CHAPTER
30

Implications of *Macrorhabdus* in Clinical Disorders

DAVID N. PHALEN, DVM, PhD, Dipl ABVP-Avian

Fig 30.1 | *Macrorhabdus ornithogaster* stained with calcofluor white M2R and viewed with ultraviolet light (380-420 nm).

History and Identification of the Organism

The information available about macrorhabdosis (aka megabacteriosis, virgamycosis, avian gastric yeast) in birds is confusing for those unfamiliar with the literature and sometimes equally as confusing for those who are. The organism was originally thought to be a yeast because of its staining characteristics.[3] Subsequently, Van Herck, et al, concluded that it was a bacterium, as they were unable to demonstrate cytoplasmic organelles or a nucleus. They did, however, show nuclear-like structures in Giemsa-stained organisms but interpreted them to be "granules."[23] Scanlan and Graham reported isolating a bacterium from the stomach of budgerigars using standard microbiological techniques. The isolated bacterium, however, was smaller than the organism in vivo and was not characterized by periodic acid-Schiff (PAS) or silver stains.[20] Attempts by other investigators to grow this organism with standard microbiological isolation techniques have been unsuccessful. However, Gerlach reported isolation of this organism on MRS medium, a medium used for growing fungi, but was unable to maintain it past a few passages.[8] Huchzemeyer, et al, also reported isolating an organism from the proventriculus of ostriches using MRS agar. This organism had the same biochemical properties as the one isolated by Scanlan and Graham, but was smaller than those seen histologically, and its ability to stain with PAS and silver stains was not reported.[9]

More recent work suggested that the "megabacterium" was, in fact, a yeast. In vivo trials showed that the "megabacterium" was susceptible to amphotericin B but not to antibacterial antibiotics.[4] It also stained strongly with calcofluor white M2R (Fig 30.1) and blancophor BA, stains that bind to chitin and cellulose, products not found in bacteria.[11,16] It grows, albeit slowly, in cell culture media supplemented with dextrose, fetal calf serum and antibacterial antibiotics. A nucleus was demonstrated by electron microscopy, and in situ hybridization with a pan eukaryote rRNA probe was positive.[17] Prior to conclusive determination of the genus and species to which the organism belonged, it was temporarily called avian gastric yeast. Tomaszewski, et al sequenced the ribosomal DNA of this organism and used this information to prove that it was a novel anamorphic ascomycetous yeast that belongs in its own new genus.[22]

Originally, it was proposed that it be named *Virgamycosis avigastricus*, but this name was not accepted. Subsequently, it has been named *Macrorhabdus ornithogaster*.[22]

SIGNS OF INFECTION

There are mixed opinions about whether *M. ornithogaster* can cause disease. Many investigators consider it to be a pathogen, while others have described it as a commensal.[8,20] The truth probably falls in between. It is clear that under some, perhaps most, circumstances, infection with *M. ornithogaster* does not result in clinical signs. It is equally clear that certain individual birds will show signs that can be attributed to infection with this organism, and that the prevalence of disease may be high in some collections and perhaps in some species of birds. Whether this represents variation in the pathogenicity of different strains of *M. ornithogaster* or differing susceptibilities of the affected birds is not known.

Host Range

Macrorhabdus ornithogaster has a wide host range and a worldwide distribution. It was first described in canaries and has subsequently been identified in captive-raised and free-ranging finches.[3,6,13,23] The prevalence of infection in budgerigar aviaries is high, and the percentage of infected birds in aviaries where it is enzootic may range from 27 to 64%, as judged by fecal shedding.[1,4,5]

Macrorhabdus ornithogaster also is commonly found in parrotlets, cockatiels and lovebirds.[5,10,14] Filippich reports that it is seen in several species of captive Australian parrots.[5] *Macrorhabdus ornithogaster* also is reported in ostriches, chickens, turkeys, geese, ducks and two species of ibis.[8,10,12,21] It is suggested that organisms thought to be *M. ornithogaster* also can infect mammals.[19] The organisms used in these experiments, however, appear to be bacteria and not *M. ornithogaster*, and there is no evidence at this time to suggest that it is a pathogen of mammals.

BUDGERIGARS

Filippich describes two clinical presentations in the budgerigar. In the acute presentation, apparently healthy birds suddenly go off feed, regurgitate ingesta (which may be blood stained), and die within 1 to 2 days. In the more common chronic form, affected birds typically appear to be hungry and spend considerable time at the food dish. Instead of eating, however, these birds are grinding their food but not ingesting it. Regurgitation is common, and fresh or dried saliva is often found on the tops of affected birds' heads. Undigested seeds may be present in the droppings. Diarrhea with or without melena also may be present. These birds go through a prolonged period of weight loss (going light) where they appear unthrifty and eventually die. If the affected budgerigar colony is sufficiently large, there will always be a few birds in the collection that will be showing these signs. Birds with clinical signs of infection are reported to have decreased packed cell volumes and low sodium, chloride, phosphate, glucose, cholesterol and aspartate aminotransferase values. When there was gastric ulceration, markedly low total protein concentrations were observed. Contrast radiography revealed, in some birds, a dilated proventriculus and an increased transit time. In one aviary, mature birds with a mean age of 2.7 years were most commonly affected.[5,6] Although clinical signs are more common in middle-aged budgerigars, infection begins very early in these birds and the author has seen large numbers of organisms in the isthmus of nestling budgerigars that were only 12 days old. It is critical to note that other diseases in budgerigars also can cause similar signs; these include candidiasis of the crop or ventriculus, a bacterial ventriculitis, trichomoniasis, enteritis, heavy metal poisoning and neoplasia of the stomach.

PARROTLETS

Parrotlets appear to have an acute onset of disease where they develop regurgitation and may have melena (D. Zantop, personal communication). Infection and disease appear to be most common in the green-rumped parrotlet, especially its color mutations.[14]

LOVEBIRDS

Regurgitation and weight loss were seen in two flocks of lovebirds. Organisms believed to be *M. ornithogaster* were found in significant concentrations in the drop-

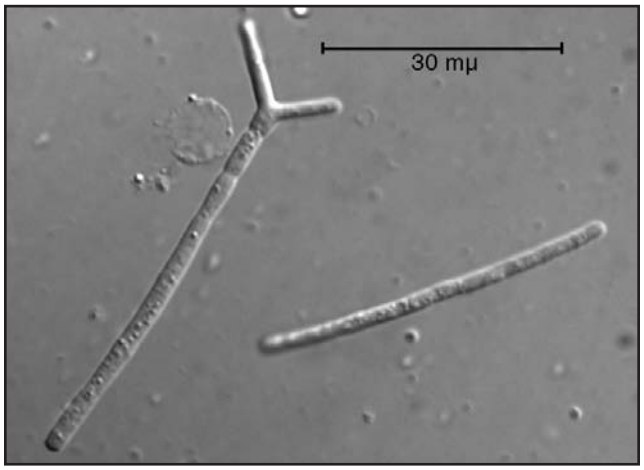

Fig 30.2 | Unstained *M. ornithogaster*. The organism on the right is typical. The Y-form on the left is rarely seen.

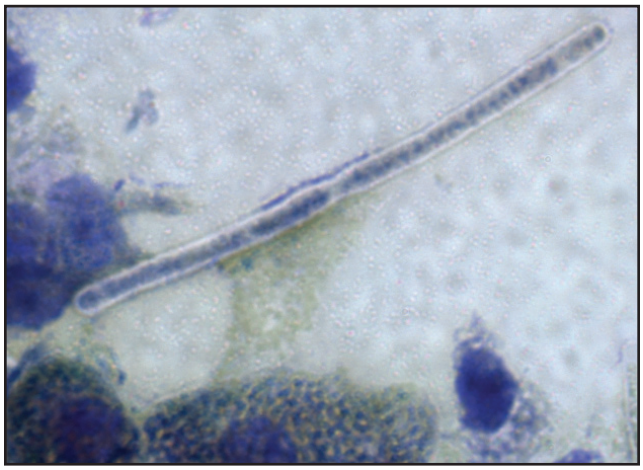

Fig 30.3 | Gram stain of *M. ornithogaster*. Only the cytoplasm stains. Often many of the organisms will stain only faintly or not at all with Gram's stain.

pings of these birds. Psittacine beak and feather disease also was found in affected birds and may have played a role in the ability of *M. ornithogaster* to cause disease in them (T. Lightfoot, personal communication, 2003). It should be noted, however, that psittacine beak and feather disease virus infection is widespread in lovebirds, so this may have been a coincidental finding.

CANARIES AND FINCHES

Signs of disease in canaries and finches are poorly defined. Most bird owners first recognize that there is a problem when a bird is found dead. Typically these birds are thin, suggesting a chronic course of disease that was unrecognized by the owner.[3,23]

OSTRICHES

Disease in ostriches associated with *M. ornithogaster* was described in 10-day-old to 12-week-old chicks. Birds appeared normal but ceased growing and lost weight. Eventually they became weak and died. Birds had soiled vents and were anemic. Diarrhea was observed in some birds while others had dry, pelleted stools. Mortality rates varied from 40 to 80% in affected flocks.[9]

CHICKENS

Two reports describe signs that were seen in flocks of chickens naturally infected with *M. ornithogaster*. In the first report, birds were stunted and prone to eat litter and pick at each other.[10] In the second report, stunting also was seen along with increased mortality and poor laying performance. All but one of the birds in the second study had significant concurrent diseases, making it difficult to know if *M. ornithogaster* acted as a primary or secondary pathogen.[21] Experimental infection with *M. ornithogaster* in white leghorn chickens did not result in clinical signs of disease. However, the feed conversion rate in infected birds was reduced compared to non-infected controls, suggesting that *M. ornithogaster* may have an important economic significance if introduced into poultry flocks.[15]

Detection

Macrorhabdus ornithogaster is a long, straight, narrow rod that is 3 to 4 μm wide and 20 to 80 μm long (Fig 30.2). It will occasionally branch, but this is rare (Fig 30.2). The longer organisms are actually chains of 2 to 4 cells, but the septations between cells are not readily observed. They are gram-positive, but many organisms will not pick up the stain or will only pick up the cytoplasm and not the thick cell wall, and therefore will stain faintly or not at all (Fig 30.3).

Similarly, *M. ornithogaster* stains poorly and variably with quick stains used for cytology.[22] Also, it has been the author's impression that the organism is easily washed off slides during the staining process.

Short of a proventricular scraping or flush, there is no definitive way to detect *M. ornithogaster* infections in the live bird. Many, probably most, sick birds with macrorhabdosis will shed large numbers of organisms in their droppings. These organisms are best observed by making an unstained wet mount of a dropping and examining it under the microscope at 100x and 400x magnifications. Reducing the diameter of the stage diaphragm will make the organisms easier to see (Fig 30.4). Shedding in birds that are not showing signs of illness is highly inconsistent. Examination of five or more droppings may be necessary to find even a few organisms, and in some birds shedding will not be detected at all. The opposite also is true, in that an occasional asymptomatic bird will shed large numbers of organisms. Additionally, fecal screening

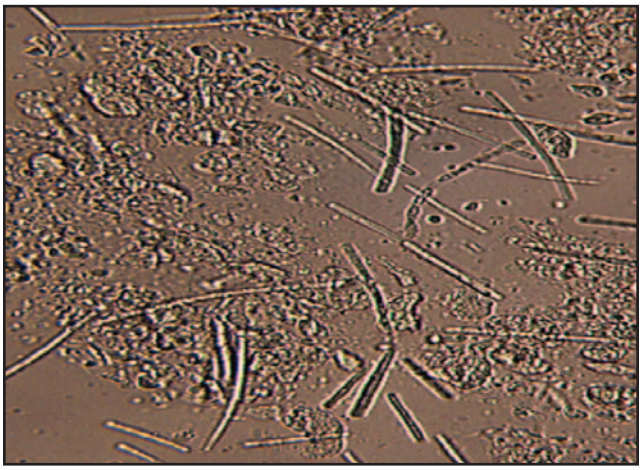

Fig 30.4 | Wet mount of a scraping from the gastric isthmus of a budgerigar with a heavy infection of *Macrorhabdus ornithogaster*. The aperture stage condenser was narrowed to increase contrast.

is complicated because feces contain debris that may be mistaken for *M. ornithogaster*.[14] *Macrorhabdus ornithogaster* stained with calcofluor white M2R is readily visualized with a fluorescent microscope with excitation barrier filters (380-420 nm) (see Fig 30.1). Unfortunately, this technique is not commonly available.[11]

Treatment Options and Interruption of the Infection Cycle

The only antimicrobial tested to date that was effective against *M. ornithogaster* is amphotericin B.[4,14] Treatment was effective only at a dose of 100 mg/kg by gavage twice a day for 30 days. A water-soluble preparation[a] when given for 14 days was not effective. A strain of *M. ornithogaster* resistant to amphotericin B has been identified in Australia.[14] It is not known how widespread resistance to amphotericin B may be. Initial reports in chickens suggested that fluconazole (100 mg/kg q 12 h) showed some promise for treating this organism. Subsequent testing showed that this dose was highly toxic to budgerigars and that mortality was seen even when the dose was reduced to 10 mg/kg q 12 h. A dose of 10 mg/kg q 24 h was less toxic but was not effective.[14] A single report suggested that nystatin was effective for treating *M. ornithogaster* in a small group of goldfinches.[4] Nystatin, however, was not effective in budgerigars in Australia.[7] Iodine preparations, lufenuron, ketoconazole, terbinafine or itraconazole also were not effective against *M. ornithogaster* in other trials.[14] *Macrorhabdus ornithogaster* shedding ceased when a *Lactobacillus* sp. was administered by gavage in treated budgerigars.[7] The birds were not necropsied, so it is not known if they were cured or just temporarily stopped shedding the organism. Previous reports suggesting that *M. ornithogaster* is susceptible to antibacterial antibiotics were erroneous, as the organism they tested was not *M. ornithogaster*.[18,20]

Evidence suggests there are mixed benefits to treating an entire flock of birds for *M. ornithogaster*. This would require that amphotericin B be given by gavage to every bird twice a day for 30 days. Additionally, it would require that the environment be extensively cleaned and disinfected; to date it is not known which disinfectants are effective against *M. ornithogaster*. With these constraints, flock treatment is not likely to result in a flock cure. Filippich, however, suggested that treatment did result in a significant reduction of birds that were shedding the organism.[6] Culling positive birds without treatment did not result in a reduction of shedding. However, it was suggested that culling positive birds after treatment might be of some benefit, as these birds may be infected with amphotericin-resistant strains.

An alternate approach to eliminating the infection from a flock is to incubator-hatch and hand-raise the young. Experimentally, it has been shown that if budgerigar eggs are pulled from the parents and cleaned, and the chicks are not allowed to have contact with the egg or infected birds after hatching, infection does not occur. Hand-feeding nestling day-old parrotlets and budgerigars and keeping them isolated from other birds is not an easy task, but may be one that a breeder is willing to do if this organism is a problem in a flock of valuable breeding birds.

Based on the virtual impossibility of treating large flocks for *M. ornithogaster*, the author recommends treatment of birds showing signs and selectively breeding birds that do not demonstrate signs of disease. Clinical experience suggests that some budgerigars may have a heritable resistance to infection (L. Filippich, personal communication, 1997).

It was thought that *M. ornithogaster* may cause a change in the pH of the stomach. While this seems very unlikely given that *M. ornithogaster* has little or no impact on the acid-secreting cells of the proventriculus, it has led some to speculate that administering agents that would acidify the stomach may be effective in its treatment. A controlled study testing this hypothesis failed to show efficacy of an orally administered acidifying agent.[4]

Findings at Necropsy

Gross necropsy findings are not specific. Birds are typically thin to emaciated and have little or no body fat. There may be ulceration of the ventriculus, proventricu-

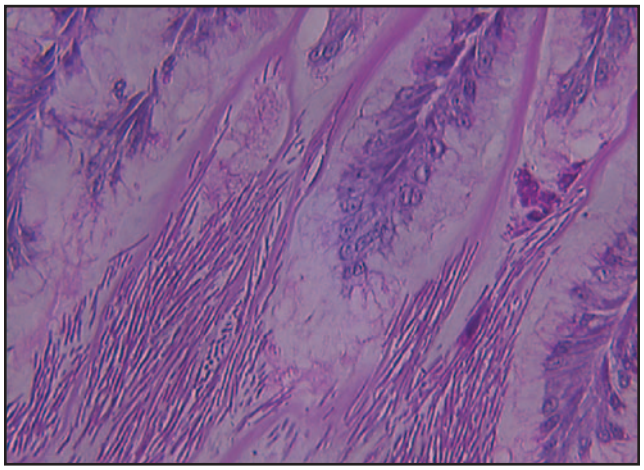

Fig 30.5 | Hematoxylin and eosin-stained section of the gastric isthmus, demonstrating the "log-jam" appearance of *Macrorhabdus ornithogaster* in situ.

lus or both. Most often the distal proventriculus and isthmus, the narrow zone of the stomach between the proventriculus and the ventriculus, is slightly dilated and has a thin wall. Perforation of the proventriculus is reported in some cases. Thick, opaque mucous secretions may cover the mucosa of the proventriculus and isthmus.[1,5,6] Microscopically, the organism is found on the surface of the glands of the isthmus and the transitional koilin. When present in large numbers, these organisms penetrate between the isthmus glands and invade the transitional koilin of the isthmus and the koilin of the ventriculus (Fig 30.5). When the organisms penetrate to the level of the isthmus glands, there is usually a significant accompanying disruption of the koilin. Additionally, isthmus glands appear to atrophy or undergo necrosis. Varying degrees of inflammation were observed in different studies. In the author's experience, inflammation in the budgerigar often is absent or minimal. If there is ulceration there will be a heterophilic response. It is normal for budgerigars, particularly nestling budgerigars, to have mild to moderate lymphoplasmacytic aggregates in the lamina propria of the proventriculus and isthmus. These findings should not be interpreted as a response to *M. ornithogaster* infection. It is possible, however, that these aggregates may become larger and more extensive in some infected birds. Baker found a significant mononuclear cell infiltrate in the mucosa of budgerigars with macrorhabdosis. Birds with chronic disease also showed evidence of goblet cell hypertrophy and fibrosis of the submucosa. Glandular cysts were seen occasionally.[1]

The microscopic lesions in infected chickens resemble those described by Baker in the budgerigar. The normal lymphoplasmacytic aggregates found in the lamina propria and submucosa are markedly expanded, resulting in a prominent widening of the folds of the glands of the isthmus.[12,15,21]

It is important to note that infections with *M. ornithogaster* are common and are often present in birds that die from other causes. Finding *M. ornithogaster* without evidence of koilin disruption and ulceration makes it unlikely that it was the cause of death.

There are reports that *M. ornithogaster* can be found in the intestinal tract and very rarely in other organs.[8] There are bacteria that can have a similar appearance to *M. ornithogaster*; therefore, if it is suspected that organisms outside the stomach are in fact *M. ornithogaster*, sections should be stained with a chitin-specific stain to prove that they are not bacteria.

Products Mentioned in the Text

a. Amphotericin-B, Megabac-S, Vetafarm, Wagga Wagga, Australia, www.vetafarm.com.au

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