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BOVINE MASTITIS CAUSED BY *Candida* Spp.: REPORT OF THREE CASES NOT RESPONDING TO ANTIBACTERIAL AGENTS

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المستخلص

التهاب ضرع الأبقار من الأمراض متعددة المسببات و الذي يؤدى إلى خسائر اقتصادية نتيجة لخفض انتاج اللبن. تعتبر منطقة شرق النيل بولاية الخرطوم من المناطق ذات المعدلات العالية من الإصابة به ذا النوع من الأمراض , حيث هناك العديد منها لا تستجيب للعلاج بمضادات البكتيريا. أجريت ه ذه الدراسة لمعرفة ما إذا كان المسبب هو بكتيريا مقاومة لمضادات البكتيريا أم مسبب آخر. تم اخ ذ عينات لبن من اربعة وثلاثين بقرة مصابة تلقت علاجا مسبقا بمضادات البكتيريا نيومايسين وسيفالوكسين {كعلاج موضعى} و البنسلين {كعلاج عام} ثم تمت زراعتها في أوساط خاصة لنمو الفطريات. ثلاثة من بين ه ذه العينات نمت بشكل واضح كمستعمرات مخاطانيق, تم عمل مسحات و صبغت بالجرام التي أوضحت وجود خمائر. تم إجراء الاختبارات التأكيدية وتم تشخيص وجود نوعين من جنس كانديدا {المبيضات} و هي كانديدا ريوقوزا (A & B) وكانديدا تارتارفورانس. بناءا على معرفتنا يعتبر عزل ه ذه الأنواع الثلاثة من ضرع أبقار مصابة بالتهاب الضرع هو الأول من نوعه في السودان. كما انه قد يكون الاول لعزل كانديدا تارتارفورانس من ضرع أبقار مصابة بالتهاب.

Abstract

The present study investigated the causative agents among 34 non responsive bovine mastitic cases for routine antibacterial agents (neomycin, cefaloxin, penicillin). Milk samples were collected cultured for fungi and isolated organisms were identified using standard

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mycological procedures. Three of the 34 cows (8.8%) revealed yeasts growth. The yeasts were identified as *Candida rugosa* (two isolates) and *Candida tartarivorans*. The study concluded that *Candida* species represent a significant role (8.8%) among not responding to antibacterial treatment dairy cows. Such unresponsive cases should be considered for regular routine fungal culture regularly in order to initiate specific antifungal treatment before udder become completely damaged.

Key words: Candida, cow, mastitis

Introduction

Mastitis in dairy cows even more, affects the production and quality of milk. It continues to be an important problem for the dairy industry, despite all the advancements in veterinary diagnosis and therapeutics. *Candida spp* may constitute part of the normal microbiota in various anatomical sites. Consequently, their involvement in pathological processes is secondary, often as a sequel to prolonged antibacterial chemotherapy, stress and/or substandard animal management practices, such as overcrowding and mal nutrition. Immune insufficiencies were important predisposing factors in human mycotic infections, but seem to have limited significance in animals (Segal and Elad, 1998). Candidal mastitis is usually self-limiting with spontaneous recovery and the infection usually has no systemic consequences. Candida species has been isolated from bovine milk with and without signs of mastitis (Segal and Elad, 1998). *Candida albicans* has been isolated from bovine udder infections (Pengov, 2002; Bourtzi-Hatzopoulou *et al.*, 2003; dos Santos and Marin, 2005; Wawron *et al.*, 2010).

Candida rugosa can be involved in/ or causes cattle clinical or subclinical mastitis and the udder infection results in a prolonged fever and inflammatory reaction in the mammary gland and associated lymph nodes (Dion and Dukes, 1982; Pengov, 2002; Bourtzi-Hatzopoulou *et al.,* 2003; dos Santos and Marin, 2005; AalbæK *et al.,* 2009; Wawron *et al.,* 2010) and rarely cause candidaemia. The infection associated with catheters and total parenteral nutrition. Because of its frequent azole-resistance the echinocandin, amphotericin and catheter discontinuation could be the recommended therapy, as the fluconazole might be substituted based on susceptibility testing and a clinical response to initial therapy (Minces *et al.,* 2009). *C. rugosa* has also been isolated from clinical samples of mastitis milk and milking machines (Keller *et al.,* 2000).

In the Sudan the isolated fungi from bovine mastitis were *Aspergillus fumigatus* and *Aspergillus niger*, while *Candida albicans* reproduced mastitis when inoculated experimentally in goats (Gibriel, 1985). *Candida albicans, Candida parapsilosis* and *Candida guilliermondii* had also been isolated from camel mastitis in Sudan (Amel, 2003). The objective of the current investigation was to determine the percentage of fungal infections among mastitic cases that do not respond to antibacterial therapy.

Materials and Methods

Investigated Animals: Thirty four lactating cows with udder infection that did not respond to local (Neomycin and cefaloxin), and systemic (Penicillin) antibacterial agents, from Sharg Alneel district, Khartoum state were investigated during this study.

Specimens Collection: Milk samples were collected from infected quarters in sterile test tubes and placed in ice bags and shipped without delay to the laboratory.

Laboratory Examination: Direct smears were stained with gram stain. Representative parts of the specimens were inoculated in duplicates onto Sabouraud Dextrose Agar (SDA) with chloromphinicol (0.05 mg/ml) and Malt Extract Agar (MEA) with chloromphinicol (0.05 mg/ml). Slopes and plates were incubated at 26ºC and 37ºC. The cultures were examined daily and observations were recorded. Further confirmatory tests were conducted using Corn Meal Agar (CMA) with tween 80, germ tube production test using equine serum, and urease test. Furthermore, sugar assimilation tests were done using API 20 kits for yeast, in accordance to to the instructions of the manufacturer (07628E-GB-2002/10, Biomerieux, France).

Results

Yeast forms were seen on direct examination with Gentian stain after de-fattening of milk. The isolates grew faster at 37ºC. After 4-5 days, creamy mucoid colonies were seen in the positive samples (8.8%). A tentative diagnosis and identification of the *Candida* species was made after examination of gram stain smears. All the positive isolates were Gram positive yeast cells. *C. rugosa* (two isolates) were round to oval in

shape while *C. tartarivorans* (one isolate) was elongate to spindle. Other morphological (Table1& Fig1) and biochemical test had been carried out (Table2).

Clinical Examination: The infected cows showed enlargement of the mammary glands and supramammary lymph nodes. The udders were painful on palpation and milk yield was reduced. Some cows revealed hardening of the mammary glands and change of the milk colour (creamy, pink and red), consistency (liquid to caseated), and some of the milk samples contained blood clots. The body temperature was normal (37° C).

Discussion

Candida rugosa is considered as one of the important fungi that could be included in/ or cause bovine mastitis (Dion and Dukes, 1982; Pengov, 2002; Bourtzi-Hatzopoulou *et al.,* 2003; dos Santos and Marin, 2005; AalbæK *et al.,* 2009; Wawron *et al.,* 2010). *C. rugosa* has also been isolated from clinical samples of mastitis milk and milking machines (Keller *et al.,* 2000). Crawshaw *et al.,* 2005 documented an outbreak of *C. rugosa* mastitis in a dairy herd after intramammary antibiotic treatment. In this investigation *C. rugosa* was isolated from Neomycin, cefaloxin (local) and Penicillin (systemic)-non responsive mastitic cows. These results authenticated the finding of Stamatakos *et al.,* 2003 who isolated of Candida spp. human mastitis from patients that suffering recurrent breast pain and swelling of the nipple/areola with nipple secretion despite treatment by the b-generation cephalosporine.

Milk constituents such as lactoferrin (Soukka *et al.,* 1992; Montagne *et al.,* 2001) can play an inhibitory role in *Candida* culture

growth. Iron-free human lactoferrin kills *C. albicans* in a dose-dependent manner (Xu *et al.,* 1999), while iron-saturated lactoferrin does not inhibit *C. albicans* growth (Kirkpatrick *et al.,* 1971). This results in the fact that the laboratory culturing of human milk for mammary candidosis is not well defined (Morrill et al, 2003). In lactating women, Candida spp. can colonize in the mammary gland as it could be included in mammary candidosis (Morrill *et al.,* 2005). Using Quil A or Freund's complete adjuvant, ovalbumin and delayed-type hypersensitivity to *C. albicans* and mycobacteria antibodies have been detected in lactating Holstein cows (Heriazon *et al.,* 2009).

Despite lack of advocate research on the fungal mastitis in Sudan; *A. fumigatus* and *A. niger* were isolated form bovine mastitis, while *C. albicans* mastitis was experimentally produced in goats (Gibriel, 1985). *C. albicans, C. parapsilosis* and *C. guilliermondii* had also been isolated from camel mastitis (Amel, 2003). To the best of our knowledge, the results obtained in the current investigation record for the first time the isolation of *C. regosa* and *C. tartarivorans* from bovine mastitic cases that did not respond to antibacterial therapy in the Sudan.

	C. rugosa (A)	*C. rugosa (B)*	*C. tartarivorans*
Colony feature	Creamy mucoid	Creamy mucoid	Creamy mucoid
Gram stain	+ve	+ve	+ve
Cell morphology	Round to oval yeast cells	Round to oval yeast cells	Elongate and spindle yeast cells
Germ tube formation	+ve	-ve	-ve
Hyphae production	+ve	+ve	+ve
Blastoconidia production	+ve	+ve	+ve
Chlamydoconidia production	+ve	-ve	-ve
Arthroconidia production	-ve	-ve	-ve

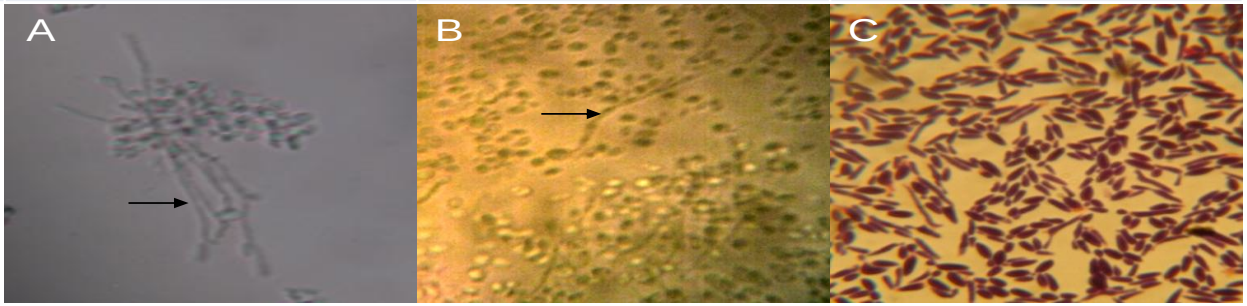

Table1&Fig1: The morphological tests reveal the colony features after five days cultivation onto both (SDA) and (MEA) media, gram stain, cell morphology, germ tube-formation, hyphae, blastoconidia, chlamidoconidia and arthroconidia production tests of the isolates. (A): Microscopic diagram showing the germ tube production by *C. rugosa (a)* after its inoculation in equine serum 37°C for 90 minute. (B): Microscopic diagram showing blastoconidia and some pseudohyphae produced by *C. rugosa (b)* after the cultivation in Corn Meal Agar (CMA) with cloromphinicol and tween 80. Because the isolation of two *C. rugosa* from two diferent cases we symbolized them by A&B. (C): Microscopic diagram showing a gram positive elongate to spindle *C. tartarivorans* cells.

Table2: Biochemical tests. Urease and sugar assimilation tests of the isolates using API 20 C AUX auxanogram kits.
The difference of the assimilation of GLY, ARA, ADO, XLT, SOR, MDG, SAC, TRE and MLZ may be due to the isolate deferences

species	Urease test	GLU	GLY	2KG	ARA	XYL	ADO	XLT	GAL	INO	SOR	MDG	NAG	CEL	LAC	MAL	SAC	TRE	MLZ	RAF
C. rugosa (A)	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	-ve	Week +ve	-ve	+ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve
C. rugosa (B)	-ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve
C. tartarivorans	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve

The abbreviation	The sugar name	The abbreviation	The sugar name	The abbreviation	The sugar name
GLU	*D-glucose*	**GAL**	*D-Galactose*	**MAL**	*D-Maltose*
GLY	*Glycerol*	**INO**	*Inositol*	**SAC**	*D-Saccharose*
2KG	*Calcium 2-ceto-Gluconate*	**SOR**	*D-Sorbitol*	**TRE**	*D-Trehalose*
Ara	*L-Arabinose*	**MDG**	*Methyl-αD-Glucopyranosid*	**MLZ**	*D-Melezitose*
Xyl	*D-Xylose*	**NAG**	*N-Acetyl-Glucosamine*	**RAF**	*D-Raffinose*
ADO	*Adonitol*	**CEL**	*D-Celiobiose*		
XLT	*Xylitol*	**LAC**	*D-Lactose (origin bovine)*		

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- *Candida* sp. 100% identical to EU056285 (closely related in *C.rugosa)* (DDBJ/EMBL/Genbank accession No. EU056285 C base No. 487-7, minus strand)
- *Candida* sp. 100% identical to DQ234791(closely related in *C.rugosa)* (DDBJ/EMBL/Genbank accession No. DQ234791 C base No. 3-483)
- *Candida tartarivorans* (DDBJ/EMBL/Genbank accession No. DQ438226 C base No. 2-572)

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