Prolonged fecal shedding of ‘megabacteria’ (*Macrorhabdus ornithogaster*) by clinically healthy canaries (*Serinus canaria*)

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*Macrorhabdus ornithogaster*, often referred to as ‘megabacterium’, is an ascomycetous yeast usually found colonizing the mucosal surface of the isthmus existing between the glandular and grinding stomach of a wide diversity of bird species. However, this yeast can also behave as an avian pathogen, therefore representing a potential threat to bird breeding. The aim of this work was to assess the prevalence and patterns of fecal shedding of *M. ornithogaster* in a colony of healthy canary birds (*Serinus canaria*) bred in captivity. Fresh fecal samples from 39 canaries (17 males and 22 females) were cultured in liquid media for *M. ornithogaster* enrichment. Only two clinically healthy females were fecal culture-positive for the yeast, which represents an overall prevalence of 5.13% in the sampled population. A close surveillance of the two culture-positive canaries, which included periodical microscopic examination of fresh stool samples, showed prolonged fecal shedding of *M. ornithogaster*. Nevertheless, both animals remained asymptomatic throughout the study period. To the best of our knowledge, this is the first study reporting the continuous shedding of *M. ornithogaster* by clinically healthy canaries.

**Keywords** *Macrorhabdus ornithogaster*, megabacteriosis, canaries, fecal shedding

**Introduction**

*Macrorhabdus ornithogaster*, often referred to as ‘megabacterium’, was long thought to be a large, rod-shaped bacterium. However, phylogenetic work has identified this microorganism as an ascomycetous yeast belonging to a novel fungal genus [1].

*M. ornithogaster* cells are typically long (20–90 μm), straight, weakly Gram-positive and periodic acid-Schiff positive rods which can form short chains [1–5]. It is usually found in the isthmus existing between the glandular and grinding stomach (proventriculus–gizzard junction) of a wide diversity of bird species, where it mainly colonises the luminal surface but can also penetrate and disrupt the koilin layer [2,3,5–8].

In pathologic situations involving *M. ornithogaster* (often termed as ‘megabacteriosis’), the whole proventricular mucosal surface is covered by a thick whitish mucus containing dense mats of yeasts [2,3,8]. Other pathologic findings include depression, chronic debilitation, atrophy of the pectoral muscle, diarrhoea, proventriculitis, and ulceration and haemorrhage of the proventricular mucosa [2,3,6,8–10]. Although acute forms of megabacteriosis have been described, mainly in budgerigars, the course of the disease is usually chronic, sometimes with intermittent periods of recovery and relapse [2].

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The postmortem diagnosis of the disease in birds is based on the observation of gross macroscopic lesions with concomitant microscopic detection of megabacteria forming dense mats in the proventricular mucus layer [8]. In live birds the yeast is usually detected in wet mounts of fresh fecal samples or a proventricular wash [10,11].

The aim of this investigation was to assess the prevalence and patterns of fecal shedding of *M. ornithogaster* in a colony of canary birds (*Serinus canaria*) bred in captivity.

Materials and methods

Animals

This study was performed with 39 canaries from a captive breeding colony composed by 40 adult pairs and about 360 nestlings. The colony was sampled during the post-breeding resting period, when adults and nestlings are mixed and kept in 40 × 60 × 120 cm cages harboring ≤12 birds each, so as to minimize stress on animals.

Each bird was handled for approximately 1 min for gross physical examination, which was performed as described by Samour [12], and sexed by inspection of the cloaca [13]. The general body condition of animals was determined by estimating the abundance of pectoral muscle and fat deposits through structural measurements and examining the surface of the keel and the abdomen [14].

Sampling

A sterile cotton-tipped swab was introduced into the cloaca of each test animal to obtain a sample of fresh feces. All animals were sampled once, and their fecal samples were immediately transported to the laboratory for mycological analysis (see below). For animals showing signs of disease, an additional swab sample was taken from the crop for microscopic observation.

Mycological analysis

Cloacal swabs were cultured for *M. ornithogaster* following the procedure described by Hannafusa et al. [6]. Culture broth was composed of Basal Medium Eagle (Gibco, Madrid, Spain) supplemented with 20% of fetal bovine serum (Gibco), 5% of sucrose (Sigma-Aldrich, Madrid, Spain), 100 IU/ml of penicillin (Gibco) and 100 μg/ml of streptomycin (Gibco) [6]. Liquid cultures were incubated for 10 days at 42°C under microaerophilic conditions, which were generated by using the GenBox Microaer system (bioMérieux, Marcy l’Etoile, France).

Canaries from which samples proved to be positive were isolated in individual cages and kept in a controlled environment for closer surveillance, which included periodical cytology of fresh fecal samples (first stools shed by the animal each day and taken early in the morning for subsequent analysis). In all cases, fecal samples (c. 4 mg) were spread onto glass slides (Menzel-Glaser, Braunschweig, Germany) prior to light microscopic observations (Nikon Eclipse E200, Tokyo, Japan) at 400 × magnification. The fecal smears were stained with methylene blue to facilitate the quantitation of yeast cells.

Data analysis

The number of yeast cells observed per fecal smear, as determined by microscopic counting (see above), was expressed according to a semi-quantitative scale (Table 1). Statistical analyses were performed with SigmaPlot v.11 (Systat Software, Inc., San Jose, CA, USA).

Results

A total of 39 canaries (17 sexed as males and 22 as females) were sampled. One animal was 3 years old, 19 were 2 years old and the remaining 19 were 1 year old. No canaries died during the sampling or the 30-day post-sampling period.

Five canaries showed disease signs at the time of sampling, i.e., two had ruffled feathers, one of which also presented haemorrhagic feces, and the remaining three had excessive saliva in their beak. Microscopic examination of crop samples for these animals was negative for megabacteria.

Samples from only two females out of the 39 test canaries were fecal culture-positive for *M. ornithogaster*, which represents an overall prevalence of 5.13% in the surveyed population. Both culture-positive animals were clinically healthy and did not manifest any sign of megabacteriosis or any other disease during the study period and up to a year after the first sampling. Nevertheless, a closer surveillance of these canaries including periodical microscopic examination of fresh stool samples (32 times for canary #1 and 26 for canary #2, with samples taken 1–7 days apart) demonstrated prolonged fecal shedding of *M. ornithogaster* (Fig. 1). For both animals, there was a wide variation in the number of *M. ornithogaster* cells excreted (Fig. 1 and Table 2), being difficult to infer a clear pattern.

<table>
<thead>
<tr>
<th>Number of megabacteria in fecal smears*</th>
<th>Level of fecal shedding</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1–10</td>
<td>1</td>
</tr>
<tr>
<td>11–25</td>
<td>2</td>
</tr>
<tr>
<td>26–50</td>
<td>3</td>
</tr>
<tr>
<td>51–100</td>
<td>4</td>
</tr>
<tr>
<td>101–200</td>
<td>5</td>
</tr>
<tr>
<td>&gt; 200</td>
<td>6</td>
</tr>
</tbody>
</table>

*Number of *M. ornithogaster* cells per sample (c. 4 mg feces).
Table 2  Shedding of *Macrorhabdus ornithogaster* by fecal culture-positive canary birds.

<table>
<thead>
<tr>
<th>Animal (sex)</th>
<th>n</th>
<th>Range (no. yeast cells/slide)</th>
<th>Level of <em>M. ornithogaster</em> shedding*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canary #1 (F)</td>
<td>32</td>
<td>0 – &gt; 200</td>
<td>Range: 0 – 6</td>
</tr>
<tr>
<td>Canary #2 (F)</td>
<td>26</td>
<td>3 – &gt; 200</td>
<td>Median: 4.5</td>
</tr>
</tbody>
</table>

*Both culture-positive canaries were females (F).*

*aNumber of fecal samples analyzed.*

*bMeasured in a 0–6 scale, as shown in Table 1.*

Nevertheless, canary #2 showed a significantly higher level of yeast shedding throughout the surveillance period than canary #1 (*P* < 0.05, Mann-Whitney rank sum test). Excretion of yeast cells was also observed in additional fecal samples obtained on day + 90 (data not shown) from these two canaries.

**Discussion**

*M. ornithogaster* is currently considered an increasingly important avian pathogen which might represent a potential threat to bird breeders [4]. However, the exact implication of this fungal species in avian disease remains unclear [2,10,11].

Megabacteriosis is widespread in colonies of some bird species, but there is extensive variation in the reported prevalence rates, which range from 22.5–64% in budgerigars [4,6,15–17] and 9–55.1% in canaries [4,8]. Furthermore, most *M. ornithogaster* infections are benign or cause little detectable disease [6,11], and healthy birds can shed yeast cells while appearing normal on physical examination whereas sick birds may not shed them continuously [2,8,11].

In this study, we detected a low prevalence (5.13%) of *M. ornithogaster* in fecal samples from canaries, which contrasts with the figures reported in the aforementioned studies. The causes for such contrasting result are unknown but multiple factors may be implicated, including differences in the populations surveyed (e.g., general condition of animals, management practices and/or male to female ratio – as megabacteria seem to be more prevalent in males [17]), the geographical distribution of *M. ornithogaster*, or the methodology used for detecting the yeast.

The initial sampling of the canary colony surveyed in this study was performed by taking a swab sample from the cloaca of each animal. As the amount of feces obtained by this procedure is usually low, to avoid false negative results we used the culture enrichment method described by Hannafusa *et al.* [6]. Nevertheless, discontinuous fecal shedding of megabacteria by some sampled canaries cannot be ruled out, which could have resulted in the underestimation of the prevalence of this yeast in the studied population. Postmortem examination of the digestive tract can reveal additional cases of *M. ornithogaster* infection in animals that do not shed yeast cells [8], but this was not possible in our case, as no canaries died during the present study.

A close surveillance of the two culture-positive canaries revealed prolonged shedding of *M. ornithogaster* during several weeks. Although the number of yeast cells shed at some sampling points was high, the animals remained asymptomatic throughout the study period. This latter observation suggests that *M. ornithogaster* might be part of the normal digestive flora of birds, as discussed by other authors [18].

In conclusion, to the best of our knowledge, this is the first study reporting the shedding of *M. ornithogaster* throughout extended time periods by clinically healthy canaries. Further studies are needed to elucidate the role of this yeast species in avian disease and, eventually, the factors which render avian hosts susceptible to megabacteriosis.

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