

J. Egypt. Vet. Med. Ass. 47, No. 1&2, 203-208 (1987)

## CRYPTOCOCCUS NEOFORMANS AS A CAUSE OF BOVINE MASTITIS IN EGYPT.

F. EL-FAR , H.A. HAMMAD AND M. REFAI\*

Animal Health Research Institute.

\* Faculty of Veterinary Medicine, Cairo University .

**SUMMARY:** Yeasts were isolated from 16 out of 60 milk samples (26.66%) collected from lactating Friesian cows. 9 of the isolates were identified as *Cryptococcus neoformans*. The remaining isolates were *Cr. albidus* (1), *Torulopsis spherica* (2), *T. species* (1) and *Rhodotorula species* (3).

### INTRODUCTION

*Cryptococcus neoformans* is the most common yeast causing severe bovine mastitis in a subacute or chronic form with subsequent serious economic losses. The first signs of infection are severe swelling and firmness of the udder and enlargement of the regional lymph nodes. In most cases, the milk yield is markedly reduced and the milk is visibly abnormal (Buxton and Fraser, 1977). This type of yeast is encapsulated, non spor-forming organism that is highly resistant to antibiotics. Cases with elevated systemic temperature and anorexia are not uncommon (Bruce and Larson, 1985).

*Cryptococcus neoformans* as a cause of bovine mastitis has been the subject of extensive studies. It was isolated from different outbreaks of mastitis in cattle by Carter and Young (1950); Stuart (1951); Pouden et al. (1952), Innes et al. (1952); Emmons (1952); Simmon et al. (1953) Galli (1954); Kauker (1955); Charlie (1955); de Gracia (1959); Funke (1960); Scholer et al. (1961); Mehnert et al. (1964); Bertschinger et al. (1964); Parisi (1965); Monga et al. (1970); Monga and Kalra (1971) and Spika and Petrovic (1976).

In Egypt, *Cr. neoformans* was isolated from 2 goats (Abdellah, 1969) and one cow (Abdel Ghani et al., 1978) suffering from mastitis. The aim of this work was to study the role of *Cr. neoformans* as a cause of mastitis in cows in Egypt .

*Received. 30. 10. 1986.*

### MATERIALS AND METHODS

Sixty milk samples were collected from lactating Friesian cows in a dairy farm suffering from mastitis. The mammary glands were hard, hot and swollen. The milk samples were greyish-white in colour ; some samples had clots, flakes and were watery while others looked normal.

The milk sampels were collected in sterile screw-capped bottles and centrifuged at 3000 rpm for 15 minutes. The supernatant was decanted and the sediment was cultured on Sabouraud's dextrose agar slant and incubated at 25°C for 48-96 hours. The yeast colonies were examined macroscopically and microscopically .

Identification was done by fermentation and assimilation of glucose , galactose, maltose, sucrose, inositol and dulcitol, nitrate assimilation hydrolysis of urea and retention of Trypan blue on My 10/20 agar for 7 days at 22-25°C . Demonstration of capsules was carried out by inoculating 48 hours culture on Sabouraud's dextrose agar, suspended in sterile physiological saline, in amounts of 0.2-0.3 ml intramuscularly in the thigh of white swiss mice. Fresh smears were prepared after 12-24 hours from the site of injection and mixed with India ink, spread on a slide, covered with a cover slip and examined under the microscope.

### RESULTS

Yeasts were isolated from 16 out of 60 milk samples (26.66%). Identification of positive isolates by fermentation and assimilation was done on the basis of the table published by ( Leonor and Carey, 1979).

As shown in (Table 1), *Cryptococcus* isolates were recovered from 10 samples (16.6%) 9 isolates were identified as *Cr. neoformans* and one as *Cr. albidus* .

*Torulopsis* species could be isolated only from 3 cases ( 5 % ).. Two isolates were identified as *Torulopsis spherica* and the other one was *Torulopsis* species. The remaining 3 isolates were identified as *Rhodotorula* species ( 5 % ).. All *Cr. neoformans* isolates showed a clear capsule when mixed with India ink (Fig. 1).

Table 1: Identification of isolated yeasts from milk samples.

Yeasts isolated	No. of isolates	% to total samples
Cryptococcus	10	16.60
Cr. neoformans	09	15.00
Cr. albidus	01	01.66
Torulopsis	03	05.00
T. spherica	02	03.33
T. species	01	01.66
Rhodotorula species	03	05.00

Fig. 1: Smear showing capsule of *Cryptococcus neoformans* stained with Indian ink (100 x 10X).

## DISCUSSION

Isolation of *Cr. neoformans* from milk of lactating Friesian cows can be considered as an important finding. Although this yeast has been isolated from pigeon droppings in different parts of Egypt (Refai et al., 1983) and from droppings of rats, chicken and soil in various poultry farms (Refai and Kotb unpublished data) it has been rarely recovered from animals. It seems that native breeds of cattle and buffaloes are probably less susceptible than Friesian cows. In the present work yeasts were recovered from 16 out of 60 Friesian cows (26.66%) and *Cr. neoformans* alone was isolated from 9 cows (15%). The milk samples from which *Cr. neoformans* was isolated appeared normal. As this milk may be consumed unpasteurized or used for manufacturing of cheese or yogurt, infection of consumers would be a high possibility. Yossef and Refai (1986) have reported 4 cases of fatal Cryptococcosis in man. However, the source of infection in those cases was obscure.

This study should draw the attention to the possible role of animals and birds in transmitting *Cr. neoformans* to man which in fact needs further extensive studies.

## REFERENCES

- 1 . Abdallah, I.S. (1969): Study of sheep mastitis as caused by *Cryptococcus neoformans* in Egypt. *Egypt. Vet. Med. J.* 17 , 203 - 216 .
- 2 . Abdel-Ghani, M., El-Shrif, M.T. and Abdel-Hamed, S. (1978): Prevalence of mycotic mastitis among sheep and goats in Egypt. *J. Egypt Vet. Med. Ass.* 38 (3), 85 - 90.
- 3 . Bertsching, H., Schweizer, R. and Scholer, H.J. (1964): Hefen als Mastitiserreger beim Rind. *Schweizer Arch. Tierheilkunde* 106 , 183 - 192.
- 4 . Bruce, L. and Larson, B.L. (1985): Lactation. Mastitis and the immune system of the mammary gland. Published by The Iowa State University Press/ Ames.

F. El-Far, et al.,

- 5 . Buxton, A. and Fraser, G. (1977): Animal Microbiology. Immunology Bacteriology, Mycology. Diseases of Fish and Laboratory Methods. Blackwell Scientific Publications. Oxford London Edinburgh Melbourne.
- 6 . Carter, H.S. and Young, J.L. (1950): Note on isolation of *Cryptococcus neoformans* from a sample of milk. J. Path. Bact. 62, 271 - 274 .
- 7 . Charlie, N.B. (1955): Cryptococcosis in animals. J. A.V.M.A. 125-132 .
- 8 . De-Gracia Aira A. (1959): Mycotic mastitis in cows in Spain caused by *Cryptococcus neoformans*. Bol. cult. Cons cal. med. Esp. 6.5,6 (ref in Vet. Bull-39.2141 (1960).
- 9 . Emmons, C.W. (1952): *Cryptococcus neoformans* strains from a severe outbreak. Mycopath. et mycol- 6 , 231 - 234.
10. Funke, H. (1960): Bovine Mastitis mit Befund von Hefepilzen im Eutersekret. Nord. Vet. Med. 12, 54 - 62.
11. Galli, G. (1954): Observation and studies on mastitis caused by *Cr. albidus*. Santo skinner Vet. Ital. 5 , 587 - 604 .
12. Innes, J.R., Seibold, H.R. and Arentzen, W.P. (1952): The pathology of bovine mastitis cused by *Criptococcus neoformans*. Amer. J. Vet. Res. 13, 469 - 475.
13. Kauker, E. (1955): Uber Mastitis Blastomyceten. Berl. Munch. Tierarztl. Wschr. 68, 407 - 409 .
14. Leanor D. Halely and Carey S. Callaway (1979): Laboratory Methods in Medical Mycology. Puplished by Center for Disease Control. New Publication No. (CDC) 79 - 8361.
15. Mehnert, B., Gedek, W. and Ernst, K. (1964): Hefen als Mastitisserreger beim Rind. Zbl. Vet. Med. A. 11 , 96 - 121.
16. Monda, D.P., Mohapatra, L.N. and Kalra, D.S. (1970): Bovine mastitis caused by *Cryptococcus neoformans*. Indian J. Med. Res. 58 , 203 - 205.

*Cryptococcus Neoformans As a Cause of Bovine .....*

17. Monga, D.P. and Kalra, D.S. (1972): Prevalence of mycotic mastitis among animals in Haryana. *Indian J. Animal Sci* **41**, (9), 813 - 816.
18. Pouden, W.D., Anderson, I.M. and Jaeger, R.F. (1952): A severe mastitis problem associated with *Cryptococcus neoformans* in large dairy herd. *Amer. J. Vet. Res.* **13**, 121 - 128.
19. Parisi, E.A. (1965): Contribution to the study of mastitis of cows in Northern Greece. *Epistimonik Epetiris K tiniatr. School Thes. Saloniki.*
20. Refai, M., Taha, M., Selim, S.A., Elshabouri, F. and Youssef, H. (1983): Isolation of *Cryptococcus neoformans*, *Candida albicans* and other yeasts from pigeon droppings in Egypt. *Sabouraudia* **21**, 163 - 165.
21. Scholer, H.J., Schneider and Bertschinger, H.M. (1961): Nachweis von *Cryptococcus neoformans* und anderen Hefen aus der Milch von Kuhen mit Mastitis. *Path. Microbiol., Basel*, **24**, 803 - 813.
22. Simon, J., Nichols, R.E. and Morse, E.V. (1953): An outbreak of bovine Cryptococcosis. *J. Amer. Vet. Med. Ass.* **122**, 31 - 35.
23. Spika, M. and Petrovic, D. (1975): High incidence of mycotic mastitis in cattle. *Gehauftes Auftreten von Hefemastitiden beim Rind Zbl. Vet. Med.* **22**, 5, 353 - 361.
24. Stuart, P. (1951): An outbreak of bovine mastitis from which yeast were isolated and attempts to produce the condition experimentally. *Vet. Res.* **63**, 314.
25. Youssef, H. and Refai, M. (1986): Fungi and the lung. *Treatise on Research. Department of chest Diseases-Ainshams University Cairo* (1986).