Wildlife Health Australia

Macrorhabdus (Megabacteria) in wild birds in Australia

Fact sheet

Introductory statement

Macrorhabdus ornithogaster is an anamorphic ascomycete yeast that is only found in the stomach of birds in a narrow zone (isthmus) between the proventriculus and the ventriculus (Tomaszewski *et al*. 2003). It has a worldwide distribution and is common in many cage bird species (Hargreaves, 1981; Tsai *et al.* 1992; Filippich and Parker 1993; Pennycott *et al*. 1998; Martins *et al*; 2006). It has also been documented in wild birds (Filippich and Parker 1994; Pennycott *et al*. 1998; Doneley 2012) and production species including the ostrich (Huchzermeyer *et al*. 1993) and the chicken (Mutlu *et al*. 1997). Many infections are asymptomatic, but infection has been associated with both morbidity and mortality particularly in cage birds (Dorrestein 1980; Baker 1985). It can be found in some species of wild Australian birds, but its reservoir, the extent of host range, and its impact in wild Australian birds remains unknown. Given that *M. ornithogaster* can infect chickens and given that it is present in wild Australian birds, there is a concern that it could impact the free-range poultry industry. *Macrorhabdus ornithogaster* has previously been known as “Megabacteria”.

Aetiology

Classification of the causative agent: Family (unnamed at this time), subfamily (unnamed at this time), genus (*Macrorhabdus*). *Macrorhabdus ornithogaster* is an anamorphic ascomycete that is the only member of its genus. It is only distantly related to other genera of ascomycete yeasts (Tomaszewski *et al*. 2003). From the time of its first microscopic description until it was characterized genetically in 2003, it was thought to be a bacterium (van Herck *et al.* 1984). Therefore, much of literature relating to this organism can be only found by searching under its original name, “Megabacteria.”

Natural hosts

Macrorhabdus ornithogaster has a unique morphology, staining characteristics and appearance in histological sections of the isthmus (Hargreaves 1981; van Herck et al. 1984). However, it can resemble long filamentous bacteria and many reports of infection do not provide any detail to its microscopic appearance. As a result,

the hosts reported in this document are only those that come from sources where an adequate description of the organism has been provided.

Psittacine species: The full spectrum of psittacine species susceptible to *M. ornithogaster* is not known, but is likely to be substantial and it is likely that infections have been seen in many species but have not been reported. To date it has been reported in birds from all geographic origins including but not restricted to lovebirds (*Agapornis* spp.) which are African species, Indian-ring necked parakeet (*Psittacula krameri*), an Asian species, parrolettes (*Forpus* spp.) which are South American species, the New Zealand red-crowned kakariki (*Cyanoramphus novaezelandiae*) and a wide range of Australian species including the budgerigar (*Melopsittacus undulates*), cockatiel (*Nymphicus hollandicus*), king parrot (*Alisterus scapularis*), red-winged parrot (*Aprosmictus erythropt*erus), sulphur-crested cockato*o* (*Cacatua gtalerita*), galah (*Cacatua roseicapilla*), white-tailed black cockatoo (*Calyptorhynchus latirostris*), Bourke's parrot (*Neophema bourkii*), scarlet-chested parrot (*Neopehma splendida*), princess parrot (*Polytelis alexandrae*), superb parrot (*Polytelis swainsonii*), mulga parrot (*Psephotus vafrius*) and the rainbow lorikeet (*Trichoglossus*) (Filippich and Parker 1993, Hanka *et al*. 2010; Piasecki *et al*. 2012).

Macrorhabdus ornithogaster has only been documented in wild parrots in Australia and not those from other geographic locations. Wild parrots identified with infection with *M. ornithogaster* include the galah and sulphur-crested cockatoo (Filippich and Parker 1993; Phalen *et al.* 2007; Doneley 2012).

Passerine species: *Macrorhabdus ornithogaster* was first described in captive canaries (*Serninus canaria*) and is widespread in them. It has been found to infect a wide range of European, African and Australian finches in captivity. These include but are not limited to the European goldfinch (*Carduelis carduelis*), the green finch (*Carduelis chloris*), and sisken (*Carduelis spinus*), Begalese finch (*Lonchura domestica*), Pictorella finch (*Heteromunia pectoralis*), grey singing finch (Sirnus leucopygius) and the Australian finches, zebra finch (*Taeniopygia guttata*), Gouldian finch (*Erythrura gouldiae*), and painted fire-tail finch (*Emblema picta*) (Filippich and Parker, 1993, Filippich and Parker, 1994b). It has been identified in wild European finches (Pennycott 1998) and in feral European goldfinches in Australia (Filippich and Parker, 1994a). It has also been detected in a wild corvid and thrush species in Poland (Piasecki *et al*. 2012).

Poultry: *Macrorhabdus ornithogaster* has been described in chickens in Europe (Mutlu *et al*. 1997; Schulze and Heidrich 2001; Hanka *et al*. 2010) North America (Behnke and Fletcher 2011), South America (Martins *et al*. 2006) and in Australia (Phalen *et al*. 2007) and chickens can readily be infected with it. Other captive-raised gallinaceous birds that have been documented to be infected with *M. ornithogaster* include the Japanese quail (Coturnix japonica), grey partridge (*Perdix perdix*), Chukar partridge (*Alectoris chukar*), and the turkey (*Meleagris gallopavo*) (Martins *et al*. 2006; Jansson *et al.* 2008; Hanka *et al.* 2010). It has also been reported to infect domestic ducks (Hanka *et al.* 2010).

Other species: Infections with a *M. ornithogaster*-like organism has been described in two species of ratite, the ostrich (*Struthio camelus*) (Huchzermeyer *et al.* 1993) and the greater rhea (*Rhea americana*) (Martins *et al*. 2006), but has not been genetically characterized. The same is true for the pigeon (*Columbia livia*) (Hanka *et al*. 2010; Piasecki et al. 2012).

There are reports of organisms resembling *M. ornithogaster* in the upper respiratory track of a cat and a dog (Cook 2000; Huchzermeyer 2000). Images of the organism were not provided in these reports. Given the pH requirements for *M. ornithogaster* and its need for a microaerophilic environment to grow (Hanafusa *et al*. 2007), it is unlikely that the observed organisms were *M. ornithogaster*. Attempts to infect mice have been unsuccessful, again suggesting that it cannot survive in mammals (Hanafusa *et al.* 2013).

World distribution

Macrorhabdus ornithogaster has been reported from captive birds in Europe, North and South America, Japan and Australia (Hargreaves, 1981; Tsai *et al.* 1992; Filippich and Parker 1993; Pennycott *et al*. 1998; Martins *et al*; 2006). However, given that it is common in captive-raised budgerigars, canaries, and other species of finches and these birds are kept as pets around the world, it is likely that it has a fully worldwide distribution. It has only been found in wild birds in Europe (Pennycott 1998) and Australia (Filippich and Parker 1993, Doneley 2012). However, the presence of it in backyard poultry in the USA (Behnke and Fletcher 2011), suggests that a wild bird or environmental source may also be present in North America.

Occurrences in Australia

Macrorhabdus ornithogaster is widespread in avicultural species of parrots and finches. It is particularly common in budgerigars, lovebirds, canaries, zebra finches, and green finches and European gold finches. It has been seen in wild galahs, sulphur-crested cockatoos, and the feral European goldfinch on the eastern coast of Australia (Filippich and Parker 2004; Phalen *et al.* 2007; Doneley 2012). Its ultimate distribution in wild species is not known. There is a single report of a *M. ornithogaster* infection in a backyard chicken (Phalen *et al.* 2007). See "Statistics" below for records contained within the National Wildlife Health Information System.

Epidemiology

Transmission:

An environmental source for *M. ornithogaster* has not been found and the main reservoir appears to be infected birds. Many infected birds never show signs of disease but still shed the organism (Phalen *et al*., 2002; Kheirandish and Salehi 2011). It is likely that most infections result from faecal-oral contamination from sick or subclinical birds shedding it in their faeces. In a controlled trial in chickens, infection was found to pass from experimentally infected chicks to uninfected chicks housed with them (Phalen and Moore, 2003). It is also possible that altricial nestlings are infected when they are fed regurgitant by their parents. Worldwide dissemination is likely to have occurred as the result of the trade in cage birds (particularly canaries and budgerigars).

*Macrorhabdus ornithogaste*r can survive in the environment in culture media at room temperature for at least 24 hours (Bradley *et al.* 2005). How long it can survive in faeces in the natural environment is not known.

Incubation period

It is likely that colonization of the isthmus begins immediately upon exposure and heavy growth can be detected in experimentally infected birds by two weeks after infection. The time between infection and the development of signs, if they are going to occur at all, may range from a few weeks to years. Budgerigars infected with *M. ornithogaster* generally do not develop signs until they are two to three years old, while illness in ostriches has been reported in growing chicks (Baker 1985; Simpson 1992; Filippich and Parker 1994).

Morbidity

Infection prevalence can range from a few percent to 100% in captive birds (Filippich and Herdrikz 1998, Phalen 2005; Phalen *et al.* 2007). Little is known about the prevalence of infection in wild birds. In most instances, at least in cage birds, although many birds in a collection are infected only a small percentage of these birds will show signs of illness. In wild galahs in Australia, *M. ornithogaster* infections are seen regularly, but in a small number of recently fledged birds (Doneley 2012).

Mortality

Birds with clinical signs that are not treated die. Mortality rates in infected flocks can vary significantly (Baker 1985; Filippich 1993; Huchzermeyer and Henton 2000). Budgerigar and finch flocks typically only experience a low level of mortality, while near 100% mortality was observed in infected flocks of ostrich (Huchzermeyer and Henton 2000).

Clinical signs

Signs in cage birds include weight loss, vomiting, diarrhoea, appearing to eat but not ingesting food, whole seeds in the droppings, lethargy and being fluffed up (Baker 1985, Filippich and Parker 1993). Less commonly, birds may demonstrate melena. In ostrich chicks a stunting and runting syndrome is described (Huchzermeyer and Henton 2000). The impact on chickens is unclear. Experimentally infected birds showed a reduced rate of gain, but no outward signs of illness (Phalen and Moore 2003). Production chickens with natural infections all showed a variety of signs, but all flocks were impacted by a range of disease agents and it was not possible to determine which if any of the signs were caused by *M. ornithogaster* (Mutlu *et al*. 1997; Schulze and Heidrich 2001; Hanka *et al*. 2010; Behnke and Fletcher 2011). Finches are often found dead, but are thin suggesting that they had been ill, but this illness was not observed.

Diagnosis

Diagnosis of infection is made by finding organisms in the droppings. Large numbers of organisms are often found in birds with signs of disease, but birds with disease caused by *M. orithogaster* infection may not be shedding organisms and apparently healthy birds may be shedding many organisms (Filippich 1994, Phalen *et al*. 2002)

Macrorhabdus ornithogaster in the faeces is best demonstrated by mixing the faeces with 20 times their volume of saline, waiting 10 seconds for the larger debris to settle and doing a wet preparation of the surface film. Organisms can be seen with the stage diaphragm reduced to improve contrast using the 10X objective on the microscope. Organisms are strait-walled with rounded ends and are typically 3 to 4 micrometres wide and 20 to 80 micrometres long. Occasionally, branching (Y-shaped) forms are found in the faeces, but these are rare (Phalen 2005).

They can also be identified in faeces that are stained with quick stains used for cytology and with the Gram stain. They stain better and are more likely to stick to the slide if the slide is heat-fixed. They may stain incompletely with both stains, but are generally considered to be Gram positive. The cell wall contains chitin and they will stain with the Calcafluor white MR2 viewed with ultraviolet light (380-420 nm). They are also Periodic Acid Shift positive (Tomaszewski *et al.* 2003)

A PCR-based assay for infection is available in the USA. Data on the sensitivity of this assay as compared to faecal examination has not been reported (Veterinary Medical Diagnostics, Milford, Ohio).

Clinical pathology

There is a single report on haematological changes in budgerigars with *M. ornithogaster* infection. These birds exhibited a leukocytosis (Henderson et al. 1998). It is likely that most birds with chronic infection and exhibiting signs will have an anaemia of chronic disease and likely to be hypoproteinaemic.

Pathology

Gross findings: Most birds will have significant pectoral muscle atrophy. Budgerigars and finches with *M. ornithogaster* infections may have a thickened wall to the proventriculus and gastric isthmus and have increased mucous in the lumen of the proventriculus. Ulceration of the proventriculus and less commonly the ventriculus may also occur. A scraping of the isthmus will reveal the organism, which may occur in large numbers (van Herck 1984; Baker 1985; Schmidt et al 2003).

Histological findings: When found as an incidental finding it will be found growing on the surface of the gastric isthmus and at times penetrating between the glands. The faintly eosinophilic organism grows densely and has been described as looking like a "log-jam." In mild infections there may not be any associated inflammation. In advanced cases the numbers of organisms increase and infection may extend into the proventriculus and into the koilin layer of the ventriculus. Organisms may penetrate deep between the glands in the isthmus and deep into the koilin, ulceration may occur. Inflammation is predominately lymphoplasmacytic, but may become heterophilic if there is ulceration (van Herck 1984; Baker 1985; Schmidt et al 2003).

Differential diagnoses

The signs associated with *M. ornithogaster* infection are not specific and can occur with many other diseases, including trichomoniasis and giardiasis, bacterial and other fungal infections of the crop and stomach, helminth infections of the digestive tract, Bornavirus infection, crop and gastric foreign bodies and heavy metal poisoning to name a few.

Laboratory diagnostic specimens

Macrorhabdus ornithogaster is shed in the faeces. How long it persists in the faeces after they have been produced is not known. Histologically, it is found in the isthmus of the stomach and this tissue should be submitted for sectioning.

Laboratory procedures

Macrorhabdus ornithogaster is readily grown in culture using cell culture media (e.g., Basal Eagle's Medium Eagle) adjusted to pH 3-4, containing 20% foetal bovine serum, and 5% glucose or sucrose under microaerophilic conditions at 42 C (Hanafusa *et al*. 2007).

Treatment

Amphotericin B, nystatin, and low toxic antifungal chemicals (e.g. sodium benzoate**)** have all been shown to have some degree of efficacy against *M. ornithogaster*. A preparation of water soluble Amphotericin B (Vetafarm, Wagga Wagga, NSW) has been tested in budgerigars and stopped their shedding of the organism (Gestier http://www.vetafarm.com.au/pages/Megabacteria-in-Australian-Budgerigars.html). As will all water

soluble medications, the dosage rate in the treated bird will depend on how much water they consume and some species of birds drink little water. Amphotericin B as a pure product is available from multiple sources and can be made into a suspension. It was found to be effective when treating chickens at 100 mg/kg orally twice a day for 14 days. Nystatin has been found to be effective when administered in water at 3,500,000 IU/l of drinking water for 2 days, then 2,000,000 IU per litre for 28 days (Kheirandish and Salehi 2011). This study was well controlled and after treatment, a cohort of birds was euthanized and proven to be free of infection. There is some evidence to suggest that some strains of *M. ornithogaster* are resistant to nystatin or amphotericin B (Filippich and Perry, 1993) Sodium benzoate, potassium benzoate, and sodium sorbate prevent *M. ornithogaster* growth *in vitro* (Bradley et al 2005). Treatment attempts in a flock of budgerigars using sodium benzoate were effective, but because of high ambient temperatures birds were drinking an increased amount of water and some died, possibly from sodium toxicity (Hoppes 2012). Until additional controlled trials are done with this or other low toxic chemicals, their use cannot be recommended.

Prevention and control

In captive birds, examining the faeces of incoming birds will detect many, but not all infected birds. Treating all birds with amphotericin for at least two weeks, may be an effective means of preventing the introduction of infection to a colony. eventing exposure of aviary birds to wild birds can reduce the risk of infection. The natural source of this organism in the wild is not known and thus control in wild populations is not possible. Hand raising chicks from incubator-hatched chicks will also break the infection cycle (Moore *et al*. 2001).

Surveillance and management

Wildlife disease surveillance in Australia is coordinated by Wildlife Health Australia. The National Wildlife Health Information System (eWHIS) captures information from a variety of sources including Australian government agencies, zoo and wildlife parks, wildlife carers, universities and members of the public. Coordinators in each of Australia's States and Territories report monthly on significant wildlife cases identified in their jurisdictions. NOTE: access to information contained within the National Wildlife Health Information System dataset is by application. Please contact admin@wildlifehealthaustralia.com.au.

As an “Interesting or unusual disease”, cases of *M. ornithogaster* in wild birds should be reported to the general wildlife surveillance system through the state and territory wildlife coordinators (see www.wildlifehealthaustralia.com.au) or be captured by the zoo-based surveillance system. There are currently no formal targeted surveillance programs for *M. ornithogaster* in wild birds. In the captive situation, avian veterinarians routinely screen faecal samples for *M. ornithogaster* and other pathogens as part of the new bird examination and as part of a work up for sick birds.

The biggest management concern is that aviary-raised rare species of birds, particularly finches, could be released in efforts to supplement wild stocks and that *M. ornithogaster* could be unknowingly introduced into a naïve population.

Statistics

The bulk of the knowledge of the prevalence of this infection in captive and wild birds comes from limited published surveys. The National Wildlife Health Information System (eWHIS: www.wildlifehealthaustralia.com.au, accessed 10 November 2013) contains information on a small number of cases of megabacteriosis identified in wild birds in Australia as part of general surveillance activities. The

majority of cases (n = 17) are from QLD (n = 10), NSW (n = 6) and WA (n = 1) from galahs (n = 13) and little corellas (*Cacatua sanguine*; n = 2). Infection has also been reported in rainbow lorikeets (n = 1) and a pied currawong (*Strepera versicolor*; n = 1). A review of cases in the Australian Registry of Wildlife Health found 9 cases of *M. ornithogaster* infection from 1999 to 2014. Infection was found in a wild galah with a chronic wasting disease and 7 captive birds. They included a king quail (*Coturnix chinensis*), a zebra finch, a double-barred finch (*Taeniopygia bichenovii*), a plum-headed finch (*Neochmia modesta*), two red-browed finch (*Neochnia tempor*alis) and a chestnut-breasted manikin (*Lonchara castaneothorax*) which are all native Australian species. Lastly, infection was detected in a Java sparrow (*Lonchura oryzivoma*) an Indonesian species.

Anecdotal reports suggest intermittent "outbreaks" of *M. ornithogaster* may have occurred: in galahs in south-east Queensland associated with *Spironucleus* spp (formerly *Hexamiter* spp.) infection from October to March each year coinciding with the spring breeding season (Doneley unpublished), and; a single report from Broome in West Australia from primarily northern rainbow lorikeets (*Trichoglossus haematodus rubritorquatus*) that occurred between August 2005 and January 2006 (Baird unpublished). WHA is keen to hear from anyone with confirmed cases or laboratory diagnoses from these types of outbreaks. Similarly, we are keen to hear from anyone with confirmed diagnoses in wild birds that extends the host range or geographical distribution of infection.

Research

There is a need to determine the full host range and prevalence of infection in native Australian parrots and finches *in situ*. The sensitivity of the faecal examination as a means of determining infection needs to be assessed. There is also a need to assess the efficacy of amphotericin, nystatin, and low toxic antifungal chemicals across a range of species of birds and multiple isolates of *M. ornithogaster*. Modelling to determine the potential impact and significance of *M. ornithogaster* in Australian wild birds would be useful. The significance of infection with *Spironucleus* spp. in expression of disease in galahs and other Australian native psittacine species. An increased surveillance of free-range chickens for infection would be beneficial to determine if infection is occurring and becoming more common.

Human health implications

Although there have been several claims that that *M. ornithogaster* can infect a mammalian species these have either been inadequately documented or subsequently been found to have been infections organisms that were not *M. ornithogaster*. Additionally, infection trails in mice were unsuccessful (Hanafusa *et al*. 2013). Therefore, at this time, there is no apparent health risk for humans or other mammals.

Conclusions

Macrorhabdus ornithogaster is widespread in aviculture in Australia and around the world. It has also been identified in a limited number of free-ranging native and feral species in Australia. The impact of this organism on wild populations of birds is not known and merits additional study. Given that reports of infection in chickens are increasing, *M. ornithogaster* may also pose a threat to the health of free-range chickens.

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To provide feedback on this fact sheet

We are interested in hearing from anyone with information on this condition in Australia, including laboratory reports, historical datasets or survey results that could be added to the National Wildlife Health Information System. If you can help, please contact us at admin@wildlifehealthaustralia.com.au.

Wildlife Health Australia would be very grateful for any feedback on this fact sheet. Please provide detailed comments or suggestions to admin@wildlifehealthaustralia.com.au. We would also like to hear from you if you have a particular area of expertise and would like to produce a fact sheet (or sheets) for the network (or update current sheets). A small amount of funding is available to facilitate this.

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