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ISOLATION AND IDENTIFICATION OF DERMATOPHILUS BACTERIA FROM THE SKIN LESIONS OF CATTLE

M. A. Mannan[1], M. S. R. Khan[1], M. M. Rahman[1], F. Begum[2] and M. Z. Uddin[1]*

Department of Microbiology and Hygiene, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

ABSTRACT

The study was conducted to isolate the *Dermatophilus* species from the skin disease of cattle in and around BAU campus during the period from May 2008 to April 2009. A total of 105 skin diseased cattle head were examined where 68 were male and 37 were female ranging from 1 month to12 years of ages. 11 (10.476%) patients were found to have typical lesions of Bovine Dermatophilosis. The rural household farm, young, male and the indigenous cattle were more susceptible. The *Dermatophilus* species were found Gram positive, branching and filamentous typical form of mycelial elements in Giemsa's staining. It was less visible in Gram's staining. The isolated agents produced only acid from dextrose but did not produce acid and gas from lactose, mannitol, maltose and sucrose. The catalase and the urease test were positive but indole test was negative. β-hemolysis also appeared in early stage on blood agar. The isolated agents were more sensitive to Amoxicillin (70%) followed by Ampicillin (60%) and Cephalexin (60%). Kanamycin (10%) and Ciprofloxacin (10%) was the lowest sensitive to it.

Keywords: *Dermatophilus* species, cattle, skin lesions, identification, isolation

INTRODUCTION

Dermatophilus bacteria cause Dermatophilosis in different kinds of animal including human beings. Approximately 10% of cattle are affected in West and Central Africa and in some Caribbean islands. It is commonly called cutaneous streptothrichosis in cattle, goats and horses. It is termed as lumpy wool disease in sheep when the wooly areas of the body are affected (Msami *et al.* 2001). Bovine Dermatophilosis is caused by *Dermatophilus congolensis,* is a facultative anaerobic actinomycete. Its chronic form is associated with tick infestations (*Amblyomma variegatum*). Tick control can reduce the prevalence of Dermatophilosis (Ali *et al.* 2003). Hides and skins of our country possess export sector to develop our economic condition and to release poverty from Bangladesh. That is why it is essential to know how we can protect these valuable goods. The prevalence, clinical features and treatment of Bovine Dermatophilosis in Bangladesh were studied only based on clinical observation (Nooruddin and Khaleque, 1986). The detailed confirmation of the *Dermatophilus* species based on the cultural properties, morphology, biochemical test and antibiotic sensitivity test was not sufficient. For better prevention and control strategy of bovine Dermatophilosis, isolation, identification and characterization of the causal agent is a prerequisite. Therefore, the present study was conducted to isolate and identify the *Dermatophilus* species from the skin lesions of cattle in and around BAU campus of Mymensingh District.

MATERIALS AND METHODS

Period and place

This study was carried out in the Department of Microbiology and Hygiene, Faculty of Veterinary Science, BAU, Mymensingh during the period from May 2008 to April 2009.

Sample collection

The samples from 105 cattle suffering from skin disease were collected in consideration to Dermatophilosis. The samples comprising of skin scraping, scabs, crusts and plucked hair (Carter and Changappa, 1993) were collected with proper aseptic measures. Swabs were collected by sterile swab sticks applying gentle rubbing.

Culture media and reagents used

The specimens were inoculated into liquid and solid media chronologically. Nutrient broth (NB), Nutrient agar (NA), Blood agar (BA), Eosin methylene blue (EMB) agar, MacConkey (MC) agar and Salmonella-Shigella (SS) agar media were used (Yardley, 2004).

*Corresponding author's e-mail : 1971bangla@gmail.com.

Isolation and identification of the bacteria

The test tubes containing collected skin sample in nutrient broth were incubated at 37°C for 24 hours. The subculture were also made on NA, BA, EMB agar, MC agar and SS agar media and incubated at 37°C for over night. The identification of the organisms was performed by the tests as described by Gordon (1964), Silva *et al.* (2003), Yardley (2004) and Anon (2007). Based on morphological and staining characteristics, Hemolytic activities on blood agar, biochemical characteristics and antibiotic sensitivity test, the bacteria were isolated and identified.

Staining method for *Dermatophilus* species

A smear was prepared from bacterial colony for Gram's staining according to the method described by Anon, 2007 and observed under microscope.

Fermentation reaction with five basic sugars

The carbohydrate fermentation test was performed by inoculating a loopful of nutrient broth culture into the test tubes containing five basic sugars (dextrose, maltose, sucrose, lactose and mannitol) and incubated at 37^0C for 24 hours. Acid production was indicated by the change of colour of media from pink to yellow and gas production was indicated by the appearance of gas bubbles in the inverted Durham's tubes (Gordon, 1964).

Catalase test

The catalase test was performed by using one-half loopful of bacterial growth and mixed with a drop of Superoxol (30% hydrogen peroxide) on a glass slide and finally formations of bubbles were observed (Silva *et al.*, 2003).

Urease production test

The isolated organisms were inoculated in urea agar slant and incubated at 37^0C for four hours. After the incubation, the test tubes were examined for any change or reaction in the media. The samples having no change in the slant was considered as negative for urease production by the organisms (Merchant and Packer, 1967).

Indole production test

The test was carried out according to Merchant and Packer (1967). The organism was grown in peptone water for 48 hours. Then an amount of 0.5 ml of kovac's reagent was added to 2 ml of culture, mixed thoroughly and examined after 1 minute. The appearance of red layer either on the surface or in any part or whole of the medium was considered as positive test and no red colour observed indicate negative test.

Antibiotic sensitivity test

In vitro antibiotic sensitivity test were done using disc diffusion test following the method of Gordon (1964). Depending on the area of the zone diameter for individual antibiotic disc, it was recorded as sensitive, intermediate and resistant. The following antibiotics that were tested against selected organisms with their disc concentration.

Table 1. The antibiotic discs with concentration used against *Dermatophilus* species

Name of the antibiotics	Disc concentration (µg / disc)
Ampicillin (A)	18
Erythromycin (ER)	15
Cephalexin (CP)	30
Amoxicillin (AX)	10
Ciprofloxacin (CI)	05
Kanamycin (KA)	30
Chloramphenicol (CK)	30
Sulphamethaxzol & Trimethoprim (SXT)	24
Tetracycline (TE)	30

Statistical analysis

Data were analyzed by Chi-square (χ^2) test to observe the significant influence of various parameters on the disease using Statistical Package for Social Science, SPSS Version 13.0 (Coakes *et al.,* 2006).

RESULTS AND DISCUSSION

Prevalence of bovine dermatophilosis

A total number of 105 cattle with skin disease were examined in this research work and 11 (10.476%) patients be found to have typical lesions of Bovine Dermatophilosis which was very similar to the findings of Soltyas (1965) who reported 11.21% prevalence in cattle in Sudan. The results of prevalence study of Dermatophilosis in cattle are presented in Table 2. A higher prevalence of Dermatophilosis was reported in rural household farm (11.842%) than that of the intensive dairy farm (6.896%) that correlated with the observation of Berhanu and Woldemeskel (1999). The highest prevalence in rural household farm might be due to poor management system, malnutrition and no regular vaccination compared to intensive dairy farm.

Table 2. Prevalence of dermatophilosis under farm and rural condition

Management system	Animal examined	Animals infected	%
Intensive dairy farm	29	2	6.896
Rural household farm	76	9	11.842**
Total	105	11	10.476
Chi-square test (P-value)	0.000		

** Significant at $p<0.01$.

Cultural properties on growth media

Development of turbidity and formation of sediment occurred in nutrient broth in case of positive cases. All strains of *Dermatophilus* species grew well aerobically at 37^0C on nutrient agar. The bacterial colonies became visible in 24-48 hours. Initially they were small with 1mm in diameter and grey-yellowish colour. After 3-4 days, the bacterial colonies could reach to 3mm in diameter, they had a rough surface and a yellow-golden pigmentation (Fig. 1). At 24 hours, aerobic culture on blood agar plates incubated at 37^0C typically showed tiny (0.5 to 1.0 mm), round to square or irregular, grayish-white, raised, very rough granular colonies, adherent and usually pitting the medium (Fig. 2). Upon further incubation, these generally became yellowish and then developed orange colour in the more crowded areas where β-hemolysis also appeared in early stage that was supported by Gordon (1964) and Anon (2007). No growth of the test organisms were found on EMB agar, SS agar and MC agar media.

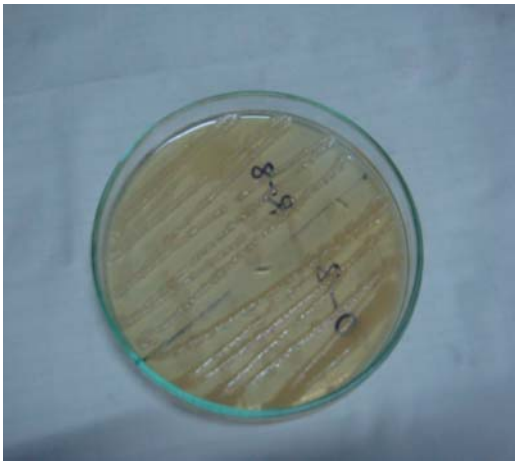

Fig. 1. Nutrient agar media showing yellowish colonies.

Fig. 2. Blood agar media showing hemolysis.

Microscopic examination of smear stained

In Giemsa's staining, the causal agent (*Dermatophilus* spp.) was Gram positive, branching, filamentous, rod, aerobic and typical form of the organisms composed of fine mycelial elements. In Gram's staining, these bacteria were less visible. Only branching and filamentous shaped were seen (Fig. 3) that was supported by the report of Jones *et al*. (1997) and Msami *et al.* (2001).

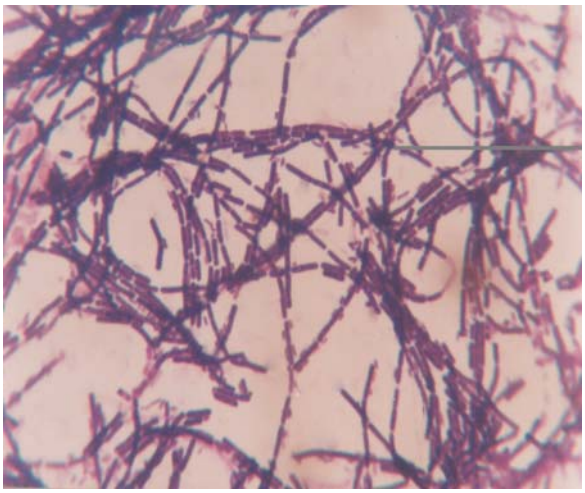

Fig. 3. Gram positive, branching filamentous, rod shaped *Dermatophilus* species in Gram's staining (x100).

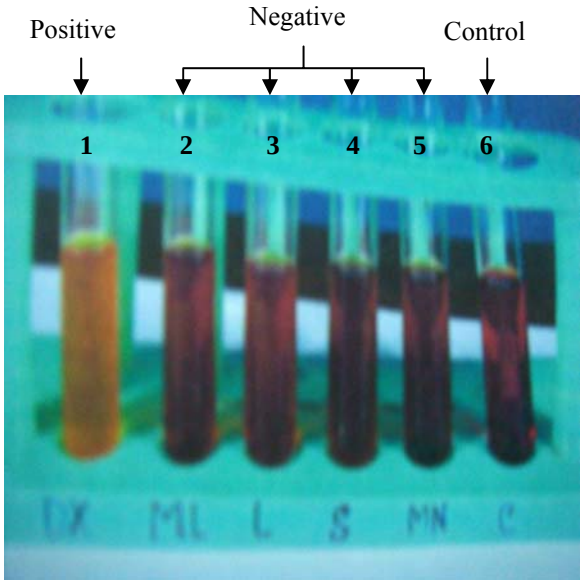

Fig. 4. Sugar fermentation test showing production of only acid from dextrose but not fermented maltose, lactose, sucrose and mannitol (1=Dextrose, 2=Maltose, 3=Lactose, 4=Sucrose, 5=mannitol and 6=Control).

Biochemical properties

D. congolensis produced only acid from dextrose but did not produce gas. The causal agents did not produce acid and gas from lactose, mannitol, maltose and sucrose (Fig. 4 and Table 3). Observation of bubbles in catalase test indicated the positive results and these bacteria showed the positive urease test by changing the colour of media. The isolated bacteria did not produce any red layer either on the surface or in any part or whole of the medium and gave the negative indole test that was supported by Merchant and Packer (1967).

Table 3. Results of the biochemical test of the isolated *Dermatophilus* species

Products	Basic five sugars				
	Dextrose	Lactose,	Mannitol	Maltose	Sucrose
Acid	+	-	-	-	-
Gas	-	-	-	-	-

+ = Fermentation, - = No fermentation.

Results of antibiotic sensitivity test

The antibiotic sensitivity and resistance patterns were studied against differof antiiscs following the method of Oduye (1976) and the results are presented in Table 4. The *Dermatophilus* species were 70% sensitive to Amoxicillin (AX) followed by 60% to Cephalexin (CP) and Ampicillin (A) due to Gram positive bacteria (Fig. 5). On the other way, 80% were resistant to the Kanamycin (KA) followed by 70% to Ciprofloxacin (CI).The results of antibiotic sensitivity test were nearly similar to Hemobade *et al.* (1979) and Aning (1996). The variation of antibiotic sensitivity and resistance patterns on same groups of antibiotic against the isolated *Dermatophilus* species might be owing to genomic variations, chromosomal or plasmid mutation of the organisms and repeated use of antibiotics.

Table 4. Results of the efficacy of antibiotic sensitivity and resistant pattern of the isolated *Dermatophilus* species from affected cattle

	A (%)	AX (%)	CP (%)	KA (%)	CI (%)	CK (%)	ER (%)	SXT (%)
Sensitive	60	70	60	10	10	20	30	40
Intermediate	20	20	30	10	20	30	50	40
Resistant	20	10	10	80	70	50	20	20

A = Ampicillin, CI = Ciprofloxacin, KA = Kanamycin, AX = Amoxicillin, CK = Chloramphenicol, SXT = Sulphamethaxzol & Trimethoprim, CP = Cephalexin, ER = Erythromycin.

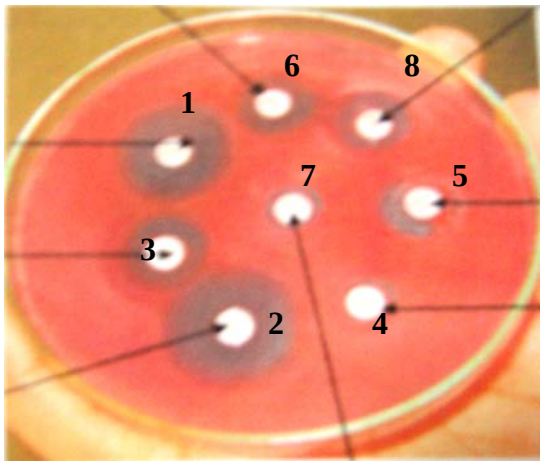

Fig. 5. Antibiotic sensitivity and resistant pattern of *Dermatophilus* species isolated from skin lesions of Cattle.

Fig. 6. Alopecia, erosion and wetting appearance observed in neck region of the body of a crossed heifer.

Clinical observations

Inspection of the skin surface revealed a partially dried and serous exudate which was perforated by hair. As the disease progressed, these materials formed crusts on the skin. In such instances, the skin crusts appeared moist and hyperemic (Fig. 6) with minor hemorrhage occurring in some cases on the forequarters and neck or along the back. In conclusion, it could be said that the serological study, molecular characterization and detection of immune responses in cattle might be necessary for specific isolation and characterization of *Dermatophilus congolensis.* But these studies were hardly possible due to some limitation. The prevalence of Dermatophilosis observed in and around BAU campus was 10.476%. Therefore, they have economic impact on the livestock production. So, proper preventive measures should be taken.

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