ECRONICON OPEN ACCESS

EC MICROBIOLOGY
Research Article

Prevalence of *Cryptococcus neoformans* in Excreta, Wood and Air of an Aviary

Mahendra Pal*

Narayan Consultancy of Veterinary Public Health and Mycology, Gujarat, India

***Corresponding Author**: Mahendra Pal, Narayan Consultancy of Veterinary Public Health and Mycology, Gujarat, India.

Received: September 28, 2017; **Published:** November 06, 2017

Abstract

Recent years have shown global interest in the study of *Cryptococcus neoformans*, which has emerged as an important life-threatening agent of human and animal mycosis. A total of 18 samples, which included 10 parrot excreta, 4 wooden scrapings from cages, and 4 wooden dusts from perches, were examined for the presence of *C. neoformans* by employing standard mycological techniques. In addition, 6 Petri dishes 3 each of Pal sunflower seed medium and Sabouraud agar were exposed in the environment of an aviary. The pathogen was isolated from 6 parrot's droppings and 4 wooden scrapings (2 cages and 2 perches). The yeast was also recovered from the air of an aviary on 2 exposed plates. All the isolations of *C. neoformans* from excreta, wooden materials and air were achieved only on Pal sunflower seed medium by observing light to dark brown pigmented colonies of *C. neoformans*. However, no isolation of *C. neoformans* could be made on Sabouraud medium because of rapid contamination with fast growing moulds. The microscopic morphology of all the isolates of *Cryptococcus neoformans* in Narayan stain revealed circular to oval, single or budding yeast cells with thin capsules. The findings of this investigation clearly demonstrated that *C. neoformans* is prevalent in the environment of an aviary, and Pal sunflower seed agar proved a very good selective medium for the rapid screening of environmental materials. Therefore, the wider application of Pal sunflower seed medium would enable the mycologist in ecological, epidemiological, and diagnostic studies of *C. neoformans*. It is emphasized that further studies on the ecological niche of this pathogenic yeast should be conducted with diverse types of environmental substrates throughout the world.

Keywords: *Avian Excreta; Cryptococcus neoformans; Environment; Narayan Stain; Pal Sunflower Seed Medium; Public Health; Wooden Material*

Introduction

Cryptococcus neoformans, a heterothallic basidiomycete, aerobic, Gram positive, encapsulated yeast, is the principal cause of cryptococcosis in humans as well as in a wide variety of animals [1]. The unprecedented upsurge in cryptococcal infection began in the early 1980 after the discovery of HIV. Currently, cryptoccocosis is recognized as a major life-threatening mycotic disease in AIDS patients [2]. It is estimated that cryptococcal infections is responsible for one million cases and 620,000 deaths every year in HIV/AIDS patients in sub-Saharan Africa [3]. The fungus is ubiquitous in distribution and has been recovered from a diverse types of natural substrates such as soil, pigeon excreta, parrot droppings, other avian faeces (budgerigar, canary, cockatoo, lorikeet, lory, macow, munia birds, peach fronted conure), bat guano, fruits, vegetables, and wood [4-14]. However, pigeon excreta serves as the chief saprobic reservoir of *C. neoformans*, at it has been frequently recovered from pigeon droppings in many countries of the world [6,9,14-19]. It has been mentioned that *C. neoformans* can survive in old and dry pigeon droppings in sheltered humid dark sites for 20 years [20]. As respiratory tract is the prime portal of entry to *C. neoformans*, the visit to such contaminated areas may pose a risk to the immunocompromised hosts, particularly the HIV/AIDS patients [2,18,20]. The paucity of information on the environmental prevalence of *C. neoformans* in aviaries, prompted the present to investigate the natural occurrence of *C. neoformans* in the environment of an aviary by employing Pal's sunflower seed agar, as a selective medium.

Materials and Methods

The investigation was conducted on January 16, 2017 at the private small aviary in a nearby village of Bharauch, Gujarat, India. A 34 - year- old male bird enthusiast kept about 10 parrots separately in a wire net fitted wooden enclosure in his house. All enclosures had wooden cages, perches, water, and feed utensils. About 5g of old and dried parrot droppings was obtained with the help of wooden spatulas and placed in clean polythene bags. Sterilized sand paper was used for the collection of wood dust from wooden cages and wooden perches. About 5 - 10 mg of wood scrapings was directly collected in sterilized disposable Petri dishes by rubbing the sand paper 10 to 15 times on 4 boxes and 4 perches [6,11]. In order to avoid the environmental exposure of *C. neoformans*, a facemask was used while collecting the samples of bird excreta and wooden cages. One gram of avian excreta was suspended in sterilized glass bottle containing 9 ml of sterile physiological saline (0.85% NaCl) supplemented with chloramphenicol (10 mg/ml). The mixture was left at room temperature for about 15 - 20 minutes, then shaken manually for 4 - 5 minutes, and later incubated at 37°C for one hour [19]. An aliquot of 0.1 ml of supernatant from the suspension was inoculated onto duplicate plates of Sabouraud dextrose agar with chloramphenicol (0.05 mg/ml) and Pal medium, which contained pulverized sunflower seed 4.5g, agar 2.0g, chloramphenicol 50 mg, distilled water 100 ml [1]. The pour plate method was employed to recover *C. neoformans* from the wooden materials [11]. The former medium was incubated at 37°C, and the later was kept at 25°C. Each Petri dish was examined daily for yeast growth, and the number of colonies showing brown pigment was counted. The sub-culture of brown pigmented colony was made on APRM medium for further confirmation [14]. The new medium contained 4.0 of marigold dried flower, 2.0g agar, 50 mg chloramphenicol, and 100 ml distilled water. The strains growing at 37°C with capsule, urease positive, and negative for potassium nitrate and lactose, can be identified as *C. neoformans*. The microscopic morphology of all the isolates was studied in Narayan stain (4 ml of glycerin, 0.5 ml of 3% aqueous solution of methylene blue, 6 ml of dimethyl sulfoxide, and 4 ml of glycerin) [21].

Results

Cryptococcus neoformans was easily isolated from 10 of the 18 environmental samples (Table 1). The positive specimens originated from 6 parrot excreta, 2 wooden cages, and 2 wooden perches (Table 1). In addition, the pathogen was also demonstrated in the air of an aviary on two exposed plates. All the isolations were made only on Pal sunflower seed medium at 25°C by observing few light brown to dark brown pigmented colonies of *C. neoformans* (Table 1). In contrast, the yeast could not be recovered from any of the environmental materials on Sabouraud medium, as all of the inoculated plates were badly contaminated with fast growing moulds that masked the growth of *C. neoformans*. The number of colonies, which grew from environmental substrates on Pal sunflower seed medium varied from 5 to 27. All the isolates of *C. neoformans* grew well on Sabouraud medium at 37°C, hydrolyzed urea, but failed to utilize lactose, and potassium nitrate. The wet mount preparations of all isolates in Narayan stain under light microscopy showed many spherical to few oval thinly encapsulated yeast cells, with and without budding. The cultural, morphological and physiochemical findings confirmed that all the isolates were *C. neoformans* [1].

S.N. Types of specimens	Isolation on *Cryptococcus neoformans* on	
	Sabouraud agar	**Pal medium**
1. Parrot excreta	0*/10**	6/10
2. Wooden cages	0/4	2/4
3. Wooden perches	0/4	2/4
Total	0/18	10/18

Table 1: *Comparative efficacy of Sabouraud agar and Pal's sunflower seed medium for the isolation of Cryptococcus neoformans from environmental materials.*

**Number of samples examined*

***Number of specimens positive for Cryptococcus neoformans*

Discussion

Although *C. neoformans* was first recovered from peach juice by Sanfelice in 1894 in Italy, the environmental source of *C. neoformans* was unknown until Emmons from USA first isolated this fungus from the soil and droppings of the pigeon (*Columbia livia*) in 1951, and 1955, respectively [22-24]. Later, this observation was substantiated by many investigators from various parts of the world [8,14-18,25,26]. The recovery of *C. neoformans* from the bird excreta and wooden materials of an aviary confirmed the earlier observations that avian habitat serves as an important saprobic reservoir for this opportunistic pathogen [12,13,27,28]. The results of this investigation indicated that the isolation of *C. neoformans* from environmental sources is extremely difficult on conventional media like Sabouraud agar. Therefore, a differential medium should be employed to study the prevalence of *C. neoformans* in saprobic environment. The success of recovering the pathogen from the environmental samples was because of the application of selective agar (Pal sunflower seed medium). The development of brown colored colonies on Pal's medium within 3 - 4 days at 25°C helped in the rapid isolation and quick presumptive identification of the yeast from the environmental sources. The usefulness of Pal sunflower seed agar as an excellent medium for rapid isolation and identification of this zoopathogenic yeast from clinical as well as environmental substrates has been reported by several other workers [16,17,26,18,29]. Recently, an experiment conducted by Katiyar and others (2012) to assess efficacy of five media namely Tobacco agar, Mustard seed agar, Niger seed agar, Sunflower seed agar, and Henna agar conclusively established that sunflower seed medium was the best medium with the mean day of growth and pigment production being 1.25 and 2.8, respectively [30]. Considering the fact that Pal sunflower seed medium is highly sensitive, very sensitive, readily available, easy to prepare and less expensive than other nutrient media, its routine application is recommended in microbiology and public health laboratories, particularly in poor resource countries, which do not have good laboratory facilities for the isolation and identification of *C. neoformans*.

The public health significance of environmental exposure to avian excreta, particularly the pigeon droppings, is well documented in the literature [1,31-33]. The presence of this zoopathogenic yeast in the environment of an aviary is an important observation and should be viewed from public health point of view, as *C. neoformans* is emerged as an important opportunistic pathogen producing life threatening disease in immunocompetent and immunocompromised hosts particularly, HIV/AIDS patients throughout the world [1,2,10,18,20]. It is pertinent to mention that 80 % of patients with pulmonary crptococcosis reveal history of environmental exposure mainly to pigeon droppings [20]. It is, therefore, emphasized that immunocompromised persons should use face to prevent the inhalation of infectious cells of *C. neoformans* while working in aviary, zoological park, bird hospital, historical building etc, especially during the cleaning of the excreta. The decontamination of infected aviary with formalin is highly imperative to check the spread of infection in susceptible subjects [1]. It is advised that any pet bird keeper with respiratory/nervous disorders should be thoroughly investigated mycologically for cryptococcal infection.

Acknowledgements

The author wishes to thank the Bird Keeper who helped in the collection of the environmental samples for this study. The Laboratory help of the staff of Welfare Hospital and Research Centre, Bharauch, Gujarat, India is also thankfully acknowledged. Thanks are also due to Anubha for computer assistance.

Funding

No research funding.

Conflict of Interest

No conflict of interest.

Bibliography

1. Pal M. "Veterinary and Medical Mycology". 1st Edition. Indian Council of Agricultural Research, New Delhi, India (2007).

2. Pal M., *et al*. "Cryptococcosis: A major life-threatening mycosis of immunocompromised hosts". *Indian Journal of Social and Natural Sciences* 1.1 (2012): 19-28.

3. Park BJ., *et al*. "Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS". *AIDS* 23.4 (2009): 525-530.

4. Ajello L. "Occurrence of Cryptococcus neoformans in soils". *American Journal of Hygiene* 67.1 (1958): 72-77.

5. Pal M and Mehrotra BS. "Studies on the isolation of Cryptococcus neoformans from fruits and vegetables". *Mykosen* 28.4 (1985): 200-205.

6. Pal M. "Studies on the epidemiology of cryptococcosis". Post Doctorate Dissertation. Institute of Tropical Medicine, Antwerp, Belgium (1986).

7. Pal M. "Cryptococcus neoformans var. neoformans and Munia birds". *Mycoses* 32.5 (1989): 250-252.

8. Pal M., *et al*. "Isolation of saprophytic Cryptococcus neoformans". *Japanese Journal of Veterinary Science* 52.6 (1990): 1171-1174.

9. Ruiz A., *et al*. "Isolation of saprophytic Cryptococcus neoformans from Puerto Rico: distribution and variety". *Mycopathologia* 106.3 (1989): 167-170.

10. Swinne D., *et al*. "AIDS- associated cryptococcosis in Bujumbura, Burundi: an epidemiological study". *Journal of Medical and Veterinary Mycology* 29.1 (1991): 25-30.

11. Pal M. "Natural occurrence of Cryptococcus neoformans var. neoformans in wooden Canary cages". *Revista Iberoamericanade Micologia* 12 (1995): 93-94.

12. Pal M. "Prevalence of Cryptococcus neoformans in faecal matter of caged parrots". *Verh Ber Erkrg Zootiere* 42 (2005): 130-133.

13. Pal M. "Cryptococcus gattii: An emerging global mycoticpathogen of humans and animals". *Journal of Mycopathological Research* 52.1 (2014): 1-7.

14. Dave P and Pal M. "New medium "APRM" for isolation of medically important fungi from clinical and environmental samples". *International Journal of Livestock Research* 5.3 (2015a): 10-18.

15. Pal M. "First report of isolation of Cryptococcus neoformans var neoformans from avian excreta in Kathmandu, Nepal". *Revista Iberoamericana de Micologia* 14.4 (1997): 181-183.

16. Sasaki K., *et al*. "Isolation of Cryptococcus neoformans from pigeon excreta". *Japanese Journal of Veterinary Medical Association* 52 (1999): 521-524.

17. Cermeno JR., *et al*. "Cryptococcus neoformans and Histoplasma capsulatum in dove's (Columbia livia) excreta in Bolivar State, Venezuela". *Revista Latinoameicana de Microbiologia* 48.1 (2006): 6-9.

18. Pal M., *et al*. "Cryptococcosis: An enigmatic mycosis of humans and animals". *Journal of Occupational and Environmental Sciences* 3.1 (2014): 53-60.

19. Pal M. "First report of isolation of Cryptococcus neoformans from pigeon droppings in Djibouti". *Molecular Microbiology Research* 5.3 (2015): 1-3.

20. Pal M and Dave P. "Cryptococcosis: An emerging airborne mycosis of global concern". *Air and Water Borne Diseases* 5 (2016): 127.

21. Pal M. "Efficacy of Narayan stain for morphological studies of mould, yeasts and algae". *Revista Iberoamericana de Micologia* 21.4 (2004): 219.

22. Emmons CW. "Isolation of Cryptococcus neoformans from soil". *Journal of Bacteriology* 62.6 (1951): 65-690.

23. Emmons CW. "Saprophytic source of Cryptococcus neoformans associated with pigeon (Columbia livia)". *American Journal of Hygiene* 62.3 (1955): 227-232.

24. Sanfelice F. "Contributo allamorfologia e biologiadei blastomicetichesis vilupanonoisucchi di alcunifrutti". Annals of Ig. R. Univ. Roma 4 (1894): 463-495.

25. Pal M. "Saprobic reservoirs of Cryptococcus neoformans". *Indian Journal of Public Health* 22 (1978): 327-328.

26. Pal M and Baxter M. "Isolation of Cryptococcus neoformans from the environment using a simplified sunflower seed medium". *Proceedings of New Zealand Microbiological Society* 29 (1985): 155-158.

27. Hashemi SJ., *et al*. "Prevalence of Cryptococcus neoformans in domestic birds referred to veterinary clinics in Tehran". *European Journal of Experimental Biology* 4.1 (2014): 482-486.

28. Dave P and Pal M. "Primary cutaneous mycosis in an immunocompetent parrot keeper due to Cryptococcus neoformans". *Molecular Microbiology Research* 5.4 (2015b): 1-3.

29. Baro T., *et al*. "First identification of autochthonous Cryptococcus neoformans var gattii isolated from goats with predominantly severe pulmonary disease in Spain". *Journal of Clinical Microbiology* 36 (1998): 458- 461.

30. Katiyar R., *et al*. "Comparison of different media for the pigment production of Cryptococcus neoformans". *Journal of Clinical and Diagnostic Research* 5.6 (2011): 1187-1189.

31. Littman ML. "Cryptococcosis (Torulopsis): Current concepts and therapy". *American Journal of Medicine* 27 (1959): 976-998.

32. Wegner HH and Staib F. "Fatal cryptococcosis in a pet bird enthusiast. Case report: Contribution to the pathology, diagnosis and epidemiology of cryptococcosis". *Zentrablat Bakteriology Hygiene A* 256.2 (1983): 231-238.

33. Pal M. "Pulmonary mycosis in a pigeon handler due to Cryptococcus neoformansvar neoformans". 2nd International Conference on Cryptococcus and Cryptococcosis. Milan, Italy (1993): 119.

Volume 13 Issue 1 November 2017