doi:10.5455/vetworld.2013.64-67

ORIGINAL RESEARCH

Evidence of Cryptococcosis in cattle in Zaria Kaduna state, Nigeria

Emmanuella N. Akange[1], Clara N Kwanashie[2], Mohammed Bisalla[3], Nicodemus M Useh[3], Emmanuel O. Ngbede[2]

1. Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, University of Agriculture Makurdi, PMB 2373 Makurdi Benue State, Nigeria ; 2. Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria. PMB 1045 Samaru Zaria, Kaduna State Nigeria; 3. Department of Veterinary Pathology, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, PMB 1069 Zaria, Kaduna State, Nigeria.;
Corresponding author: Emmanuella N. Akange email:aveseakange@hotmail.com
Received: 30-06-2012, Accepted: 14-07-2012, Published online: 17-12-2012

How to cite this article:
Akange EN, Kwanashie CN, Bisalla M, Useh NM and Ngbede EO (2013) Evidence of Cryptococcosis in cattle in Zaria Kaduna state, Nigeria, *Vet World* 6(2): 64-67. doi: 10.5455/vetworld.2013.64-67

Abstract

Aim: Cryptococcosis is azoonotic infection caused by fungal of the *Cryptococcus neoformans* complex comprising of *C. neoformans* and *C. gattii*.The disease affects humans and animals worldwide causing morbidity and mortality. This work was carried out to determine the occurrence of cryptococcal antigens and factors associated with presence of antigens in cattle in Zaria, Nigeria.

Materials and Methods: Three hundred and ninety (390) serum samples from cattle of various ages were collected from 11 farms in Zaria, Nigeria. The samples were analysed using alatex agglutination test and lateral flow assay kit which detectsthe polysaccharide capsular antigens of *Cryptococcus* species.

Results: Out of the 390 samples tested 28 (7.17%) were found to be positive using the latex agglutination test while only of these 22 (5.64%) were positive using the lateral flow assay. There was a strong correlation (r=0.939, $p=0.0002$) between the results of the latex agglutination test and the lateral flow assay. There was no statistically significant difference ($p>0.005$) in positivity for cryptococcal antigens between sex, age and sex, though, there was a statistically significant difference ($p<0.05$) in positivity between management systems i.e. semi-intensive and intensive farming systems.

Conclusions: The epidemiological value of this report lies in its demonstration that the risk of cattle and humans infection with cryptococcosis exist in farms in Zaria. The presence of this pathogen among these cattle poses an economic threat to the livestock industry due to the mastitis it causes. It also poses a significant public health threat because of its zoonotic nature and the increasing population of immunocompromised individuals. Large scale studies to determine specific risk factors and the role of the environment and experimental studies to determine what governs the transition from nasal colonisation to infection are recommended.

Keywords: cattle, cryptococcus antigens, lateral flow assay, latex agglutination, Nigeria, sera, Zaria.

Introduction

Cryptococcosis is a fungal disease of man and domestic animals, caused by the *Cryptococcus neoformans* complex [1]. The complex is divided into two species: *C. neoformans* and *C. gattii* [2]. Each of these species comprised of four molecular types; VNI - VNIV and VGI – VGIV for *C. neoformans* and *C. gattii*, respectively and several serotypes. The species are further divided into varieties; *C. neoformans* into two varieties: var. *grubii* (serotype A, VNI and VNII) and var. *neoformans* (serotype D, VNIV) with an additional hybrid (serotype AD, VNIII), while *C. gattii* is comprised of two serotypes (serotypes B and C) and molecular types VGI, VGII, VGIII and VGIV [1, 3, 4].

Cryptococcus neoformans has been described as a yeast species causing spontaneous mycosis in a wide variety of animal species and man [5]. Mammals are the species mostly affected but some invertebrates and reptiles have been reported to have been affected by cryptococcosis [6]. Infections are sporadic and occasional with no definite pattern of occurrence. Inhalation of environmental yeast may cause upper or lower respiratory tract infection depending on the immune/health status of the animal or the virulence of the inhaled yeast [7]. Cryptococcosis in animals is often characterized by upper respiratory symptoms, subcutaneous nodules, pneumonia, central nervous system or ocular disorders, lymphadenopathy, or subcutaneous nodules [8]. In cattle *C. neoformans* causes mastitis and this infection usually occurs by introduction into the skin or mucous membranes of the mammary gland via unhygienic or dirty milking equipment, hands and environments. A case of meningioencephalitis was recently reported in a 5-year old bull [9].

C. neoformans has been isolated from various locations in nature, in bird feces, and detritus of trees [10]. The environmental habitat and nature of this yeast makes it easy for a wide variety of animals to become infected by exposure to environmental sources of the organism.

In animals factors that predispose to infection have been studied extensively in cats, feline immuno-deficiency virus a lenti virus similar to human HIV

Table-1. Occurrence of cryptococcal antigens among cattle sampled from some farms in Zaria, Kaduna State, Nigeria using latex agglutination test and lateral flow assay.

No. animals tested	No. positive
390	28
390	22

r= 0.9394 p=0.0002

Table-2. Age, sex and breed distribution of occurrence of cryptococcal antigen among cattle sampled in 11 farms in Zaria, Kaduna State, Nigeria.

Variables	No. positive (%)	Total no. of cattle tested	*p value*
Sex			
Males	5 (7.69)	65	0.7955
Females	23 (7.08)	325	
Age			
6mths – 2 yr	6 (8.00)	75	0.8330
> 2 yrs	22 (6.98)	315	
Breeds			
Local	16 (5.59)	286	0.1123
Exotic	2 (15.38)	13	
Crosses	10 (10.99)	91	
Management			
Intensive	7	46	
Semi intensive	21	344	

virus is said to explain the high susceptibility of this species to *C. neoformans* and co-infection with feline leukaemia virus has also been incriminated [11]. The relationship between *C. neoformans* and birds is slightly different from that of mammals. It was described in 1974 that pigeon excrements are an important reservoir of the yeast but pigeons themselves do not come down with the disease [12]. It has also been isolated in a wide variety of domestic and wild birds [13,14]. Because of the nature of this fungus and its abundance in soil samples, it can be presumed that herbivorous or grazing animals will be more likely to get infected and re-infected because of their higher rates of exposure to environmental sources of the yeast. In most situations the possibility that a fungus may be responsible for disease is not explored by most veterinarians in Nigeria especially because of the long duration needed for culture and confirmation, and the tendency to shy away from the long process and exorbitant cost of treatment. This possibility may be explored in pet animals but not so much in food animals where treatment might not be cost effective except for prize animals. This study was carried out to determine the status of *Cryptococcus* among cattle in Zaria with a view of determining its role in chronic mastitis cases and the role cattle may play in its spread to other animals and humans.

Materials and Methods

Three hundred and ninety serum samples collected from apparently healthy cattle of various ages and breeds in eleven farms in Zaria, Nigeria were tested for cryptococcal antigens. A latex agglutination and lateral flow assay kit which detects cryptococcal antigens in serum and cerebrospinal fluid obtained from Immuno-Mycologics Inc. Norman Oklahoma were used to test the sera samples. The assays were preformed as recommended by the manufacturer and both test (latex agglutination and lateral flow) were run concurrently with the positive and negative controls provided by the manufacturers. Briefly, the latex agglutination test was performed by first pre-treating of the serum samples using pronase (300µl serum:50µl pronase) and heated at 56ºC in a water bath, and a drop of pronase inhibitor was added to stop the reaction before testing. 25µl of positive and negative control were added to separate rings of the slide and 25µl of detection latex was added to each ring and mixed using an applicator stick. 25µl of pre-treated serum was then added to 25µl of cryptococcal latex on separate rings on the slide. The slide was rocked slightly by hand for about 5 minutes and read.

Briefly, the lateral flow assay was performed by adding one drop of specimen diluent to 40µl of serum in a test tube. The lateral flow assay strip was then inserted into the diluted serum in the test tube. The strip was removed after 10 minutes and observed for the formation of a visible test line formed between the gold conjugated anti-cryptococcal antibodies and cryptococcal antigens in serum. Samples were identified based on age, sex, breed and management system under which such cattle were raised. Personal interviews were conducted with the farm managers, veterinarians and herdsmen on each of the farms visited. Chi square and Fisher's exact test were used to analyze the data and values of $p<0.05$ was considered statistically significant. The results were presented in tabular form.

Results

Out of the 390 samples tested, 28 (7.17% prevalence) tested positive for cryptococcal antigens using the latex agglutination test and 362 (92.83%) tested negative. Of the 28 samples which tested positive with the latex agglutination only 22 (5.64%) tested positive with the lateral flow assay. There was a strong correlation (78.57%, $r=0.9394$ and $p=0.0002$) between the two tests (Table-1).

Out of the 390 cattle sampled 65 were males while 325 were females. Five (7.69%) and 23 (7.08%) of the males and females were positive for the cryptococcal antigen respectively. There was no statistically significant difference ($p=0.7955$; $p>0.05$) in positivity between males and female (Table-2).

Three hundred and fifteen of the cattle sampled were adults(greater than two years of age) out of which 22 (6.98%) were positive for cryptococcal antigen. Seventy five were young animals (6months – 2yr), out of these 6 (8.00%) positive for cryptocoocal antigen. There was no statistically significant difference

(p=0.8330; p>0.05) in positivity between the age groups (Table-2).

Two hundred and eighty six of the cattle sampled where local (Zebu) breeds of cattle out of which 16 (5.59%) were positive for the cryptococcal antigen. Two (15.38%) out of the 13 exotic breeds and 10 (10.99%) out of the 91 cross breeds sampled were positive for the cryptococcal antigen. There was no statistically significant difference *(p=0.1123; p>0.05*) in positivity between the breeds sampled (Table-2).

Of the 390 cattle sampled 46 were raised under intensive system while 344 were raised under semi-intensive system. Out of the 46 cattle raised under intensive system of management 7 were positive while out of the 344 cattle raised under semi-intensive system 21 were positive (Table-2).

Discussion

The presence of the Cryptococcal antigens in these apparently healthy cattle is an indication of natural exposure to the agent. Cryptococcal antigens have been shown to be present in animals without presentation of clinical signs [15]. It is possible the animals have been infected via inhalation of feed contaminated with the cryptococcal basidiospores or skin inoculation in the case of mastitis. It has been reported as an important cause of mastitis, cerebral cyptococoma, granulomatous pneumonia [16,17]. The disease also produces varying clinical signs in immunocompromised humans [18-20]. The absence of a statistically significant difference in positivity between age groups, sex and breeds sampled is an indication that all ages are at risk of infection with the disease. Though immunocompromised animals, the young and aged have been reported to be more prone to mycosis [20]. The strong correlation between the result of the latex agglutination test and the lateral flow assay is an indication that such point of care tests will provides fast and easy methods of eliminating or confirming *Cryptococcus* as cause of disease in respiratory or other infections such as mastitis which are unresponsive to antibiotic treatment.

The difficulty of diagnosis and treatment of most systemic fungal infections in domestic animals rest mainly on the inability of early detection of disease. Symptoms of mycoses in animals are mostly vague and mimic other viral and bacterial infections. Domestic animals (both pet and food) animals remain a sentinel population for human infections especially nomadic cattle or cattle moving out to graze which can transfer this fungus to and from grazing or watering areas to other cattle which in turn continue the spread. *Cryptococcus* species must be monitored in domestic animals to track geographic distribution of *Cryptococcus* species and the conditions which lead to a development of disease or clearance.

Conclusion

Cryptococcus antigens where found in cattle in Zaria, Nigeria. The epidemiological value of this report lies in its demonstration that the risk of cattle and humans infection with *Cryptococcosis* exist in farms in Zaria. This is of serious implication as it has been reported as a cause of un treatable mastitis and granulomatous lesions. Though transmission between animal-animal or animal-humans is said to be uncommon, the potential for and danger of such spread to other animals and humans working with these cattle cannot be rule out. There is need for caution in the use of systemic steroid in the treatment of animals. There is a need for *Cryptococcus* to be included as a differential of mastitis cases unresponsive to antibiotics in Nigeria. Also there is need for active surveillance for this disease so as to understand its epidemiology in this environment and an improved awareness and knowledge of the importance and characteristics of *Cryptococcosis* among veterinarians and mycologist. There is need to control pigeons populations around farms, reduce their contact with animals or the feeds as well as reduce animal contact with the feces of pigeons especially during grazing .

Author's contribution

All authors contributed equally. All authors read and approved the final manuscript.

Acknowledgements

Authors are thankful to The head, Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, University of Agriculture Makurdi, Makurdi Benue State, Nigeria for providing necessary facilities.

Competing interests

Authors declare that they have no competing interests.

References

1. Litvintseva, A., Thakur, R., Reller, L.B. and Mitchell, T.G. (2005). Prevalence of clinical isolates of *Cryptococcus gattii* serotype C among patients with AIDs in sub-saharan Africa. *J. Infect. Dis.*, 192: 888-892.
2. Lui, G., Lee, N., Ip, N., Choi, K.W., Tso, Y.K., Lam, E., Chau, S., Lai, R. and Cockram, C.S. (2006). Cryptococcosis in apparently immunocompetent patient. In *Q. S. Med.*, 99: 143-151.
3. Bicanic, T. and Harrison, T.S. (2004). Cryptococcal meningitis. *Br Med. Bull.*,72: 99-118.
4. Lester, S.J., Malik, R., Bartlett, K.H. and Duncan, C.G. (2011). Cryptococcosis: update and emergence of *Cryptococcus gattii*. *Vet. Clin. Pathol.*, 40: 4e17.
5. Torres-Rodríguez, J.M., Morera, Y. and Baró-Tomás, T. (2004). Spontaneous infection of animals with *Cryptococcus neoformans* and *Cryptococcus gattii*. *Mikologia Lekarska*, 11: 303-307.
6. Miller, W.G., Padhye, A.A., Van Bonn, W., Jensen, E., Brandt, M.E. and Ridgwa, S.H. (2002).Cryptococcosis in a bottlenose dolphin (*Turiopsis truncates*) caused by *Cryptococcus neoformans* var *gattii*. *J. Clin. Microbiol.*, 40: 721-724.
7. Dora, J.M., Kelbert, S., Deutschendorf, C., Cunha, V.S., Aquino, V.R., Santos R.P., Goldani, L.Z. (2006). Cutaneous cryptococcosis due to *Cryptococcus gattii* in immuno-competent hosts: case report and review. *Mycopathologia*, 161: 235-238.
8. Datta, K., Bartlett, K.H., Baer, R., Byrnes, E., Galanis, E., Heitman, J., Hoang, L., Leslie, M.J., Macdougall, L., Magill,

S.S., Morshed, M.G., Marr, K.A. and The *Cryptococcus gatti* working group of the Pacific North West.(2009). Spread of *Cryptococcus gattii* into Pacific Northwest region of the United States. *Emerg. Infect. Dis.*, 15(8): 1185-1191.
9. Riet-Correa, F., Krockenberger, M., Dantas, A.F. and Oliveira, D.M. (2011). Bovine cryptococcal menin goencephalitis. *J. Vet. Diagn. Investig.*, 23: 1056-1060.
10. Casadevall, A. and Perfect, R.J. (1998).*Cryptococcus* and cryptococcosis. First edit. American Society of Microbiology press, 1998. ISBN 1-55581-107-8.p. 547.
11. O'Brien, C.R., Krockenberger, M.B., Wigney, D.I., Martin, P. and Malik, R. (2004).Retrospective study of feline and canine cryptococcosis in Australia from 1981 to 2001: 195 cases. *Med. Mycology*, 42: 449-460.
12. Swinne-Desgain, D. (1974). The pigeon as reservoir of *Cryptococcus neoformans*. *Lancet*, 304: 842-843.
13. Irokanulo, E.O.A., Makinde, A.A., Akuesgi, C.O. and Ekwonu, M. (1997). *Cryptococcus neoformans* isolated from droppings of captive birds in Nigeria. *J. WildlifeDis.*, 33: 343-345
14. Criseo, G., Bolignano, M.S., De Leo, F. and Staib, F. (1995). Evidence of canary droppings as an important reservoir of *Cryptococcus neoformans*. *Zentralblatt Fuer Bakteriologie*, 282: 244-254.
15. Flatland, B., Greene, R.T. and Lappin, M.R. (1996). Clinical and serologic evaluation of cats with cryptococcosis. *J. Am. Vet. Med. Assoc.*, 209: 1110-1113.
16. Fava, C.D., Levey, F.L., Scannapieco, E.M., Cunha, E.M.S. (2011). Cryptococococcal pneumonia and meningitis in a horse. *J. Equine Vet. Sci.*, 31: 693-695.
17. Oruc, E. (2005). Meningoencephalitis Tuberculosa in a Holstein Cow. *Vet. Pathol., 42: 856-858.*
18. Pal, M. and Mehrotra, B.S. (1983). Cryptococcal mastitis in diary animals. *Mykosen*, 26: 615-616.
19. Lemos, L.S., Santos, A.S.O., Viera-Da-Motta, O., Texeira, G.N. and Carvalho, E.C.Q. (2007). Pulmonary Cryptococcus in a slaughtered sheep: anatomopathology and culture. *Vet. Microbiol.*, 125: 350-354.
20. Nayak, J.B., Brahmbhatt, M.N., Savalia, C.V., Chatur, Y. A. and Bhong, C.D. (2010). Cryptococcosis in man and animals. *Vet. Comm.*, 5: 32-36.

****