworm cases in cattle and buffaloes. Fifty diseased animals (cattle no; 30 and Buffalo no 20) had been treated with both preparations. The clove oil used as pure oil as topical spray 3 times daily for 10 successive days on the affected lesions. The culture filtrate vaccine used as suspension injected S/C with dose of 5ml and second dose 10 days later in. The results indicate strong antifungal action of clove oil and complete healing of ringworm lesions within 2 weeks as well as culture filtrate vaccine was effective therapy as emergency treatment in injectable form.

**Keywords:** Ringworm; Clove oil; Culture filtrate; Dermatophytosis; Trichophyton; Microsporum; Epidermophyton; Ectothrix; Eugenol; Endothrix; Cows; Buffaloes; Actidion

## Introduction

Ringworm or known as dermatophytosis is contagious fungal disease caused by dermatophytes species affecting both animals and human that mainly damage the superficial layer of skin, hair, nails and claw [1]. Only, three genera (Trichophyton, Microsporum and Epidermophyton) are known to be pathogenic for both human and animals [2]. Recently the high incidence of fungal infection mainly attributed to intensive use of immunosuppression drugs in man and poorly maintenance condition surrounding animals [3]. The diseased animals, infected fomites and contaminated environment act as main source and reservoir for infection among susceptible hosts through direct or indirect contact [4]. The disease prevalent more predominant in poorly closed contact housed animals which help in disease dissemination [5].

In particularly, the warm humid climate countries as in Africa and Egypt are favorable for spore formation and consequently highly infection [6]. Dermatophytosis mainly observed in young calves, which have high skin PH and immune system is ill developed [7]. The increasing in resistance to antifungal drugs had led for emergence of new compounds to avoid the toxic effects of traditional antifungal drugs. For a long time, Clove oil was widely used as antiseptic, analgesic and anesthetic

agent especially in dental practice [8]. Moreover [9] had been reported significant and strong antifungal efficacy of Clove oil active ingredient (Eugenol) in treatment of most fungal species including dermatophytes. Recently vaccination model appears to be of value in control and treatment of dermatophytosis in cattle [10]. So, this study aimed to evaluate the antifungal effect of both Clove oil and local prepared culture filtrate vaccine in treatment of dermatophytosis in cows and buffaloes calves under field condition.

## Material and Methods

### Clinical Examination and Samples Collection

Four-hundred animals were clinically examined for ringworm lesions from cows and buffaloes calves from a private feedlot farm in Minufiya governorate, Egypt. Fifty skin scrapings (50) were collected from 250 buffaloes calves and 150 cows' calves (20 and 30 skin scraping) respectively. The age ranged from 6 to 24 months. Clinical examination of affected calves showed clinical signs of ringworm (non-itched lesion of alopecia and/ circumscribed grayish-white, crusty, raised lesions) in ear and around eyes. Each sample was divided into two parts. One part was used for direct microscopic examination. The second

part was cultured on Sabouraud's dextrose agar (SDA) containing (Chloramphenicol 50mg/L and actidion (sigma) 0.5g/L) with addition of thiamin and inositol and incubation at 37 °C up to 3 week with daily examination.

### Mycological Examination

#### Direct Microscopical examination of samples

According to [11]. Briefly, one drop of 20% KOH in a glass slide with skin scraped sample and covered by cover slide with gently heating and left for 1 hour. Then examined under microscope or detection of (hyphea and spores around (ectothrix) or within the hairs (endothrix).

The identification of dermatophytes: through macroscopical identification of colony morphology as described by [12,13] that include the rate of growth, color, texture of the colony or consistency. While Microscopical morphology of the isolates was done by using wet mount preparation [14].

#### Culture filtrate

It is prepared according to [15] The isolated strain of

*T.verrucosum* was inoculated into Erlenmeyer flasks of 500ml containing 300ml of Sabouraud,s dextrose broth supplemented with thiamine and inositol, incubated at 37 °C for 4-6 weeks. The submerged growth of *T. verrucosum* was harvested and filtrated with 0.45um Millipore filter. Then mixed with adjuvant (Montanide) by equal amount and stored at 4 °C.

#### Antifungal Activity of Clove Oil

**Essential oil:** Clove oil was obtained from pharmacology department, National Research Center, Doki, Giza, Egypt, which dissolving in Tween80.

#### Field Treatment of Dermatophytosis by Colve Oil and Culture Filtrate Vaccine in Infected Cases

The clove oil used as pure oil as topical spray 3 times daily for 10 successive days on the affected lesions in (15 cases in cows and 8 cases in buffaloes). The culture filtrate vaccine used as suspension injected S/C with dose of 5 ml and second dose 10 days later in (10 cases in cows and 7 cases in buffaloes). Five cases from each species were left without treatment to serve as control cases.

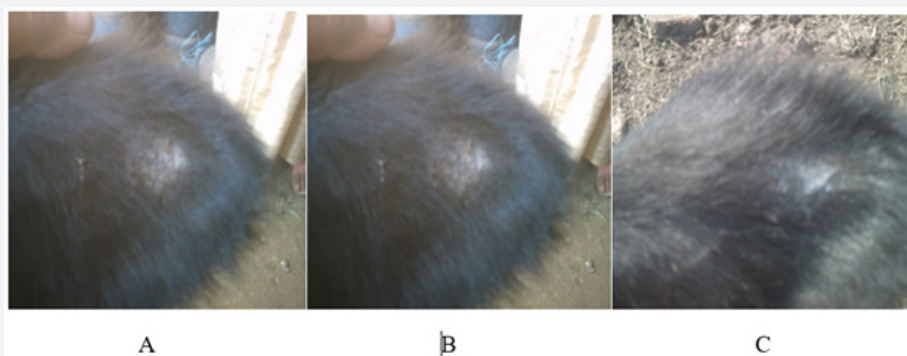
### Discussion

**Table 1:** Prevalence of dermatophytosis in cattle and buffaloes in relation to KOH and culture examination.

Total Number	Positive in Clinical Examination	%	Positive in KOH	%	Positive in Culture	%	
Cattle	150	30	20	22	73.3	14	46.7
Buffaloes	250	20	8	12	60	9	45
Total	400	50	12.5	25	-	17	-

**Table 2:** Results of treatment of dermatophytosis by clove oil and culture filtrate in cows and buffaloes.

Animal Species	Infected Cases	No of Animals Treated by Clove Oil	No of Cured Animals	%of Cured Animals	No of Animals Treated by Culture Filtrate	No of Cured Animal	%of Cured Animal
Buffaloes	20	8	7	87.5%	7	5	71.4%
cows	30	15	12	80%	10	7	70%



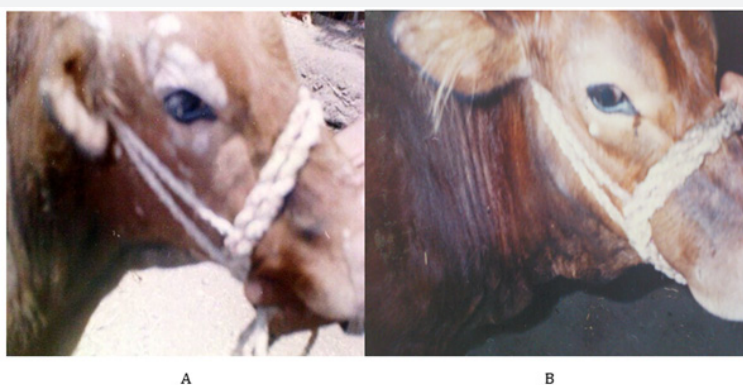
**Figure 1:** Treatment of ringworm by s/c injection of culture filtrate suspension in buffaloes and cows.  
 A. Ear of buffalo calf (1.5 year) with ringworm lesion, round area of hairless, grayish-white color lesion.  
 B. After first dose of culture filtrate injection, begin of hair growth, diminish area of alopecia.  
 C. 3week after first dose of culture filtrate, hair grow up and disappear of area of alopecia.

Cattle ringworm is the most common skin disease affecting animals and zoonotic to human. The resistance to antifungal drugs, costs of treatment, control obstacles and public health concern emerging importance for discovery of new compounds

of broad spectrum antifungal and low side effects for control of animal ringworm such as vaccination [16]. The overall prevalence of ringworm in cattle and buffaloes in this study was 12.5 % as showed in Table 1. Nearly prevalence rate of

10.75% in calves in Iran was estimated by [1]. While higher prevalence rates reported by [17] of 32.8% and 30.4% in calves respectively. Variation in prevalence rates may related to some factors as management, environmental condition, age and immune status of animal [18]. Table 1 clarified that result of KOH 20% examination in cattle and buffaloes were 73.3% and 60% respectively. This in constant with previous study of [19,20] proved that KOH 20% used as good diagnostic tool for ringworm examination. This study used culture filtrate vaccine in field animals suffering from ringworm lesions as injection. The results illustrated a good and significantly improvement and curing of the ringworm lesions by 2 doses of 5 ml s/c at 10 days intervals and hair completely grow up within 3 weeks after first dose in (7 and 10 buffaloes and cows' calves respectively) with curing rate 71.4% and 70% respectively as in Table 2 and Figure 1. Similar result of [21] confirmed the importance and beneficial efficacy of culture filtrate vaccine in treatment of bovine ringworm. Moreover [22] indicated the ability of dermatophytes vaccine as prophylactic measure in disease control. These also

supported by early studies of [23,24] they analyzed the results of the application of *T. verrucosum* vaccine on animals showing clinical signs of ringworm infection which illustrated high recovery rate as therapeutic and immunoprophylactic agent. On the other hand, clove oil was applied daily for 10 successive days in (8 and 15 buffaloes and cows' calves respectively). Healing of lesion was observed in (87.5% and 80% respectively) as shown in Table 2 and Figure 2. Clove oil from *Syzygium aromaticum* and eugenol have been widely used in folk medicine and have antiseptic, analgesic and anesthetic characters. Previous study of [25] proved that Clove oil and its active principle (eugenol) have tested for antifungal activity in animal models. Lo'pez et al. [26] found that clove oil possesses significant scavenging inhibition of dermatophytes species [27]. Ahlam et al. [28] through destructive changes in cell membrane composition. This supported by [29] concluded that clove oil has strong antifungal effect against dermatophytosis in animal models and such effect due to components such as Eugenol.



**Figure 2:** Field treatment of ringworm by clove oil.

- A. Calf of 1year suffering from ringworm in face and around eyes before clove oil application.  
 B. After 10 days treatment with clove oil, complete healing of lesion.

### Conclusion

This study spot highlights on new compounds that can be used in treatment of ringworm in cattle and buffaloes. Clove oil had a highly antifungal activity against dermatophytes infection as a local spray. Moreover, culture filtrate vaccine exhibited strong fungicidal action on ringworm cases in cattle and buffaloes calves.

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