ISSN 2310-4090

International Journal of Scientific Footprints Open Access

Fungi Associated With Cases of Bovine Mastitis in Khartoum State, Sudan

Abubaker A. El Ayis, Amani B. A, Shadia A. Lazim & Intisar A. M. Osman

College of Veterinary Medicine, Bahri University-Sudan

Keywords:
Candida; Aspergillus; Fungus; Mastitis; Khartoum.

Correspondence:
Abubaker A. El Ayis. College of Veterinary Medicine, Bahri University-Sudan

Funding Information:
No funding information provided.

Manuscript History:
Received: October 2016
Accepted: January 2017

International Journal of Scientific Footprints 2017; 5(1): 1 - 8

Abstract

Of 130 mastitic milk samples collected from different localities of Khartoum State, 32 samples were positive for fungal growth after examination by cultural, morphological, biochemical methods. The isolates were confirmed using VITEK2 identification system. The incidence of fungal bovine mastitis was 24.6%. The yeast and yeast like fungi isolated were Candida *albicans*, Geotrichum *capitatum* and Saccharomyces *cerevisiae*. The mold isolates were Aspergillus fumigatus and Aspergillus *niger*. The results of the present study suggest that Candida spp. and Aspergillus *spp*. Are the predominant fugi implicated with bovine mastitis in Khartoum State. Good hygiene and sanitation practices of animal farm and judicious use of antibiotics will lower incidence of bovine mycotic mastitis.

Introduction

Fungal infection probably introduced with contaminated intramammary infusions or teat cup liners. Establishment of the infection is encouraged by damage to the mammary epithelium and stimulated by antibiotic therapy; for example Candida spp. Utilize penicillin and tetracyclines as sources of nitrogen. A fever is accompanied by a severe inflammation of the quarter, enlargement of the supramammary lymph nodes, and a marked fall in milk yield. The secretion consists of large yellow clots in a watery milk cistern and there is no invasion of the mammay gland itself. Usually the disease is benign and spontaneous recovery following in about a week is the rule, with infection by Aspergillus fumigatus or A. nidulans there are multiple abscesses in the quarter. These are surrounded by granulation tissue, but the milk ducts are generally unaffected. Many other yeasts including Saccharomyces spp. Pichia spp have also caused mastitis in cattle [1].

Severity of mycotic infection of mammary glands depends upon the species of the fungus involved as well as the percentage of infectivity [2].

Mycotic mastitis is usually sporadic but it may occasionally reach epidemic status if the circumstances are favorable for its spread throughout the herd. The frequency of mycotic mastitis varies: in some herds it is almost unheard of, whereas in others 20 – 25 % of the

mastitis cases are reported to be caused by yeast or molds. In more extensive studies, the frequency of mycotic mastitis has been established to be 1-4% of all mastitis cases [3].

The most common fungal species which cause mycotic mastitis include *Candida spp, Sacchromyces spp*, and occasionally *Aspergillus fumigatus* [4].

Although there are many reports of fungal involvement in bovine mastitis around the world, still very little work done in SudanThe objective of this research work was to assess the incidence of mycotic mastitis in Khartoum State, Sudan. Also to determine genera and species of yeasts and molds which cause the disease condition?

Materials and Methods

Area of the study

A total of 130 bovine mastitic milk samples were collected from farms of the three localities in Khartoum State in Sudan. In Bahri and East Nile localities samples were collected from Parlor 2 and 3, Shigla farms, Selate farms, Mygoma Farms and Helat kuku farms. In Omdurman locality, samples were collected from El Ruduan farms and Gabal Toureia Farms. In Khartoum and Gabel Awleia localities samples were collected from El Saig farms, Soba farms and El Azhari farms.

Sampling Procedure

Before collection of milk samples from the tested cows, the udder was thoroughly cleaned with soap and water, rubbed dry, and the teat area was rubbed thereafter with a piece of cotton soaked in 70% alcohol . The first stream of milk was discarded. Samples were collected from mastitic udder into sterile bottles. The collected samples were put in ice box containing ice and transported to the laboratory. In most cases the time between collection and arrival to the laboratory was 1-2 hrs. In the laboratory mastitic milk samples were kept in a deep-freezer. All samples were examined on the next day. On the next day mastitic milk samples were removed from the deep–freezer and left on the bench to thaw.

Isolation, Identification and Characterization of Yeast, Yeast like Fungi and Molds

Saboraud's dextrose agar plates were prepared and sterilized according to the manufacturer instructions. For the primary isolation of fungi, a loop full milk sample was streaked onto Saboraud's dextrose agar using sterile wire loop. The cultures were incubated aerobically at 37C^{o} and examined for growth at 24, 48 and 72 hours and at weekly intervals for 4 weeks after which the plates showing no growth were considered negative. The colonies were picked up and re-streaked on another SDA plate to get the pure cultures. These fungal isolates were studied for their cultural and morphological characteristics [5]. The morphological characteristics were noted after staining with Gram's and Lacto phenol cotton blue stain. Isolates were identified by different sugar fermentation test. Briefly, 1% solution of different sugar solutions were dispensed into separate tubes and fungal isolates were inoculated in these tubes. The tubes were then incubated at 37oC for 24-48 hours. The results were indicated by the change of colour [6].

The yeast isolates recovered from the milk samples were identified based on \their colony character, microscopic morphology, presence or absence of capsules, growth at 25°C with cycloheximide, growth on cornmeal agar containing Tween 80, germ tube test, urease at 25°C, growth in Sabouraud’s dextrose broth,

sugar fermentation and sugar assimilation test employing various sugars.

Identification of Yeast and Yeast like Isolates Using Vitek2 Identification System

The VITEK2 (BIOMERIEUX, France) test was performed as described by [7]. Pure colonies were suspended in 3.0 ml sterile saline with turbidity of 0.50- 0.63 MacFarland. The tested samples and the (yeast card) identification cards were placed into a cassette. The cassette was placed into a vacuum chamber station inside the VITEK2 analyzer machine in which YST cards were inoculated with fungal suspensions. The inoculated cards were passed by a mechanism, which cut off the transfer tube and sealed. The cassette was manually inserted in the VITEK2 reader-incubator modules at 35.5oC and every card automatically subjected to a kinetic fluorescence measurement every 15 minutes. The results were interpreted by the ID-YSTB database after the incubation period.

Statistical Analysis

Statistical analysis was done through Microsoft office Excel 2007.

Results and Discussion

A total of 130 bovine mastitic milk samples were collected from different localities of Khartoum State. 32 samples were positive for fungal growth. The incidence of fungal mastitis was 24.6% (table 1).

Yeast and Yeast like Fungi Isolated from Mastitic Milk Samples

According to the cultural characteristics, microscopic morphology, presence or absence of capsules, growth at 25°C with cycloheximide, growth on cornmeal agar containing Tween 80, germ tube test, urease at 25°C, growth in Sabouraud's dextrose broth, sugar fermentation and sugar assimilation test employing various sugars and VITEK2 results, a total of 32 fungi isolated from bovine mastitic milk samples. Yeast and yeast like fungi represented 65.6% of the isolated fungi. Yeast and yeast like fungi included *Candida albicans* (46.9%), *Geotrichum candidum* (3.1%) and *Saccharomyces cerevisiae* (15.6%). Molds represented 34.4% of the isolated fungi. Molds included *Aspergillus fumigates (*15.6%) and *Aspergillus niger* (18.8%) (table 2).

Identification of Yeast and Yeast like Fungi Using Vitek2 System

According to the interpretation of the results after using VITEK2 identification system, yeast and yeast like isolates scored high probability percentages ranged from 96.00% to 99.00% (table 3).

Table 1 Number of Bovine Mastitic Milk Samples Positive for Fungal Growth

Farm	No. of samples	Positive Fungal Growth
Parlor 2	12	2
Parlor 3	10	3
Shigla	15	2
Selate	10	1
Mygoma	12	4
Helat kuku	13	3
El Ruduan	10	3
Gabal Toureia	15	6
El Saig	11	4
Soba	10	2
El Azhari	12	3
Total	130	32 (24.6%)

Table 2 Number and Percentage of Fungi Isolated from Mastitic Milk

Fungal species	Number	Percentage
Candida albicans	15	46.9%
Geotrichum capitatum	1	3.1%
Saccharomyces cerevisiae	5	15.6%
Aspergillus fumigates	5	15.6%
Aspergillus niger	6	18.8%
Total	32	

Table 3 Number of Bovine Mastitic Milk Samples Positive for Fungal Growth

Test	*Candida albicans*	*Geotrichum candidum*	*Saccharomyces cerevisiae*
(LysA)	-	-	+
(lMLTa)	+	+	+
(leuA)	-	-	+
(ARG)	-	-	-
(ERYa)	+	+	-
(GLYLa)	+	+	+
(TreA)	-	-	+
(BANG)	-	-	-
(ARBa)	-	-	+
(AMYa)	-	-	-
(dGALa)	+	+	+
(GENa)	-	-	+
(dGLUa)	+	+	+
(LACa)	-	-	-
(MadGa)	-	-	-
(dCELa)	+	+	+
(GGT)	-	-	-
(dMALa)	+	+	+
(dRAFa)	-	-	+
(NAGA1)	-	-	-
(dMNEa)	+	+	+
(dMELa)	+	+	+
(aMLZa)	-	-	-
(lSBEa)	+	-	-
(lRHMa)	-	+	_
(XLTa)	+	+	+
(dSORa)	+	+	+
(SACa)	+	+	+
(URE)	-	-	-
(AGLU)	-	+	+
(dTURa)	-	+	+
(dTRE)	+	+	+
(NO3a)	-	-	-

Table 3 (continued)

Test	*Candida albicans*	*Geotrichum capitatum*	*Saccharomyces cerevisiae*
(lARAa)	-	+	+
(dGATa)	-	+	-
(ESC)	-	-	-
(lGLTa)	-	+	-
(dXYLa)	+	+	+
(LATa)	-	-	-
(ACEa)	+	+	+
(CITa)	+	+	+
(GRTas)	-	-	-
(lPROa)	-	+	+
(2KGa)	-	-	-
(NAGa)	-	-	-
(dGNTa)	-	-	-
Probability	99.0%	96.0%	98.0%

In this study the incidence of fungal agents in clinical cases of mastitis was 24.6%. Higher rates of incidence 29, 27% and 34% were reported by [8]. Molds (34.4%) and Yeasts and yeast like (65.6%) were isolated in this study. [4] mentioned that, the frequency of mycotic mastitis in Nordic countries varied: in some herds it was almost unheard of, whereas in others 20-25% of the mastitis cases were reported to be caused by yeasts and molds.

Candida albicans (46.9%) was the most frequently isolated yeast and yeast like fungi from mastitic milk samples and this agree with the findings of [9]. Other researchers have also reported Candida spp. as a causative agent of bovine mastitis [10-11]. Incidence of Aspergillus spp. in clinical cases was found to be 34.4%. Other researchers have also reported Aspergillus spp as a causative agent of bovine mastitis [12-13].

Saccharomyces cerevisiae (15.6%) also isolated in this study from mastitic milk. [14] reported that *Sacchromyces spp* is one of the most common fungal species which cause mycotic mastitis.

A higher percentage of isolation of fungi from clinical cases of mastitis reveals that the due to unhygienic conditions of the animal sheds supporting the growth of fungal spores and hyphae in the vicinity of lactating animals, thereby increasing the probability of fungal spores and yeast cells to enter into the udder parenchyma which provides the best environment for the growth of these fungi [15].

Twenty yeast and yeast like fungi were selected randomly and inserted into VITEK2 automated identification system. The isolates scored high probability percentages; they fulfill the requirements for identification as yeast and yeast like fungi by

VITEK2 system according to [16] and [17]. According to the present findings Vitek2 as an automated system provided very good and trustable accuracy and reproducibility. Actually its developed methods are used for identification of fungal samples that conventional methods can't recognize properly.

Conclusion

This study clearly revealed the high incidence rate of fungal mastitis (24.6%). The study also revealed that *Candida albicans* is the predominant fungal spp. isolated from bovine mastitic milk samples in Khartoum State. Moreover, *Candida albicans* is involved in bovine mastitis.

Other fungi found in mastitic milk samples in this study included: *Geotrichum capitatum*, *Saccharomyces cerevisiae*, *Aspergillus fumigates and Aspergillus niger*, which are responsible for bovine mastitis. According to frequency of isolation, Aspergillus spp. came in second place to *Candida albicans* as cause of bovine mastitis followed by *Saccharomyces cerevisiae*.

Vitek2 as an automated system for fungal identification provided very good and trustable accuracy and reproducibility.

Further studies should be carried out to investigate the predisposing factors related to the incidence of bovine fungal mastitis and to identify different causes of bovine mastitis. Further studies should include a survey of more animals in different farms and an extensive study of the significance of different microorganisms in bovine mastitis. Moreover the serotyping of isolates obtained from different areas should be given more attention.

Acknowledgement

Authors are thankful to University authorities for providing necessary facilities to carry out this research.

References

[1] Barnett JA, Payne RW, Yarrow D, editors. *Yeasts: Characteristics and Identification*, 3rd ed. Cambridge University Press, New York. 2000.

[2] Blowey, R. and Edmondson, P. Mastitis control in dairy herds, 2nd edition. (2010) CAB international, Cambridge, MA, 02139, USA, p- 55.

[3] Cárdenes-Perera CD, Torres-Lana A, Alonso-Vargas R, Moragues-Tosantas MD, Pontón-San Emeterio J, Quindós-Andrés G, *et al.* Evaluation of API ID 32C and VITEK-2 to identify Candida dubliniensis. Diagn Microbiol Infect Dis. 2004; 50(3):219-21.

[4] David H. P. (2005). Microbial Identification Using the Biomerieux Vitek2 System, biomerieux, MO, USA. Online published doc on net, biomerieux official website., https:// apiweb.biomerieux.com.

[5] Dworecka-Kaszak, B., Krutkiewicz, A., Szopa, D., Kleczkowski, M., and Bieganska, M. (2012) High Prevalence of Candida Yeast in Milk Samples from Cows Suffering from Mastitis in Poland, The Scientific World Journal, vol. 2012, Article ID 196347. doi:10.1100/2012/ 196347.

[6] Elad, D., Shpigel, N. Y., Winkler, M., Klinger, I., Fuchs, V., Saran, A. *et al*. (1995) Feed contamination with Candidakrusei as a probable source of mycotic mastitis in dairy cows. JAVMA; 207:620–2.

[7] Gupta, P.R.K., Kalra, D.S., Verma, P.C. and Lodha, B.C. (1981). Final Technical Report on studies on mycotic mastitis in domestic animals with particular reference to its incidence, Pathology and diagnosis, HAU, Hissar.

[8] Pengov, A. (2002) Prevalence of Mycotic mastitis in cows. Acta Veterinaria (Beograd), 52: 133-136.

[9] Quinn, P. J.; Markey, B. K.; Leonard, F. C.; Fitzpatrick, E. S.; Fanning, S. and Hartigan, P. J. (2011). Veterinary Microbiology and Microbial diseases.Virginia. Willey – Blackwell. U.S.A.

[10] Radostits, O. M.; Gay, C.C.; Hinchcliff, K. W. and Constable, P. D. (2012). Veterinary Medicine, A text book of the diseases of cattle, sheep, pigs and goats. 10th ed. Philadelphia. U.S.A.

[11] Shetty, N.; Hill, G. and Ridgway**,** G. L. (1998). The Vitek analyser for routine bacterial identification and susceptibility testing: protocols, problems, and pitfalls;

Journal of Clinical Pathology., 51:316- 323.

[12] Simaria, M.B. and Dholakia, P.M. (1986). Incidence and diagnosis of mycotic mastitis in Cattle. Indian Journal of Animal Science, 56 (10): 995-1000.

[13] Spanamberg A., Wűnder Jr E.A., Pereira D.I.B., Argenta J., Sanches E.M.C., Valente P., Ferreiro L.: Diversity of yeast from bovine mastitis in Southern Brazil. Rev Iberoam Micol 2008, **25**, 154-156.

[14] Stephan, R., Senczek, D., Muller, C. and Feusi, C. (2000) Isolierung von Listeria spp. and Aspergillus fumigatus-zwei Fallberichte aus der Mastitis diagnostik.Schweiz. Arch. Tierheilkd, 142:387–390.

[15] Tarfarosh, M. A. and Purohit, S. K. (2008) Isolation of Candida spp. fromMastitic cows andMilkers.Vet. Scan, 3: 14-18.

[16] Van Custem, J. and F. Rochette. (1991). Mycoses in domestic animals. Jassen Research Foundation. P. 55-56.

[17] Williamson, J. H. and Di Menna, M. E. (2007) Fungi isolated from bovine udders, and their possible sources. NZ Vet J, 55:188–90.