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Antifungal Susceptibility of Dermatophytes Isolated from Domestic Calves in Thamar, Yemen

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Abstract: This research aimed to isolate and identify some dermatophytes which causing ringworm disease in calves in Thamar government, Yemen Republic and evaluate antifungal susceptibility of isolates against some common chemical compounds ($CuSO_4$+CaO, Formaldehyde and Hydrogen Peroxide and Amphotericin B was used as standard antifungal agent). A total of 139 samples were collected from head, chest and limbs of calves which infected by ringworm disease. Dermatophytes Test Medium (DTM) was used to differ between dermatophyte and non dermatophyte fungi. Only 11 isolates were considered dermatophytes. Macro and microcharacteristics were studied to identify the dermatophyte isolates. All isolates were identified as Trichophytonverrucosum. The identification of the isolates were confirmed by Assiut University Mycological Center (AUMC), Egypt and were given AUMC NO. 2407. *In vitro* antifungal susceptibility test was performed according to Committee for Clinical Laboratory Standards for filamentous fungi (NCCLS M38-A) guidelines. The study concluded that *T. verrucosum* could be major causative agent of ringworm disease in calves in Thamar, Yemen. Of all the farms, the farms of government sector had the highest ringworm disease prevalence rate. *In vitro* activity of Formaldehyde solution (10%) against *T. verrucosum* isolated from calves was more than mixture of cupric sulphate with calcium oxide and hydrogen peroxide. It could be used as antifungal drugs to treat ringworm disease in calves in Yemen.

Key words: Dermatophytes, calves, NCCLS, *in vitro*, antifungi

INTRODUCTION

The term ringworm or tinea is used to describe some of the superficial mycoses diseases and dermatophytosis. It is one of the commonest fungal skin diseases in human or animals (Bruner *et al*., 1981; Kelly, 1984; Blood *et al*., 1983). This disease generally infects cow and calves (3-10 months of age) than any other animals (Gudding *et al*., 1991; Rybnikar *et al*., 1991; Gudding, 1996). The reason of increasing the infection in calves is referred to the pH of the skin that is mild acidic to neutral (5.5-7) in calves while it is acidic (4) in cow (Blood *et al*., 1983). Incubation period of ringworm disease extends 2-4 weeks. The recovery from the infection occurs in some cases within 2-4 months (Fathi and AL-Samarai, 2000). Blood *et al*. (1983) and Kelly (1984) reported that the 80% of human infections especially baby infections coused by *Trichophyton* sp. transmitted from calves and *Microsporum canis* transmitted from dogs, also they found that the infections transmitted from animals to human are more virulence than the infections transmitted from human to human. The incidence of dermatophytosis varies according to climate and with the natural reservoirs. However, the pattern of the species of dermatophytes involved in dermatophytosis may be different in similar geographical conditions, both in humans and animals and it has been related, among other factors to the decline in the incidence of animal ringworm in these areas or the degree and closeness of animal to human contact. In Jordan, several species of *Microsporum* and *Trichophyton* were isolated and identified from calves and horses by Al-Ani *et al*. (2002) also Rhaymah (1999) isolated some species of *Microsporum* and *Trichophyton* from calves in Iraq. In the same context, the earlier species were isolated from human who live near the calves' farm (Maslen, 2000; Roman *et al*., 2001). Swai and Sanka (2012) reported that the major causative of skin diseases in cow is *Trichophyton verrucosum*. Terbinafin oil has ability to treat ringworm disease in calves while some researchers used Butenafin oil to treat ringworm in Guinea pigs and

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experimental mice (Arika *et al*., 1992; Jessup *et al*., 2000). Numbers of antifungal drugs are used to treat ringworm disease in animals such as itrakenazol, ketaconazol, voriconazol and tricosan (Inouye *et al*., 2001; Decun *et al*., 2001). Galuppi *et al*. (2010) found that fluconazole is inefficient *in vitro* against the dermatophytes isolated from animals, humans and environment while econazole and enilconazole were the most effective than another antimycotic drugs.

The aim of the present study was to isolate and identify dermatophytes which causing ringworm diseases in Thamar, Yemen and evaluate the efficiency of common antifungal agents against isolated dermatophytes.

MATERIALS AND METHODS

Samples: Three different farms in Thamar were chosen for these studies that are Rsabah farms, military economic corporation farm and Nihmy farm. The samples were collected from head, chest and limbs of calves which infected by ringworm disease. The samples were collected and treated according to Jungerman and Schwartzman (1972) and Muller *et al*. (1983).

Isolation of fungi: The samples were cultivated and isolated on Sabouraud Dextrose Agar (SDA) with Chloramphenicol (100 μ mL^{-1}) and Cycloheximide (50 μ mL^{-1}) (SDA CC) according to English (1980) and Fathi and AL-Samarai (2000).

Identification of fungi: A direct microscopic test was carried out according to Weitzman and Summerbell (1995) and Perea *et al*. (2001). Macro and micro charactrisitic were studied after cultivation on SDA according to Fathi and AL-Samarai (2000). Dermatophytes Test Medium (DTM) was used to differ between dermatophytes and non dermatophytes fungi according to Lennette *et al*. (1985). The confirmatory identification was performed in Assiut University Mycological Center (AUMC), Egypt.

In *vitro* antifungal susceptibility test: Three common chemical compounds were used for this test. The first one is the mixture of compound of 0.8 mg mL^{-1} of cupric sulphate ($CuSO_4.5H_2O$) and 0.8 mg mL^{-1} of calcium oxide (1:1), the second compound of 180 mg mL^{-1} of hydrogen peroxide (H_2O_2) andthe third compound of 39 mg mL^{-1} of formaldehyde (H.CHO) were used. These chemical compounds commonly used to treat ringworm disease by farmers. The Minimal Inhibition growth (MIC) and Minimal Fungicidal Concentration (MFC) were determined according to Committee for Clinical Laboratory Standards for filamentous fungi (NCCLS M38-A) guidelines (NCCLS, 2002). The results were compared with amphotericin B as standard antifungal agent.

Statistical analysis: A Completed Random Design (CRD) was applied in this experiment. Statistical analysis of data was performed by analysis of variance (SAS, 2002).

RESULTS AND DISCUSSION

Isolation and identification: The results in Fig. 1 indicated that the total 139 isolates (Dermatophtes and non dermatophytes) have been isolated from calves infected by ringworm disease in three different farms. A significant differences ($p<0.05$) showed between the farms in the number of fungal isolates. A highest number of fungal isolates was isolated from Rsabah farms. A health care and hygiene play major role in the reducing skin infections between animals while the spirant between animal especially in Winter induces the infection (In Winter, the animals converge with each others for heating) (Hanlin and Uiloa, 1979; Muller *et al*., 1983).

DTM was used to differ between dematophytes and non dermatophytes. Eleven dematophyte isolates were obtained from 139 isolates (Fig. 2). The percentage of non dermatophyte isolates showed significant increases compared to the percentage of dermatophytes and this result agreed with Gromadzki *et al*. (2003). DTM contains phenol red indicator to detect a secondary alkaline metabolites that produce them the dermatophytes whereas the non dematophytes produce a secondary acidic metabolites. The changes of the yellow color medium to red indicate that the isolates are dematophytes.

A dermatophyte identification criteria were studied to identify the dermatophyte isolates, those characteristics

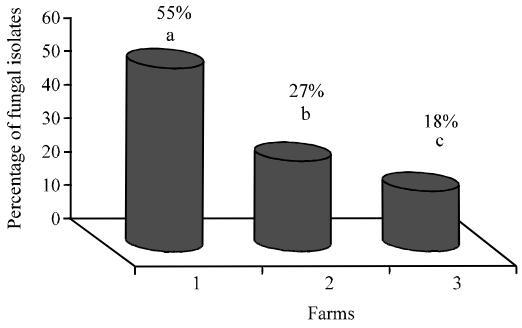

Fig. 1: Percentage of fungi isolated from calves in Thamar, Yemen. Farm 1 = Rsabah, farm 2 = Military Economic Corporation and farm 3 = Nihmy. The percentage with different letters notification are significant at $p<0.05$

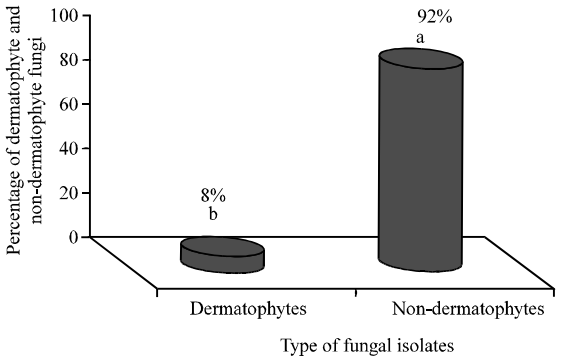

Fig. 2: Percentage of dermatophyte and non dermatophyte fungi isolated 3from calves in Thamar. The percentage with different letters notification are significant at $p<0.05$

Table 1: Identification of dermatophyte isolates (10 isolates) in Assiut University Mycological Center, Assiut, Egypt

Identification	*AUMC No.	Sample No.
Trichophyton verrucosum	2407	1
Isolates identified by researcher is 10 isolates	▲	

*AUMC No: Cultures are deposited in the culture collection of Assiut University Mycological Center; ▲ = *T. verrucosum* (10)

include a gross colony on Sabouraud agar (Front, reverse and texture), microscopic features (Microconidia, macroconidia and chlamydoconidia) colony color on corn meal agar with 2% dextrose, urea dextrose agar, hair penetration test, growth rate on trichophyton agar and growth rate on boiled rice. The dermatopyte isolates were identified as *Trichophyton verrucosum*. The features of isolates were similar to that described by Campbell and Stewart (1980). The isolates were sent to Assiut University Mycological Center (AUMC) in Egypt to confirm the identification. The isolates were deposited in the culture collection and were given AUMC NO. 2407. (Table 1).

The percentage of *T. verrucosum* isolated from farms of calves is illustrated in Fig. 3. The results showed significant decreases ($p<0.05$) in the Nihmy farm (Native sector) compared to the government sector (Rsabah farms and military economic corporation farm). Even though that there are a many species have ability to cause the ringworm disease (*T. megnini*, *T. equinum*, *T. mentagrophytes* and *M. canis*) in calves but *T. verrucosum* is considered a major causative for this disease (Akbarmehr, 2011).

The minimum inhibitory concentration ($\mu g\ mL^{-1}$) of the chemical compounds (cupric sulphate with calcium oxide, hydrogen peroxide, formaldehyde and Amphotericin B required to inhibit the growth of 50% (MIC 50) and inhibit the growth of 90% (MIC 50) of *T. verrucosum* are shown in Fig. 3. The three common

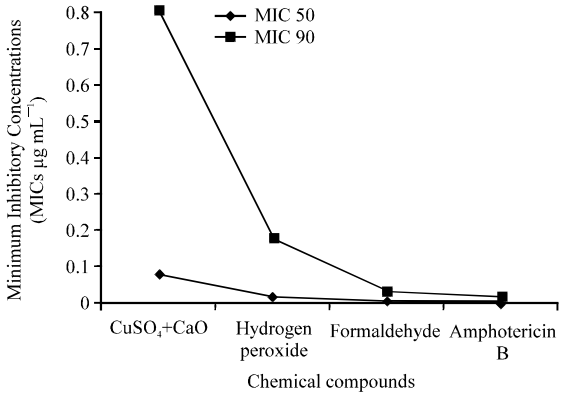

Fig. 3: Minimum inhibitory concentration of chemical compounds required to inhibit the growth of 50% (MIC 50) and Inhibit the growth of 90% (MIC 90) of *T. verrucosum* isolated from calves. Range: $CuSo_4$+CaO: 0.008-80, Hydrogen Peroxide: 0.0018-1.8, Formaldehyde: 0.00039-0.039, Amphotericin B: 0.0002-0.2

chemical compounds (cupric sulphate with calcium oxide, hydrogen peroxide and formaldehyde) inhibited the growth of *T. verrucosum*. The MIC 50 ranged from 0.0039-0.08 while the MIC 90 ranged from 0.039-0.8 $\mu g\ mL^{-1}$. Amphotericin B showed a high inhibitory activity compared to the common chemical compounds. The MIC 50 and MIC 90 of Ampotericin B were 0.002 and 0.02, respectively. Although, the effectiveness of formaldehyde is less than effectiveness of amphotericin B but it is inexpensive. Also, compare these results with study of Barchiesi *et al*. (2001) demonstrated that formaldehyde showed a high activity compared to itraconazole and posaconazole.

A many of farmers treat ringworm diseases in animals by a cheap chemical compounds such as formaldehyde, mixture of cupric sulphate and calcium oxide, potassium iodide and benzoic acid (Soares and Cury, 2001). These compounds economically are a lower cost than Amphotericin B, ketaconazol, tricomycine and griesofolvin. The present study supported results of Renner (1992) who treated ringworm diseases in calves by injection the formaldehyde solution (10%) intravenousaly.

CONCLUSION

The results of the present study reported that *T. verrucosum* was a major causative for ringworm disease in calves in Thamar, Yemen. The disease was more extensive in farms of government sector. *In vitro* activity of formaldehyde solution (10%) against *T. verrucosum* isolated from calves was more than mixture of cupric

sulphate with calcium oxide and hydrogen peroxide. It could be used as antifungal drugs to treat ringworm disease in calves in Yemen.

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REFERENCES

Akbarmehr, J., 2011. The prevalence of cattle ringworm in native dairy farms of Sarab city (East Azarbayjan province), Iran. Afr. J. Microbiol. Res., 11: 1268-1271.

Al-Ani, F.K., F.A. Younes and O.F. Al-Rawashdeh, 2002. Ringworm infection in cattle and horses in Jordan. Acta Vet. Brno, 71: 55-60.

Arika, T., M. Yokoo and H. Yamaguchi, 1992. Topical treatment with butenafine significantly lowers relapse rate in an interdigital tinea pedis model in guinea pigs. Antimicrob. Agents Chemother., 36: 2523-2525.

Barchiesi, F., D. Arzeni, V. Camiletti, O. Simonetti, A. Cellini, A.M. Offidani and G. Scalise, 2001. *In vitro* activity of posaconazole against clinical isolates of dermatophytes. J. Clin. Microbiol., 39: 4208-4209.

Blood, D.C., O.M. Radostits, J.A. Henderson, J.H. Arundel and C.C. Gay, 1983. Veterinary Medicine. 6th Edn., Bailliere Tindal and Cassel Ltd., London.

Bruner, D.W., W.A. Hagan and J.H. Gillespie, 1981. Hagen and Bruner's Infectious Diseases of Domestic Animals: With Reference to Etiology, Pathogenicity, Immunity, Epidemiology, Diagnosis and Biologic Therapy. 7th Edn., Cornell University Press, Ithaca, New York, USA., Pages: 815.

Campbell, M.C. and J.L. Stewart, 1980. The Medical Mycology Handbook. John Wiley and Sons Inc., USA., ISBN-13: 9780471047285, Pages: 436.

Decun, M., I. Tibru, I. Nichita, A. Motar and V. Burghelea, 2001. Tricosan: A new antifungal agent for treating ring worm in cattle. Revista Romana Med Vet., 3: 293-298.

English, P.M., 1980. Medical Mycology. Edward Arnold Publishers, London, UK.

Fathi, H.I. and A.G. AL-Samarai, 2000. Prevalence of Tinea capitis among school children in Iraq. Eastern Mediterranean Health J., 6: 128-137.

Galuppi, R., A. Gambarara, C. Bonoli, F. Ostanello and M.P. Tampieri, 2010. Antimycotic effectiveness against dermatophytes: Comparison of two *in vitro* tests. Vet. Res. Commun., 34: 57-61.

Gromadzki, S., R. Ramani and V. Chaturvedi, 2003. Evaluation of new medium for identification of dermatophytes and primary dimorphic pathogens. J. Clin. Microbiol., 41: 467-468.

Gudding, R., 1996. Cost-benefit considerations of vaccination against ringworm in cattle. Acta Vet. Scand., 90: 67-68.

Gudding, R., B. Naess and O. Aamodt, 1991. Immunisation against ringworm in cattle. Vet. Rec., 128: 84-85.

Hanlin, R.T. and M. Uiloa, 1979. Atlas of Introductory Mycology. Hunter Publishing Company, Winston-Salem, North Calorina, USA., pp: 3-8.

Inouye, S., K. Uchida and H. Yamaguchi, 2001. *In-vitro* and *in-vivo* anti-*Trichophyton* activity of essential oils by vapour contact. Mycoses, 44: 99-107.

Jessup, C.J., M.A. Ghannoum and N.S. Ryder, 2000. An evaluation of the *in vitro* activity of terbinafine. Med. Mycol., 38: 155-159.

Jungerman, P.F. and R.M. Schwartzman, 1972. Veterinary Medical Mycology. Lea and Febiger, Philadelphia, PA., USA., ISBN-13: 9780812103229, Pages: 200.

Kelly, W.R., 1984. Veterinary Clinical Diagnosis. 3rd Edn., Bailliere and Tindal, London, ISBN: 0812107675, pp: 22-40.

Lennette, E.H., A.A. Palons, W.J. Havser and H.J. Shacony, 1985. Manual of Clinical Microbiology. 4th Edn., Am. Soc. Microb., Washington, DC., USA., pp: 763-768.

Maslen, M.M., 2000. Human cases of cattle ringworm due to *Trichophyton verrucosum* in Victoria, Australia. Australasian J. Dermatol., 41: 90-94.

Muller, G., H.R. Kirk and D.W. Scott, 1983. Small Animal Dermatology. 3rd Edn., W.B. Saunders Company, Canada, ISBN-13: 9780721666099, Pages: 889.

NCCLS, 2002. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi. NCCLS Document M38-A. The National Committee for Clinical Laboratory Standards, Wayne, Pennsylvania, USA.

Perea, S., A.W. Fothergill, D.A. Sutton and M.G. Rinaldi, 2001. Comparison of *in vitro* activities of voriconazole and five established antifungal agents against different species of dermatophytes using a broth macrodilution method. J. Clin. Microbiol., 39: 385-388.

Renner, J.E., 1992. A new treatment for trichophytosis of cattle. Deutsche Tierarztliche Wochenschrift, 99: 208-210.

Rhaymah, M.S., 1999. Clinical and therapeutic study of ringworm in bull calves. Iraqi J. Vet. Sci., 12: 53-62.

Roman, C., L. Massai, C. Gianni and C. Crosti, 2001. Case reports. Six cases of infection due to *Trichophyton verrucosum*. Mycoses, 44: 334-337.

Rybnikar, A., J. Chumela, V. Vrzal and V. Krupka, 1991. Immunity in cattle vaccinated against ringworm. Mycoses, 34: 433-436.

SAS, 2002. SAS User's Guide: Statistics. Statistical Analysis Systems Institute, Cary, NC., USA., ISBN: 0917382374.

Soares, M.M. and A.E. Cury, 2001. *In vitro* activity of antifungal and antiseptic agents against dermatophytes isoletes from patients with tinea pedis. Brazilian J. Microbid., 32: 130-134.

Swai, E.S. and P.N. Sanka, 2012. Bovine dermatophytosis caused by *Trychophyton verrucosum*: A case report. Vet. World, 5: 297-300.

Weitzman, I. and R.C. Summerbell, 1995. The dermatophytes. Clin. Microbiol. Rev., 8: 240-259.