
ISOLATION AND IDENTIFICATION OF AVIAN GASTRIC YEAST FROM A FLOCK OF CAPTIVE BUDGERIGARS IN KERALA

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ABSTRACT

Avian gastric yeast (*Macrorhabdus ornithogaster*) is an anamorphic ascomycetous yeast which colonises the junction between proventriculus and ventriculus in many species of birds. Although a normal flora, sometimes it causes a subclinical infection and at times lead to gastric infections. The present study confirmed the presence of *Macrorhabdus ornithogaster* from faecal samples of budgerigar with symptoms like regurgitation and chronic wasting. Gram's staining of the direct smear demonstrates large number of pleomorphic Gram positive organisms. The organism could be cultured on blood agar, Sabouraud's dextrose agar and De Man Rogosa and Sharpe agar but not on MacConkey agar. The organisms were found to be negative for catalase and oxidase and did not reduce nitrate. It produced acid from maltose, sucrose, glucose, galactose and trehalose and not

from raffinose and lactose. The cultural and biochemical characters of the isolate along with clinical symptoms confirmed the case as avian gastric yeast infection.

Keywords: *Macrorhabdus ornithogaster*, Avian Gastric Yeast, Budgerigars, De Man Rogosa and Sharpe agar

1. INTRODUCTION

Macrorhabdus ornithogaster is the causative agent of avian gastric yeast infection which was earlier known as megabacteriosis. As the name indicates, they are long to filamentous rods, found at the junction between proventriculus and ventriculus of birds. The organism may appear as 20 to 80 μm long with a diameter of 2 to 3 μm in mucosal scrapings and faeces (Hannafusa *et al.*, 2007). The organism was found to be Gram positive, but only the cytoplasm gets stained (Baker, 1992). Once thought to be a bacterium, the organism was reclassified into the class of

Saccharomycetes which is an anamorphic ascomycetous yeast and it is the sole member of the genus *Macrorhabdus* (Tomaszewski *et al.*, 2003). This organism has been detected in many species of birds including budgerigars (Henderson *et al.*, 1988; Baker, 1992; Flippich and Hendrikz, 1998), canaries (Van Herek *et al.*, 1984; Marlier *et al.*, 2006), ostriches (Huchzermeyer *et al.*, 1993) turkekys (Schulze and Heidrich, 2001), chicken, quail (Pennycott *et al.*, 2003), guinea fowl, domestic pigeon, ground dove (Martins *et al.*, 2006) and in budgerigars it is one among the important cause of gastrointestinal disease (Pustow *et al.*, 2017). It may be present in clinically healthy birds as well. Transmission is mainly by faeco oral route, as the sick or subclinical birds shed the organism in faeces (Kheriandish and Salehi, 2011). Wild birds are regarded to be the possible source of infection (Schulze and Heidrich, 2001). The infection may go unnoticed most of the time and if manifested, the symptoms shown by budgerigars include severe depression, ruffled feathers, polyphagia/dysphagia, vomiting/ regurgitation, diarrhoea, the birds may be die within few days of exhibition of symptoms in acute form (Gerlach, 2001) and there will be severe weight loss termed as “going light” and eventual death in chronic form (Baker, 1992; Flippich *et al.*, 1993). At necropsy, there will be proventricular dilatation, koilin

disruption, ventriculus mucosal wall contain opaque white mucus and haemorrhage and ulceration at isthmus region (Kheriandish and Salehi, 2011). The diagnosis should be based on combination of multiple factors like history of regurgitation, weight loss, presence of large number of organisms in fresh faecal smear, isolation and identification of organism and in case of dead birds, gross lesions and histopathology will be ideal. Amphotericin B (Pustow *et al.*, 2017), nystatin and acidifying the water with organic acid are considered effective for the treatment (Kheriandish and Salehi, 2011).

2. MATERIALS AND METHODS

2.1 Collection of clinical sample

Faecal sample of a budgerigar showing symptoms of chronic weight loss and regurgitation was brought to the Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy, Kerala. Swabs were taken from the brought sample.

2.2 Direct smear examination

The faecal swab was smeared on a clean grease free glass slide, air dried and Gram's staining was done. The stained smear was then observed under oil immersion objective of the microscope for preliminary identification of organism.

2.3 Isolation of the organism

The sample was then inoculated to 7 per cent Blood agar, De Man Rogosa and Sharpe agar, Sabouraud's dextrose agar and MacConkey agar and incubated in microaerophilic condition at 37°C for 24 hours. The blood agar was further kept for about 5 days to know the haemolytic activity of the organism.

2.4 Identification of the organism

The colonies obtained were subjected to catalase, oxidase, nitrate reduction, and Voges Proskauer test. Tests were done to check the ability of the organism to ferment maltose, sucrose, glucose, galactose, trehalose, raffinose, lactose and to utilise ornithine and lysine.

3. RESULTS AND DISCUSSION

3.1 Direct smear examination

On direct smear examination of faecal sample, pleomorphic Gram positive organism were detected (Fig. 1). The organism can be easily identified from smears prepared from faecal material and mucosal scrapings because of their size. Severely affected birds will shed significant number of organisms in faeces making the primary diagnosis easy nevertheless, the process of shedding begins only after certain time and the process is not regular (Gerlach, 2001). Furthermore, the shedding

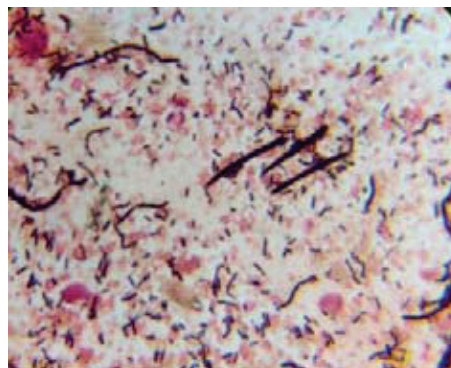


Fig. 1 Gram positive pleomorphic rods with rounded ends

in asymptomatic birds can vary from none to many. Thus a negative direct smear examination cannot be relied solely to rule out infection (Phalen, 2005).

3.2 Isolation of the organism

The organism can be cultured on blood agar (Scanlan and Graham, 1990), De Man Rogosa and Sharpe (MRS) agar (Huchzermeyer *et al.*, 1993; Gerlach, 2001) and Sabouraud dextrose agar (Martins *et al.*, 2006). The colonies on blood agar were grey, flat (Fig. 2) and produce hemolysis on incubation for up to 5 days (Fig. 3). Similar results were obtained by Scanlan and Graham (1990). The colonies on De Man Rogosa and Sharpe agar were white to yellow small, convex colonies (Fig. 4). Huchzermeyer *et al.* (1993) used MRS broth as enrichment media and MRS was then used to culture the organism. Iridescent colonies were observed after 48 h of incubation at 5 to 10 % CO₂. On Sabouraud's dextrose agar colonies were

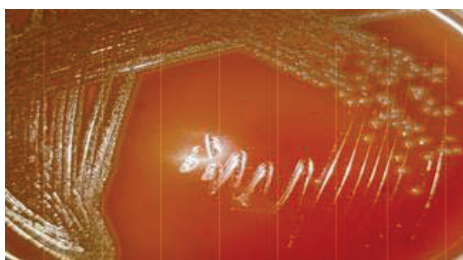


Fig. 2 Grey, flat colonies on the blood agar



Fig. 3 Flat, hemolyzing colonies with irregular edges after 5 days of incubation in blood agar



Fig. 4 White to yellow small, convex colonies in MRS agar

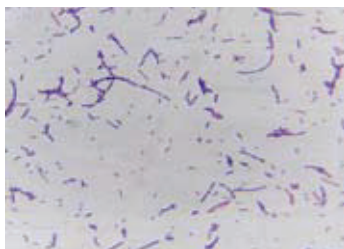


Fig.5 Gram positive pleomorphic rods small and dry. No growth could be obtained in MacConkey agar (Scanlan and Graham, 1990).

On Gram staining, the organisms were found to be Gram positive pleomorphic rods (Fig. 5). Amer and Mekky (2020)

stated that the organisms reveal change in length and diameter when subcultured.

3.3 Identification of the organism

The isolate was negative for catalase and oxidase, positive for Voges Proskauer test and did not reduce nitrate. It produced acid from maltose, sucrose, glucose, galactose and trehalose and not from raffinose and lactose. It also failed to utilise ornithine and lysine (Table. 1). The results of this study was in accordance with the results of Scanlan and Graham, 1990. Thus, cultural and biochemical characters of the isolate along with clinical symptoms confirmed the case as avian gastric yeast infection.

Table. 1 Biochemical characteristics of the organism

Biochemical Tests			
Carbohydrate Utilization Test		Decarboxylase Test	
Test	Result	Test	Result
Maltose	+	Ornithine	-
Sucrose	+		
Glucose	+		
Galactose	+	Lysine	-
Trehalose	+		
Raffinose	-		
Lactose	-		

4. SUMMARY

Macrorhabdus ornithogaster is opportunistic yeast found at the proventricular-ventricular junction in many species of birds including budgerigars

worldwide. In Kerala, this is the first report of Avian Gastric Yeast infection in budgerigars. Routine monitoring should be done to assess the prevalence of the organism and to control the spread of the disease, as it causes economic loss to the avicultures.

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REFERENCES

- Amer, M. and Mekky, H. 2020. Avian gastric yeast (AGY) infection (macrorhabdosis or megabacteriosis). *Bulg. J. Vet. Med.* **23** (4): 397-410.
- Baker, J.R. 1992. Megabacteriosis in exhibition budgerigars. *Vet. Rec.* **131**(1): 12-14.
- Filippich, L. J., OBoyle, D.A., Webb, R. and Fuerst, J.A. 1993. Megabacteria in birds in Australia. *Aust. Vet. Pract.* **23** (2): 72-76.
- Filippich, L. J. and Hendrikz, J. K. 1998. Prevalence of megabacteria in budgerigar colonies. *Aust. Vet. J.* **76** (2): 92-95.
- Gerlach, H. 2001. Megabacteriosis. In *Seminars in Avian and Exotic Pet Medicine* **10** (1): 12-19.
- Hannafusa, Y., Bradley, A., Tomaszewski, E. E., Libal, M. C. and Phalen, D. N. 2007. Growth and metabolic characterization of *Macrorhabdus ornithogaster*. *J. Vet. Diagn. Invest.* **19** (3): 256-265.
- Henderson, G. M., Gulland, F. M. and Hawkey, C.M. 1988. Haematological findings in budgerigars with megabacterium and *Trichomonas* infections associated with 'going light'. *Vet. Rec.* **123** (19): 492-494.
- Huchzermeyer, F. W., Henton, M. M. and Keffen, R. H. 1993. High mortality associated with megabacteriosis of proventriculus and gizzard in ostrich chicks. *Vet. Rec.* **133** (6): 143-144.
- Kheirandish, R. and Salehi, M. 2011. Megabacteriosis in budgerigars: Diagnosis and treatment. *Comp. Clin. Path.* **20** (5): 501-505.
- Marlier, D., Leroy, C., Sturbois, M., Delleur, V., Poulipoulis, A. and Vindevogel, H. 2006. Increasing incidence of megabacteriosis in canaries (*Serinus canarius domesticus*). *Vet. J.* **172** (3): 549-552.
- Martins, N. R. S., Horta, A. C., Siqueira, A. M., Lopes, S. Q., Resende, J. S., Jorge, M.A. 2006. *Macrorhabdus*

- ornithogaster* in ostrich, rhea, canary, zebra finch, free range chicken, turkey, guinea-fowl, columbina pigeon, toucan, chuckar partridge and experimental infection in chicken, japanese quail and mice. *Arq. Bras. Med. Vet. Zootec.* **58** (3): 291-298.
- Pennycott, T. W., Duncan, G. and Venugopal, K. 2003. Marek's disease, candidiasis and megabacteriosis in a flock of chickens (*Gallus gallus domesticus*) and Japanese quail (*Coturnix japonica*). *Vet. Rec.* **153** (10): 293-297.
- Phalen, D. 2005. Diagnosis and management of *Macrorhabdus ornithogaster* (formerly megabacteria). *Vet. Clin. North Am. Exot. Anim. Pract.* **8** (2): 299-306.
- Pustow, R. and Krautwald-Junghanns, M. E. 2017. The incidence and treatment outcomes of *Macrorhabdus ornithogaster* infection in budgerigars (*Melopsittacus undulatus*) in a veterinary clinic. *J. Avian. Med. Surg.* **31** (4): 344-350.
- Scanlan, C. M. and Graham, D. L. 1990. Characterisation of a Gram-positive bacterium from the proventriculus of budgerigars (*Melopsittacus undulatus*). *Avian Dis.* **34** (3): 779-786.
- Schulze, C. and Heidrich, R. 2001. Megabacteria-associated proventriculitis in poultry in the state of Brandenburg, Germany. *DTW. Deutsche Tierärztliche Wochenschrift.* **108** (6): 264-266.
- Tomaszewski, E. K., Logan, K. S., Snowden, K. F., Kurtzman, C. P and Phalen, D. N. 2003. Phylogenetic analysis identifies the 'megabacterium' of birds as a novel anamorphic ascomycetous yeast, *Macrorhabdus ornithogaster* gen. nov., sp. nov. *Int. J. Syst. Evol. Microbiol.* **53** (4): 1201-1205.
- Van Herck, H., Duijser, T., Zwart, P., Dorrestein, G. M., Buitelaar, M. and Van Der Hage, M. H. 1984. A bacterial proventriculitis in canaries (*Serinus canaria*). *Avian Pathol.* **13** (3): 561-572.