Infection trials in mice suggest that *Macrorhabdus ornithogaster* is not capable of growth in mammals

YASUKO HANAFUSA*, ENRICA COSTA† & DAVID N. PHALEN†

*Bacterial and Parasitic Disease Research Division, National Institute of Animal Health, Kannondai, Tsukuba, Ibaraki, Japan, and †Wildlife Health and Conservation Centre, The University of Sydney, Camden, New South Wales Australia

The objective of this study was to determine if *Macrorhabdus ornithogaster*, a yeast recovered from the junction of the proventriculus and ventriculus of the stomach of parrots and other birds, can infect mice. Fifteen healthy ARC (S) female mice (age 10 weeks) were inoculated with *M. ornithogaster* by gavage at (two different dosage rates \( n = 5 \)) or intraperitoneal injection \( (n = 5) \) (one dosage rate). They were euthanized 5 days later and examined for gross and microscopic evidence of infection. *Macrorhabdus ornithogaster* was not found to colonize the stomach, peritoneum, or viscera of the challenged mice. The results of this study show that *M. ornithogaster* is not able to infect mice by the oral and intraperitoneal routes of administration and suggest that infection in mammals is unlikely to occur.

**Keywords** *Macrorhabdus ornithogaster*, mammal, infection

Introduction

*Macrorhabdus ornithogaster* (formally known as megabacteria) is an anamorphic ascomycetous yeast. Based on phylogenetic analysis of its rDNA it is the only known member of its genus and is only distantly related to other yeasts [1]. It commonly infects budgerigars and canaries, as well as several other species of psittacine and passerine birds, ostriches and chickens. *M. ornithogaster* grows exclusively on the mucosal surface of the junction of the proventriculus and ventriculus (gastric isthmus) (Fig. 1). Most infections do not result in overt manifestations, but mild inflammatory lesions are seen histologically in clinically normal birds and a chronic wasting disease and an acute hemorrhagic gastritis are occasionally associated with infection [2].

*M. ornithogaster* is a slender and long microorganism (2 μm wide and up to 80 μm long) composed of two to six cells (Fig. 2) [1]. Filamentous bacteria and chains of large rod-shaped bacteria can superficially resemble it. However, this yeast has some very distinctive staining characteristics that distinguish it from other microorganisms. It stains positively with the Gram stain, but unlike bacteria in which the stain is found in the cell walls, the stain variably accumulates in the cytoplasm of *M. ornithogaster* and is not found in the cell wall. The yeast also stains with the Periodic Acid Shift stain and the positive response obtained with calcafluor distinguishes it from bacteria [1]. Genetically, it is readily separated from bacteria, as its ribosomal DNA can be amplified by PCR using panfungal and *M. ornithogaster*-specific primers [1].

Two reports have suggested that *M. ornithogaster* may also be able to infect mammals as well as birds. A microorganism resembling it was identified in cytological preparations of nasal mucus from a dog and a cat that had a chronic respiratory disease and the authors’ suggested that it was Megabacteria, (now *M. ornithogaster*) [3]. However, limited details of the morphology of the observed organisms were provided and they were not characterized by fungal specific stains or genetic analysis and as a consequence it was not possible to verify the authors’ conclusions. A subsequent infection study was conducted in mice using an organism isolated from the stomach of a budgerigar that was thought to be *M. ornithogaster*. At very high doses of this organism, it was found to colonize the
However, genomic analysis of this organism subsequently showed it to be a bacterium and not *M. ornithogaster* (Phalen, D.N. unpublished observation 2005).

*M. ornithogaster* is fastidious and has only recently been grown *in vitro*. To grow, it requires a microaerophilic environment and a liquid or semi-solid media of pH 3 – 4 containing bovine serum and one of several sugars. The yeast optimally grows at 42°C, somewhat more slowly at 37°C, and does not grow at room temperature [5]. Given that *M. ornithogaster* requires such restricted growth conditions, it is unlikely that the organisms seen in the respiratory secretions of the dog and cat were this microorganism.

The ability to culture *M. ornithogaster* has created an opportunity to perform controlled infection trials with pure preparations of organisms. The purpose of the study reported here was to test the hypothesis that *M. ornithogaster* can infect a mammalian species, the mouse.

**Materials and methods**

Fifteen 10-week-old outbred albino mice (Animal Resource Centre, Canning Vale, Western Australia) (S) mice were included in this study. Mice were housed in standard isolation units in a temperature controlled room and provided with food and water ad libitum. The study protocol was reviewed and approved by the University of Sydney’s Animal Ethics Committee.

*M. ornithogaster* was grown, as previously described, from scrapings of the gastric isthmus of a budgerigar (*Melospitta undulata*) that died spontaneously with an ulcerative proventriculitis [5]. Briefly, scrapings from the gastric isthmus were inoculated into Basal Eagle’s Medium, pH 3.5, containing 20% fetal bovine serum, 2% glucose and 100 IU/ml of each penicillin and streptomycin and incubated at 42°C in a microaerophilic environment. Growth of the fungus was immediate and robust and 10-fold dilutions were made every third day. The fifth passage of this isolate was used in the infection trial. Organisms were incubated for 4 days between passages. The fifth passage was used as it was the first to generate sufficient organisms to use in the infection trials. Immediately prior to inoculation, the concentration of *M. ornithogaster* was determined by counting organisms in a haemocytometer.

*M. ornithogaster* forms rods that contain between 1 and 4 nuclei. A single rod was considered as an organism in this study. Organisms were then pelleted (1000 g for 1 min) and resuspended in 0.25 ml of phosphate-buffered saline (PBS) (pH 7.4). Mice were randomly assigned to three treatment groups each containing five animals. One million and 10 million organisms were given by gavage in 0.25 ml of PBS into the oesophagus of the first (*n* = 5) and second (*n* = 5) treatment groups of mice. Ten million organisms were injected into the peritoneum of the third treatment group in a volume of 0.25 ml PBS (*n* = 5). The numbers of organisms used to inoculate the mice were chosen to maximize the chance that infection if it was possible would occur.

Each treatment group was monitored for the first 2 h following inoculation and then daily for 5 days. On the 5th day, animals were humanely euthanized as this was the day that experimental chickens were found to be infected [6]. Heart, lung, liver, spleen, kidney, oesophagus, stomach, peritoneum, mesentery, duodenum,

---

**Fig. 1** Haematoxylin and eosin stained section through the junction of the proventriculus and ventriculus (isthmus) of the stomach of a canary. *Macrorhabdus ornithogaster* are seen as tightly packed slender rod-shaped organisms that are moderately eosinophilic. In this section they fill the spaces within the mucosal glands (Arrow). This Figure is reproduced in color in the online version of *Medical Mycology*.

**Fig. 2** Gram stain of *Macrorhabdus ornithogaster* grown in culture. This organism is considered to be Gram positive, but often stains incompletely or faintly. This Figure is reproduced in color in the online version of *Medical Mycology*.
jejunum, ileum, caecum, and colon were collected, formalin-fixed, paraffin-embedded, sectioned at 4 μm and stained with haematoxylin and eosin. Sections were examined microscopically for evidence of colonization with *M. ornithogaster*. The stomach was split longitudinally. The mucosa of the tissue that was not fixed was scraped and the scrapings were examined directly under the microscope for *M. ornithogaster*.

**Results**

The mice remained healthy during the 5 days after infection. Gross lesions were not found at necropsy and scrapings of one half of the gastric mucosa were negative. Microscopically, *M. ornithogaster* was not found at any level of the digestive tract or in the internal organs of the mice from the three treatment groups. A moderate, locally extensive chronic, lymphoplasmacytic, interstitial pneumonia was present in two mice infected orally (10 million organisms) and one mouse infected by the intraperitoneal route.

**Discussion**

The history of *M. ornithogaster/megabacteria* is a convoluted one. Although this organism has always had the staining characteristics of a fungus, it was originally concluded to be a bacterium because organalles could not be readily detected by electron microscopy [7]. Scanlan and Graham also concluded that the organism was a bacterium because they were able to grow a bacterium from the stomach of budgerigars where *M. ornithogaster/megabacteria* were histologically observed [8]. However, the organism that they grew was a true bacterium and not *M. ornithogaster*.

Morphologically it did not resemble *M. ornithogaster* and the authors never used fungal stains to document that it had the *in vivo* characteristics of this fungus. More recently, genetic analysis of their isolate showed it to contain bacterial rDNA (E. Tomazewski and D. Phalen unpublished observation, 2003). Based on the methodology of Scanlan and Graham, Rossi isolated a bacterium from a budgerigar stomach and showed that this organism could infect mice. Believing that he had recovered *M. ornithogaster/megabacteria* he concluded that it could infect mammals [4].

It is conceivable that *M. ornithogaster* could grow in the mouse stomach as it has a similar pH (pH 3–4) to the optimum growth conditions for *M. ornithogaster* and it is likely that it provides a microaerophilic environment [5,9]. However, growth on respiratory surfaces or in healthy tissues would be very unlikely as their pH would be close to neutral and oxygen concentrations would be too high.

In this experiment, mice were infected with a dose of $10^6$ and $10^7$ *M. ornithogaster*. These doses are 10 and 100 times higher than the minimum dosage that caused 100% infection after five days in day-old chickens and therefore could be expected to result in infection in the mice [7]. These doses were chosen to provide the maximum opportunity for infection and yet still be in a range that would result in 100% infection in chickens (Phalen, unpublished observation, 2004). Given that infection did not occur we conclude that gastric infection of simple stomach mammals and systemic infection of mammals by *M. ornithogaster* is either unlikely or impossible.

We cannot completely rule out that had we used immunodeficient (nude) mice that infection would have been possible. However, we consider it unlikely, as the 5 days period between inoculation and apparent complete clearance of the organism would be too short for infection to have been mediated by an acquired immune response. Thus, we consider it more likely that environmental conditions such as oxygen, hydrogen ion, or nutrient concentrations more likely to have prevented infection.

A chronic interstitial pneumonia was found in three mice but *Macrorhabdus ornithogaster* was not found in these lesions. Similar lesions can be caused by the murine pneumovirus, Sendai virus, *Mycoplasma pulmonis* and *Chlamydia muridarum*. Potentially any one of these could be found in laboratory mice [10]. Given that these lesions were chronic and likely to have been present at the time of the infection we feel that they were unrelated to the treatment trial.

**Declaration of interest:** The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

This work was supported by the Wildlife Health and Conservation Centre, University of Sydney, and the Organization for Economic Co-operation and Development, Co-operative Research Programme, Paris France.

**References**

Hanafusa et al.


This paper was first published online on Early Online on 18 January 2013.