



AVIAN GASTRIC YEAST (AGY) INFECTION (MACRORHABDIOSIS OR MEGABACTERIOSIS)

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Summary

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The review on avian gastric yeast (AGY) aimed to collect and update available information about this infection from clinical point of view which can help for better understanding of the disease. *Macrorhabdus ornithogaster* (*M. ornithogaster*) is the causative agent of proventriculitis in birds. The organism is large, rod-like, classified as anamorphic ascomycetous yeast. Clinical signs may vary from acute with sudden death or chronic wasting. Diarrhoea or enteritis has also been reported in infected birds. Birds can have other concurrent infections (enteric parasites, bacteria or viruses). Gross lesions may include proventricular oedema, hyperaemia, or haemorrhage, with overproduction of mucus accumulated in the proventricular lumen. The proventriculus may be dilated with or without ulceration in mucosa. The organism can be cultured on De Man, Rogosa and Sharpe (MRS) agar, but it is easily detected in Gram's, periodic acid-Schiff (PAS) and Giemsa stained proventricular/ventricular junction, or isthmus sections. Histological changes are more prominent in the ventriculus. Affected birds have marked disruption of the koilin layer with disorganisation and degeneration, and demonstrate large numbers of yeasts with matchstick or logjam appearance. The organism can be detected by polymerase chain reaction (PCR). The disease is common in budgerigars, canaries, finches, and parrotlets. Organisms are identified retrospectively in approximately one-fourth of canaries and budgerigars. The infection has also been reported in chickens, partridges, and ostriches but mildly in chickens. The affected birds can be successfully treated with amphotericin B.

Key words: budgerigars, histologic changes, *M. ornithogaster*, proventriculitis, yeast

INTRODUCTION

M. ornithogaster is the causative organism of avian gastric yeast (AGY). *M. ornithogaster* is an anamorphic Ascomycota yeast (Tomaszewski *et al.*, 2003). It infects a wide range of bird species as chickens, turkeys, ostriches, several spe-

cies of parrots, passerine species, in addition to captive-bred and wild finches (Phalen, 2014). The organism has a worldwide prevalence and differs extensively in pathogenicity. It was first described in canaries by Van Herck *et al.*

(1984). Under some conditions, *M. ornithogaster* infection does not show any signs. Certain individual birds will show signs as a result of infection with *M. ornithogaster*. In some species of birds, the prevalence of the disease may be high. This can be attributed to variation in the pathogenicity of *M. ornithogaster* strains or variation in susceptibility of the affected birds.

AGY is a highly contagious disease where *M. ornithogaster* colonises the proventriculus close to the proventricular-ventricular isthmus of birds and has not been recognised in other parts of the body or in the environment (Tomaszewski *et al.*, 2003; Phalen, 2005; Hannafusa *et al.*, 2007). Frequently affecting budgerigars, the disease appears without any symptoms in a chronic course and even the whole flock can become infected without showing any symptoms for several weeks or months. The chronic course can be interrupted by intermittent acute episodes. The disease has a negative influence on the digestion; therefore the affected bird loses weight in spite of excessive feeding.

SYNONYMS

M. ornithogaster name is derived from Greek words macrorhabdus (meaning long rod), and ornithogaster (meaning stomach of bird). *M. ornithogaster* (Megabacterium) disease has many synonyms as megabacteriosis, macrorhabdiosis, going light (GL) (Baker, 1985), wasting disease, budgie wasting disease (Henderson *et al.*, 1988), bacteria giganticus, Megabacteria Associated Disease (MAD) (Perry, 1993), or Proventricular/Ventricular Disease (PVD) (Filippich & Parker, 1994). The commonest name, however, is avian gastric yeast (AGY) infection.

HISTORY

M. ornithogaster was recognised as a bacterium for a long time; however latest research studies affirmed that the organism was a yeast (Tomaszewski *et al.*, 2003; Hannafusa *et al.*, 2007). The organism is a large Gram-positive bacillus. It was generally noticed in the proventriculus of budgerigars like normal flora (Scanlan & Graham, 1990).

Since 2000, *M. ornithogaster* "megabacteriosis" was distinguished clinically by emaciation, prostration, loss of appetite, cachexia and death. Normal chronic course was observed in chickens, turkeys, guinea fowls while acute disease was seen in the canary (*Serinus canarius*), the zebra finch (*Taeniopygia guttata*) and budgerigars (*Melopsittacus undulatus*) (Martins *et al.*, 2006).

ECONOMIC IMPORTANCE

The infection can be subclinical and birds appear normal. Variable mortality rates were reported in infected avian species: 50% mortality in rhea, 100% in a free-range flock of guinea fowl and 40% to 80% mortality in affected ostrich flocks (Huchzermeyer *et al.*, 1993). In chickens, experimental infection with *M. ornithogaster* showed only decrease in the feed conversion rate without any clinical signs (Phalen & Moore, 2003).

CHARACTERISATION OF

M. ORNITHOGASTER

Scientific classification

M. ornithogaster is the only member of genus *Macrorhabdus*, Order Saccharomycetales, Class Saccharomycetes, Division

Ascomycota, Kingdom Fungi (Tomaszewski *et al.*, 2001).

Morphology

M. ornithogaster is an ascomycetous yeast (Tomaszewski *et al.*, 2003). The vegetative cells are motile, elongated (2 to 20 µm) and divided by fission. The cells are single or in short chains of two to four cells. It is Gram positive, but only the cytoplasm stains with Gram stain (Hannafusa *et al.*, 2007). In mucosal scrapings and in the faeces of infected birds, the organism appears as a stiff, straight rod, 20 to 80 µm long and 2 to 3 µm wide, with rounded ends. The long rods may bend slightly in a gentle curve. On a wet mount, the organisms are viewed directly as small oblong refractile structures while the nuclei, found at regular intervals, are readily seen. The nucleus was demonstrated in *M. ornithogaster* isolates from budgerigars by electron microscopy and *in situ* hybridization with a pan-eukaryote rRNA probe was positive (Ravelhofer-Rotheneder *et al.*, 2000). On electron microscopy, it had a nucleus that contained eukaryotic ribosomal DNA as reported by Ravelhofer-Rotheneder *et al.* (2000). The cell wall contains chitin: a eukaryote-specific substance as detected by Calcofluor white M2R staining, and rRNA *in situ* hybridization demonstrates that it is a eukaryote (Tomaszewski *et al.*, 2003). Motility pattern of *M. ornithogaster* was seen as cellular lateral swinging or linear forward movements (Martins *et al.*, 2006).

Staining

M. ornithogaster stained weakly basophilic by haemotoxylin and eosin (H&E) and positive periodic acid reaction (Scanlan & Graham, 1990). It stained with silver stains and the periodic acid-Schiff stain (PAS) (Dorrestein *et al.*, 1980; Har-

greaves, 1981). The organism stained strongly with stains that bind the polysaccharide chitin as blanchophor BA (Ravelhofer *et al.*, 1998) and calcofluor white M2R (Moore *et al.*, 2001). The organism was Gram-positive, PAS-positive and acidophilic in Gram's, PAS and Giemsa stained sections, respectively (Kheirandish & Salehi, 2011).

Biochemical features

The isolates were catalase-negative and oxidase-negative and did not reduce nitrate. All isolates failed to utilise arginine, lysine, ornithine or tryptophan but produced acid from glucose, galactose, levulose, maltose, melibiose, starch, and sucrose. All isolates produced acetoin from glucose and hydrolysed esculin (Scanlan & Graham, 1990).

Culture media

The bacterium is extremely pleomorphic, facultatively anaerobic and capnophilic. When subcultured on agar media, it changes particularly in both diameter and length. After incubation on blood agar for several days, the bacterium is haemolytic, forming flat colonies with irregular edges. All isolates were reported to grow on sodium azide agar but not on MacConkey agar (Scanlan & Graham, 1990). Isolation of this organism on De Man, Rogosa and Sharpe (MRS) agar medium was reported (Huchzermeyer *et al.*, 1993; Gerlach, 2001). The organism appeared to grow gradually in cell culture media enhanced with dextrose, foetal calf serum, in addition to antibiotics (Ravelhofer-Rotheneder *et al.*, 2000).

NATURAL HOSTS

AGY was reported in several avian species including canaries (van Herck *et al.*,

1984), budgerigars (Scanlan & Graham, 1990; Henderson *et al.*, 1988; Baker *et al.*, 1992; Filippich & Hendrikz, 1998; Pennycott *et al.*, 1998), ostriches (Huchzermeyer *et al.*, 1993; Baker, 1997; Marinho *et al.*, 2004), chickens (Mutlu, 1997; Schulze & Heidrich, 2001) and turkeys (Schulze & Heidrich, 2001). Also, Martins *et al.* (2006) reported the disease in ostrich, rhea, canary, budgerigar, zebra finch, industrial broiler, free range chicken, turkey, guinea-fowl, domestic pigeon, ruddy ground-dove, toucan, chucker partridge. *Macrorhabdus*-like agents were reported also in mammals as dogs, cats and laboratory mice (Cooke, 2000; Rossi, 2000).

EXPERIMENTAL HOSTS

In chickens and Japanese quails, the attempts for experimental infection by using chicken isolate revealed the presence of *M. ornithogaster* in apparently normal birds in two organs (proventriculus and liver) (Martins *et al.*, 2006). Experimental intraperitoneal infection of mice with 10^3 CFU/dose was resulted in 100% mortality (Martins *et al.*, 2006), while Hanafusa *et al.* (2013) was failed to induce infection in mice.

INCIDENCE AND DISTRIBUTION

The large rod-shaped organism could be identified in both infected and normal chickens, turkeys, quails and pigeons, rheas (*Rhea americana*), ostriches (*Struthio camelus*), canaries, zebra-finches, guinea-fowl (*Numida meleagris*) and budgerigars (Martins *et al.*, 2006). The prevalence of infection in budgerigar, parrotlet, canary, and finch aviaries was often high (Dorrestein *et al.*, 1980; Simpson, 1992; Ravelhofer *et al.*, 1998; Baker,

1985; Filippich & Herdrikz, 1998). The prevalence of *M. ornithogaster* among cockatiels, budgerigars, lovebirds and barred parakeets was: 42.4% (14/19), 29.0% (9/22), 50.0% (2/4) and 0% (0/3) respectively in Uberaba, state of Minas Gerais (Paula *et al.*, 2018). The infection was also reported in wild parrots including the galah and sulphur-crested cockatoo (Filippich & Parker, 1993; Phalen *et al.*, 2007; Doneley, 2012). In Germany, Hanka *et al.* (2010) reported the infection in birds of 16 orders through Giemsa stained impression smears of the glandular stomach surface and *M. ornithogaster* was found in different frequencies in birds of the orders Psittaci-, Passeri-, Anseri-, Galli- and Columbiformes.

M. ornithogaster has been described in chickens in Europe, North and South America, and Australia (Mutlu *et al.*, 1997; Schulze & Heidrich, 2001; Hanka *et al.*, 2010; Behnke & Fletcher, 2011; Martins *et al.*, 2006; Phalen *et al.*, 2007). Also, Japanese quails, partridges and turkeys have been reported to be infected with *M. ornithogaster* (Martins *et al.*, 2006; Jansson *et al.*, 2008; Hanka *et al.*, 2010). Hanka *et al.* (2010) reported the infection in domestic ducks.

The infection with *M. ornithogaster* does not result in disease under most circumstances. Therefore, the recognition of the organism in a sick or dead bird does not confirm that it was the cause of the bird's sickness. Predisposing factors to *M. ornithogaster*-associated disease comprise variation in *M. ornithogaster* strains, poor management in addition to genetic factors of the host (Filippich & Perry, 1993; Speer *et al.*, 2004; Filippich & Herdrikz, 1998).

TRANSMISSION

M. ornithogaster, agent of megabacteriosis, of yeast form, is frequently encountered in the normal flora of bird's proventriculus. Under nonspecific circumstances, the host-fungus balance is disturbed, and the so-called "going light syndrome" clinical signs are manifested (Queirós *et al.*, 2011). *M. ornithogaster* colonises the isthmus (narrow junction) of the proventriculus (glandular stomach) and the ventriculus (grinding stomach) of birds (van Herck *et al.*, 1984). Healthy appearing normal birds can shed the organism with faeces while diseased birds may not continuously shed. Likely, most infections result from faecal-oral contamination from sick or subclinical birds shedding *M. ornithogaster* in their faeces (Kheirandish & Salehi, 2011; Lanzarot *et al.*, 2013). If one bird feeds another, the pathogen can be spread. A chronically infected bird that seems to be healthy can introduce and spread the disease when introduced into a new flock. Birds suffering from a megabacterial infection often show secondary bacterial infections. Detection of the infection could occur in some avian species along with other infectious or disease problems, for example, endoparasites (helminths, coccidia) and ectoparasitism (order Mallophaga and/or order Acarina) (Martins *et al.*, 2006). Transmission may happen among species and is supported by the absence of a traditional biosecurity policy (Martins *et al.*, 2006).

It was suggested the implication of genetic factors as the *M. ornithogaster* prevalence was significantly greater in chicks from parents positive for *M. ornithogaster* than from *M. ornithogaster*-negative parents, even when reared by *M. ornithogaster*-negative parents (Filippich & Hendrikz, 1998; Hoppes, 2013). Infec-

tion was found to pass from experimentally infected chicks to uninfected chicks housed with them (Phalen & Moore, 2003). Wild birds were considered as possible source of infection. *M. ornithogaster* infections were identified in 13 laying hens and 4 cocks located in 14 different flocks in addition to one turkey. As all infected birds were reared under circumstances permitting contact to wild birds and *M. ornithogaster* infections have been detected earlier in wild-living green finches, wild birds were considered a potential source of infection (Schulze & Heidrich, 2001). Various conditions such as recent shipping, crowded housing, reproductive activities, mixed species aviaries are commonly reported as stressors.

CLINICAL SIGNS

Generally, signs are non-specific varying from asymptomatic carriership to dangerous illness with high mortality. Birds become emaciated and suffer from anorexia, vomiting, cachexia and death. Dropping consistency varies from slight softness to severe diarrhoea. In some cases, birds eat frenziedly, but in fact are just grinding seeds without ingestion. In the acute stage, birds die quickly within few days. In the chronic stage, they become gradually more emaciated and weakened then die within weeks or months, or recover but with retrogression after weeks or months (Gerlach, 2001; Moore *et al.*, 2001; Martins *et al.*, 2006).

Budgerigars and parrotlets

Two clinical forms of the infection were reported in budgerigars.

- Acute form

The apparently healthy birds suddenly reduce the food intake than normal, regur-

gitate ingesta (which may be blood stained), then die within 2 days (Baker, 1985; Filippich *et al.*, 1993; Moore *et al.*, 2001; Phalen *et al.*, 2002). In the green-rumped parrotlet, infection and disease seems to be widespread. Also, clinical signs are widespread in middle-aged budgerigars, the infection begins very early and large numbers of organisms were detected in the isthmus of nestling birds aged 12 days (Phalen *et al.*, 2002; Phalen, 2006).

- Chronic form

Affected birds typically seem to be hungry and settle long time at the food dish. Rather than eating, the birds are grinding the food without ingesting it. Regurgitation is frequent, so the tops of affected birds' heads usually contain fresh or dried saliva. Undigested seeds may be present in the droppings. Diarrhoea with or without melena may also be present. These birds go through a long period of weight loss (going light) looking unhealthy and finally, they die (Baker, 1992; Filippich *et al.*, 1993; Antinof, 2004; Phalen, 2006). Few birds with the chronic form show clinical signs in the large affected budgerigar colony. Most commonly, affected birds are mature birds with an average age of 2.7 years (Filippich *et al.*, 1993).

Canaries and finches

Initially, owners perceive that there is a problem when a skinny bird is discovered dead (van Herck *et al.*, 1984; Phalen *et al.*, 2002). The infection in canaries and finches likely resembles the chronic form shown in budgerigars (Dorrestein *et al.*, 1980; van Herck *et al.*, 1984; Filippich & Parker, 1994).

The avian gastric yeast (megabacteriosis) recognised by high morbidity and

mortality was reported in a flock of zebra finches, the adults being exclusively affected. Clinical signs comprised puffed and ruffled feathers, feather loss, lethargy, and raised respiratory stress (Snyder *et al.*, 2013).

Endoventricular mycosis results from yeast proliferation within the koilin of the ventriculus in finch and finch-like birds. The clinical signs vary from sudden death to weight loss and presence of undigested seeds in the droppings.

Chickens

Schulze & Heidrich (2001) demonstrated a megabacteriosis-associated proventriculitis in 13 laying hens and 4 cocks located in 14 different flocks in addition to one turkey. Birds suffered from progressive runting, high mortality as well as poor laying performance. At necropsy, the proventriculus was enlarged with thickened wall and the mucosa filled with cloudy, grey-white mucus. At the proventricular-ventricular junction mainly, petechial haemorrhages and ulcerations were observed accompanied with irregular sloughing of the necrotic koilin layer of the ventriculus. In outbreak of mortality in chickens and Japanese quails sharing the same airspace, megabacteria were observed in examined 8 out of 11 chickens and 16 out of 24 quails (Pennycott *et al.*, 2003).

White Leghorn chickens experimentally infected with *M. ornithogaster* did not show any clinical signs, only the feed conversion rate was decreased in infected birds in comparison to non-infected controls (Phalen & Moore, 2003).

Ostriches

In ostriches, cases of *M. ornithogaster* infection were reported in 10-day to 12-week-old chicks. Birds appeared normal

but stopped growing and lost weight. Finally, they became weak and died. Birds were anaemic and had soiled vents. Some birds suffered from diarrhoea, while others excreted dry pelleted droppings. Mortality rates in affected flocks ranged from 40% to 80% (Huchzermeyer *et al.*, 1993).

Pigeons

Hanka *et al.* (2010) examined a total of 1,137 birds in 16 orders for presence of *M. ornithogaster*. Most of the findings were accomplished on preparations from glandular stomach surface. *M. ornithogaster* was detected in birds of the orders Psittaci-, Passer-, Anseri-, Galli- and Columbiformes in different frequencies. The authors presented the first proof of *M. ornithogaster* infection in two diseased feral pigeons.

GROSS LESIONS

At necropsy, atrophy of pectoral muscle, accompanied with proventricular dilation was observed. Proventricular and ventricular walls became thickened containing thick white mucus of alkaline pH 7–7.3 in addition to koilin layer loosening. Haemorrhage and ulceration in the proventricular-ventricular junction are reported (Baker, 1992; Werther *et al.*, 2000; Schulze & Heidrich, 2001; Marlier *et al.*, 2006; Martins *et al.*, 2006).

HISTOPATHOLOGY

Histologically, examined Gram-, PAS-, or silver-stained proventriculus sections showed pale eosinophilic organisms present at the tips of the glands of the isthmus lined up in parallel, like logs in a logjam. As the number of organisms increased, they moved into the spaces between the glands and could be seen on the surface of

the koilin of the gizzard, and, at times, invade it. Atrophy of the glands of the isthmus and ulceration of the isthmus and koilin developed in the most severe cases. Before ulceration, little or no inflammation was present. When inflammation occurred, it was typically a lymphoplasmacytic infiltration of the lamina propria of the glands of the isthmus. A moderate thickening of the lamina propria of the isthmus glands was noted in chickens infected experimentally with *M. ornithogaster*. Dilation of the proventricular glands and disruption of the normal structure of the koilin are other lesions that may be detected (Dorrestein *et al.*, 1980; Hargreaves, 1981; van Herck *et al.*, 1984; Baker, 1985; Filippich *et al.*, 1993). *M. ornithogaster* was histologically diagnosed in the mucosal isthmus of the proventriculus and ventriculus of adult hobby chickens showing intermittent signs of enteritis (Behnke & Fletcher, 2011). The most severe histopathological lesions in budgerigars were observed in the proventriculus and gizzard, especially at the proventricular-ventricular junction, including penetration of an organism to the lumen of the superficial proventricular crypts and occasionally to deeper parts of the glands; lymphocytic plasmacytic proventriculitis, lymphocytic plasmacytic ventriculitis and disruption of the koilin layer (Kheirandish & Salehi, 2011; Powers *et al.*, 2019).

CLINICAL PATHOLOGY

Henderson *et al.* (1988) reported that budgerigars showing clinical signs of *M. ornithogaster* infection presented some haematologic changes as reduced packed cell volumes (anaemia), leukocytosis, heterophilia, monocytosis, lymphocytosis, basophilia, and thrombocytosis as well as

low phosphate, sodium, chloride, glucose, cholesterol, and aspartate aminotransferase values.

DIAGNOSIS OF *M. ORNITHOGASTER* INFECTION

The characteristics of the disease and shape of the organisms found in the ulcerated gastric mucosa suggested megabacteriosis (Martins *et al.*, 2006).

Diagnosis of *M. ornithogaster* infection is based on the evaluation of clinical signs, gross lesions and the microscopic examination of Gram or Giemsa-stained direct smears from gastric mucosa, fresh faeces, or using mini-Flotac technique and detection of long rod-shaped organisms at 400 and 1000 times magnification (Martins *et al.*, 2006; Cringoli *et al.*, 2013; Borrelli *et al.*, 2015). Birds may shed the organism intermittently, so a negative faecal examination does not exclude infection. Wet mount, modified Wright's, or Gram stain of a faecal sample often reveal *M. ornithogaster* appearing as a large, Gram-positive rod, with mottling or stippling throughout its length (Hoppe, 2013). Examining five droppings from each bird will increase the chance of finding *M. ornithogaster*. Definitive diagnosis of megabacteriosis is most consistently demonstrated by histopathology and fresh smear of the proventricular mucus (Kheirandish & Salehi, 2011). Sullivan *et al.* (2017) compared polymerase chain reaction (PCR) of cloacal swab samples and faecal Gram's stain (FGS) for diagnosis of active *M. ornithogaster* shedding in a captive flock of budgerigars, where 57% sampled birds were positive by PCR and 24 – by FGS. All FGS-tested birds were positive on PCR, but the overall percent agreement for the two methods was only 67%.

The experimental infection of mice using the chicken isolate may be an indication of the pathogenicity of the isolate (Martins *et al.*, 2006).

Direct detection of *M. ornithogaster* cells in faeces by PCR (Phalen, 2014) and by culture methods using cloacal cotton-tipped swabs (Lanzarot *et al.*, 2013). In budgerigars, PCR was reported to be more preferable than FGS in diagnosis of *M. ornithogaster* (Sullivan *et al.*, 2017).

Isolation and identification

M. ornithogaster lives at the junction between proventriculus and ventriculus. Isolation of organism occurs mainly from crop wash, fresh faecal samples by sterile cotton-tipped swab or scraping of the gut lining. In case of crop wash and fresh faeces, samples are mixed with 20 times of their volume of saline. After 10 seconds, a wet preparation of the surface film was ready for preparation. Organisms can be seen by the 10× objective on the microscope. Fresh impression smears of gut mucosa are prepared onto glass slides for observation after staining by Giemsa and Gram's methods under light microscopy (Hannafusa *et al.*, 2007; Lanzarot *et al.*, 2013).

Molecular diagnosis

Genetic characterisation for *M. ornithogaster* is considered the proper method for diagnosis due to difficulty in its culturing. So, PCR application on tissue lesions of proventriculus and its contents from dead birds lead to a final diagnosis of *M. ornithogaster*.

PCR by pan-fungal DNA primer sets was applied for amplification of extracted rDNA from purified cells. Specific primer sets amplified only rDNA extracted from isthmus scrapings of an infected bird, but not rDNA extracted from a non-infected

bird or other control DNA. The sequence was assured to be obtained from the purified organism by *in situ* rRNA hybridization using a specific probe. Phylogenetic analysis of sequences of the 18S rDNA and domain D1/D2 of 26S rDNA demonstrated the organism to be previously undesignated anamorphic ascomycetous yeast appointing a new genus (Tomaszewski *et al.*, 2003).

There are two sets of the PCR primers targeting 18S rDNA of *M. ornithogaster*. The first PCR primer set (AGY1/SM2) is selected as a specific PCR test for *M. ornithogaster*; the forward primer being AGY1 5'-GGACTTATATTACTAGTCAGATGG-3' (positions 620–643). AGY1 does not match with any other reported fungi sequences and the reverse primer: Sm2 5'-CAATACGCCTGCTTTGAACACTC-3' (positions 761–783) (Razmyar *et al.*, 2016).

The second PCR set (SM1/SM2) used for sequencing analysis of *M. ornithogaster* comprised the forward primer, Sm1 5'-ATCTGGTT-GATCCTGCCAGTAGTC-3' (positions 2–25) and the reverse primer, Sm2 (5'-CAATACGCCTGCTTTGAACACTC-3' (positions 761–783) (Tomaszewski *et al.*, 2003).

DIFFERENTIAL DIAGNOSIS

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.

DISEASE CONTROL AND TREATMENT

M. ornithogaster control and treatment are very difficult for avian clinicians, as subclinical infections without clear clinical signs can happen in many birds. It is practically difficult to keep extensive aviaries free from the disease (Filippich *et al.*, 2004). Numerous antimicrobial and antifungal medications are not efficient in treating the disease as iodine arrangements, lufenuron, nystatin, fluconazole, ketoconazole, itraconazole, and terbinafine (Filippich *et al.*, 1993; Phalen *et al.*, 2002; Phalen, 2005). Amphotericin B is considered the best drug for treatment. It is given orally two times every day for a month at a dose of 100 mg/kg (Phalen *et al.*, 2002; Phalen, 2005). As megabacteria develop in an alkaline environment, another treatment technique based on increasing the proventricular fluid acidity by oral administration of apple vinegar or grapefruit juice can be used (Gerlach, 2001). On the other hand, Püstow & Krautwald-Junghanns (2017) applied a medication with amphotericin B (100 mg/kg PO q12 h) for 4 weeks in positive infected budgerigars. The reported results were unacceptable and stressful for the birds because of the handling and the long treatment duration.

In a pilot study, amphotericin B was administered at 100 mg/kg twice daily for 30 days in two naturally infected bird species (*Melopsittacus undulatus* and *Agapornis roseicollis*) and at 25 mg/kg and 100 mg/kg twice daily for 10 days in experimentally infected chickens. The retrospective analysis indicated treatment failure in 80.4% of 36 cases, but significantly decreased *M. ornithogaster* burden, followed by profound rebound effect of the number of organisms shed in the faeces. The findings proved treatment failure in 3

scenarios and indicated that treatment efficacy of amphotericin B against *M. ornithogaster* was poor (Baron *et al.*, 2019).

In a colony of zebra finches, correction of the light cycle treatment with amphotericin B improved dramatically the health of the birds and baseline mortality returned to normal (Snyder *et al.*, 2013).

Strains of *M. ornithogaster* were reported to be amphotericin B resistant in Australia (Filippich & Perry, 1993).

For moderate improvement of the flock mortality, oral application of nystatin medication in the feed in addition to vinegar administration in the drinking water for 3 weeks was recommended, followed by 500 mg/L sodium benzoate in the drinking water is administered orally for 4 weeks (Madani *et al.*, 2014).

Successful treatment of megabacteriosis in a canary was obtained with nystatin (Scullion & Scullion, 2004). The rate of mortality decreased and reached zero in budgerigars treated with nystatin (Kheirandish & Salehi, 2011). Probiotics are also thought to be of use through promoting health generally and by helping lower digestive tract pH through the production of lactic acid.

The mini-Flotac system enables the counting of yeast cells under microscope which is more obvious than the wet mount or Gram stain. So, evaluation of the effectiveness of the applied treatment can be based on yeast counting by this quantitative examination (Borrelli *et al.*, 2015).

PREVENTION

It is believed that as *M. ornithogaster* is spread faecally, it would be prudent to maintain good hygiene in bird's cages or house by thorough cleaning and disinfection with daily droppings elimination.

Common utilisation of waterers may be another source of disease transmission that can be controlled by good thorough house cleaning. It is also thought that disease transmission can occur through natural behaviour of the birds feeding each other. The *M. ornithogaster* infection was reported in both captive and wild psittacine and passerine species. Therefore, it is important to prevent cross infection between species. Strict hygiene measures should be undertaken when dealing with any sick bird.

Many AGY-infected birds are asymptomatic but still are shedding the organism (Phalen *et al.*, 2002), therefore it is important to screen all new birds especially parrots admitted into aviaries from wildlife so that infected birds can be isolated from AGY negative birds.

Proper and hygienic hand raising chicks from incubator-hatched chicks will also break the infection cycle (Moore *et al.*, 2001). Experimentally, it appeared that the disease did not happen if budgerigar eggs are collected from their parents, cleaned and the produced chicks are kept away from contact with the egg or infected birds (Moore *et al.*, 2001).

CONCLUSION

M. ornithogaster infection is prevalent worldwide in aviculture. In Egypt, there are no available data about the impact of this yeast on aviculture. Studies are required to illustrate the role of this yeast in avian disease and, eventually, the factors which render avian hosts susceptible to megabacteriosis.

REFERENCES

- Antinoff, N., 2004. Diagnosis and treatment options for megabacteria (*Macrorhabdus*

- ornithogaster*). *Journal of Avian Medicine and Surgery*, **18**, 189–195.
- Baker, J. R., 1985. Clinical and pathological aspects of “going light” in exhibition budgerigars (*Melopsittacus undulatus*). *The Veterinary Record*, **116**, 406–408.
- Baker, J. R., 1992. Megabacteriosis in exhibition budgerigars. *The Veterinary Record*, **131**, 12–14.
- Baker, J. R., 1997. Megabacteria in diseased and healthy budgerigars. *The Veterinary Record*, **140**, 627.
- Baron, H. R., K. C. L. Leung, B. C. Stevenson, M. S. Gonzalez & D. N. Phalen, 2019. Evidence of amphotericin B resistance in *Macrorhabdus ornithogaster* in Australian cage-birds. *Medical Mycology*, **57**, 421–428.
- Behnke, E. L. & O. J. Fletcher, 2011. *Macrorhabdus ornithogaster* (Megabacterium) infection in adult Hobby chickens in North America. *Avian Diseases*, **55**, 331–334.
- Borrelli, L., L. Dipineto, L. Rinaldi, V. Romano, E. Noviello, L. F. Menna, G. Cringoli & A. Fioretti, 2015. New diagnostic insights for *Macrorhabdus ornithogaster* infection. *Journal of Clinical Microbiology*, **53**, 3448–3450.
- Cooke, S. W., 2000. Role of Megabacteria in mammals [letter]. *The Veterinary Record*, **146**, 444.
- Cringoli, G., L. Rinaldi, M. Albonico, R. Bergquist & J. Utzinger, 2013. Geospatial (s) tools: Integration of advanced epidemiological sampling and novel diagnostics. *Geospatial Health*, **7**, 399–404.
- Doneley, B., 2012. Weight loss syndrome in juvenile free-living galahs (*Eolopus roseicapillus*). In: *Proceedings of the Annual Conference of the Australasian Association of Avian Veterinarians and Unusual and Exotic Pet Veterinarians*, Australia, pp. 9–11.
- Dorrestein, G. M., P. Zwart & M. N. Buitelaar, 1980. Problems arising from disease during the periods of breeding and rearing canaries and other aviary birds. *Tijdschrift voor Diergeneeskunde*, **105**, 535–543.
- Filippich, L. J. & R. A. Perry, 1993. Drug trials against Megabacteria in budgerigars (*Melopsittacus undulatus*). *Australian Veterinary Practitioner*, **23**, 184–189.
- Filippich, L. J., D. A. O’Boyle, R. Webb & J. A. Fuerst, 1993. Megabacteria in birds in Australia. *Australian Veterinary Practitioner*, **23**, 72–76.
- Filippich, L. J. & M. G. Parker, 1994. Megabacteria and proventricular/ventricular disease in psittacines and passerines. In: *Proceedings of the Annual Conference of the Association of Avian Veterinarians*, Reno, Nevada, USA, pp. 287–293.
- Filippich, L. J. & J. K. Hendrikz, 1998. Prevalence of megabacteria in budgerigar colonies. *Australian Veterinary Journal*, **76**, 92–95.
- Filippich, L. J., B. Speer, L. V. Powers & D. Phalen, 2004. Diagnosis and treatment options for megabacteria (*Macrorhabdus ornithogaster*). *Journal of Avian Medicine and Surgery*, **18**, 189–195.
- Gerlach, H., 2001. Megabacteriosis. *Seminars in Avian and Exotic Pet Medicine*, **10**, 12–19.
- Hanafusa, Y., E. Costa & D. N. Phalen, 2013. Infection trials in mice suggest that *Macrorhabdus ornithogaster* is not capable of growth in mammals. *Medical Mycology*, **51**, 669–672.
- Hanka, K., K. Koehler, E. F. Kaleta, D. Sommer & E. Burkhardt, 2010. *Macrorhabdus ornithogaster*: Detection in companion birds, poultry and pigeons, morphological characterization and examination of *in vitro* cultivation. *Der Praktische Tierarzt*, **91**, 390–395.
- Hannafusa, Y., A. Bradley, E. E. Tomaszewski, M. C. Libal & D. N. Phalen, 2007. Growth and metabolic characterization of *Macrorhabdus ornithogaster*. *Journal of Veterinary Diagnostic Investigation*, **19**, 256–265.

- Hargreaves, R. C., 1981. A fungus commonly found in the proventriculus of small pet birds. In: *Proceedings of 30th Western Poultry Disease Conference and 15th Poultry Health Symposium*, Davis, California, USA. University of California at Davis, pp. 75–76.
- Henderson, G. M., F. M. D. Gulland & C. M. Hawkey, 1988. Haematological findings in budgerigars with megabacterium and trichomonas infections associated with ‘going light’. *The Veterinary Record*, **123**, 492–494.
- Hoppes, S. M., 2013. Macrorhabdus in Budgerigars. In: *Pacific Veterinary Conference*. <https://cvma.net/wp-content/uploads/2014/10/FungalParrots.pdf>. (29 May 2019, date last accessed).
- Huchzermeyer, F. W., M. M. Henton & R. H. Keffen, 1993. High mortality associated with megabacteriosis of proventriculus and gizzard in ostrich chicks. *The Veterinary Record*, **133**, 143–144.
- Jansson, D. S., C. Brojer, R. Mattsson, R. Feinstein, T. Morner & C. H. Segerstad, 2008. Mycotic proventriculitis in gray partridges (*Perdix perdix*) on two game bird farms. *Journal of Zoo and Wildlife Medicine*, **39**, 428–437.
- Kheirandish, R. & M. Salehi, 2011. Megabacteriosis in budgerigars: Diagnosis and treatment. *Comparative Clinical Pathology*, **20**, 501–505.
- Lanzarot, P., J. L. Blanco, S. Alvarez-Perez, C. Abad, M. T. Cutuli & M. E. Garcia, 2013. Prolonged fecal shedding of ‘megabacteria’ (*Macrorhabdus ornithogaster*) by clinically healthy canaries (*Serinus canaria*). *Medical Mycology*, **51**, 888–891.
- Madani, S. A., A. Ghorbani & F. Arabkhaaei, 2014. Successful treatment of macrorhabdiosis in budgerigars (*Melopsittacus undulatus*) using sodium benzoate. *Journal of Mycology Research*, **1**, 21–27.
- Marinho, M., M. V. Meireles & A. V. G. Souza, 2004. Determination of the gastrointestinal microflora of ostriches (*Struthio camelus*) from the northwest area of the São Paulo state, Brazil, submitted to necropsy. *Arquivos do Instituto Biológico São Paulo*, **71**, 267–271.
- Marlier, D., C. Leroy, M. Sturbois, V. Delleur, A. Poulipoulis & H. Vindevogel, 2006. Increasing incidence of megabacteriosis in canaries (*Serinus canaries domesticus*). *The Veterinary Journal*, **172**, 549–552.
- Martins, N. R. S., A. C. Horta, A. M. Siqueira, S. Q. Lopes, J. S. Resende, M. A. Jorge, R. A. Assis, N. E. Martins, A. A. Fernando, P. R. Barrios, T. J. R. Costa & L. M. C. Guimarães, 2006. *Macrorhabdus ornithogaster* in ostrich, rhea, canary, zebra finch, free range chicken, turkey, guinea-fowl, columbina pigeon, toucan, chukkar partridge and experimental infection in chicken, Japanese quail and mice. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, **58**, 291–298.
- Moore, R. P., K. F. Snowden & D. N. Phalen, 2001. A method of preventing transmission of so-called ‘megabacteria’ in budgerigars (*Melopsittacus undulatus*). *Journal of Avian Medicine and Surgery*, **15**, 283–287.
- Mutlu, O. F., S. Seckin, R. A. Ravelhofer Hildebrand & F. Grimm, 1997. Proventriculus in fowl caused by megabacteria. *Der Praktische Tierarzt*, **25**, 460–462.
- Paula, I. H. de, F. P. Linhares, C. Y. Kanayama, S. P. de Carvalho, E. R. Bittar, M. T. G. Campos & J. F. F. Bittar, 2018. Megabacteria (*Macrorhabdus ornithogaster*) in psittacids kept in commercial establishments in the municipality of Uberaba – MG. *PUBVET*, **12**, 1–4.
- Pennycott, T. W., H. M. Ross, I. M. McLaren, A. Park, G. F. Hopkins & G. Foster, 1998. Causes of death of wild birds of the family Fringillidae in Britain. *The Veterinary Record*, **143**, 155–158.
- Pennycott, T. W., G. Duncan & K. Venugopal, 2003. Marek’s disease, candidiasis and megabacteriosis in a flock of chickens (*Gallus gallus domesticus*) and Japanese

- quail (*Coturnix japonica*). *The Veterinary Record*, **153**, 293–297.
- Perry, R. A., 1993. Megabacteria associated disease. In: *Proceedings of Avian Diagnostics Refresher Course for Veterinarians*, Post Graduate Committee University of Sydney, **221**, 13–19.
- Phalen, D. N., 2005. Diagnosis and management of *Macrorhabdus ornithogaster* (formerly megabacteria). *Veterinary Clinics of North America: Exotic Animal Practice*, **8**, 299–306.
- Phalen, D. N., 2006. Implications of *Macrorhabdus* in clinical disorders. In: *Clinical Avian Medicine*, vol II, eds G. J. Harrison, T. L. Lightfoot, Palm Beach, FL: Spix Publishing, Inc, pp. 705–710.
- Phalen, D. N., 2014. Update on the diagnosis and management of *Macrorhabdus ornithogaster* (formerly megabacteria) in avian patients. *Veterinary Clinics of North America: Exotic Animal Practice*, **17**, 203–210.
- Phalen, D. N., E. Tomaszewski & A. Davis, 2002. Investigation into the detection, treatment, and pathogenicity of avian gastric yeast. In: *Proceedings of the 23rd Annual Conference of the Association of Avian Veterinarians*, Monterey, USA, pp. 49–51.
- Phalen, D. N. & R. P. Moore, 2003. Experimental infection of white-leghorn cockerels with *Macrorhabdus ornithogaster* (Megabacterium). *Avian Diseases*, **47**, 254–260.
- Phalen, D. N., Y. Hanafusa, E. Costa & A. Bradley, 2007. Further investigation into the biology of *Macrorhabdus ornithogaster*. In: *Proceedings of Annual Conference of Association of Avian Veterinarians Australian Committee*, Melbourne (Victoria), Australia, pp. 75–78.
- Powers, L. V., M. A. Mitchell & M. M. Garner, 2019. *Macrorhabdus ornithogaster* infection and spontaneous proventricular adenocarcinoma in budgerigars (*Melopsittacus undulatus*). *Veterinary Pathology*, **56**, 486–493.
- Püstow, R. & M. E. Krautwald-Junghanns, 2017. The incidence and treatment outcomes of *Macrorhabdus ornithogaster* infection in budgerigars (*Melopsittacus undulatus*) in a veterinary clinic. *Journal of Avian Medicine and Surgery*, **31**, 344–350.
- Queirós, T. S., P. R. Carvalho & M. C. G. Pita, 2011. Megabacteriosis: *Macrorhabdus ornithogaster* in bird – review. *PUBVET*, **5**, 1080.
- Ravelhofer, K., R. Rotheneder, M. Gareis, R. Suttner, O. Wolf, R. Matiello & J. Kusters, 1998. Megabakteriosen bei verschiedenen Vogelspezies. XI. DVG Tagung über Vogelkrankheiten, München, 95–104.
- Ravelhofer-Rotheneder, K., H. Engelhardt, O. Wolf, R. Amann, W. Breuer & J. Kusters, 2000. Taxonomic classification of “megabacteria” isolates originating from budgerigars (*Melopsittacus undulatus* Shaw, 1805). *Tierärztliche Praxis*, **28**, 415–420.
- Razmyar J, AR Movassaghi & M Rezaee. 2016. An outbreak of severe *Macrorhabdus ornithogaster* infection in common canaries (*Serinus canaries domesticus*), Molecular and pathological assay. *Iranian Journal of Veterinary Science and Technology*, **8**, 64–68.
- Rossi, G., 2000. Possibility of infecting mammals with megabacteria isolated from birds [letter]. *Veterinary Record*, **147**, 371–372.
- Scanlan, C. M. & D. L. Graham, 1990. Characterization of a gram-positive bacterium from the proventriculus of budgerigars (*Melopsittacus undulatus*). *Avian Diseases*, **34**, 779–786.
- Schulze, C. & R. Heidrich, 2001. Megabacteria associated proventriculitis in poultry in the state of Brandenburg, Germany. *Deutsche Tierärztliche Wochenschrift*, **108**, 264–266.
- Scullion, F. T. & M. G. Scullion, 2004. Successful treatment of megabacteriosis in a canary (*Serinus canaria*) with nystatin. *The Veterinary Record*, **155**, 528–529.

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- Simpson, V. R., 1992. Megabacteriosis in exhibition budgerigars. *The Veterinary Record*, **131**, 203–204.
- Snyder, J. M., D. M. Molk & P. M. Treuting, 2013. Increased mortality in a colony of zebra finches exposed to continuous light. *Journal of the American Association for Laboratory Animal Science*, **52**, 301–307.
- Speer, B., D. Phalen, L. Powers, L. Filippich & N. Antinoff, 2004. Diagnosis and treatment options for megabacteria (*Macrorhabdus ornithogaster*). *Journal of Avian Medicine and Surgery*, **18**, 189–195.
- Sullivan, P. J., E. C. Ramsay, C. B. Greenacre, A. C. Cushing, X. Zhu & M. P. Jones, 2017. Comparison of two methods for determining prevalence of *Macrorhabdus ornithogaster* in a flock of captive budgerigars (*Melopsittacus undulatus*). *Journal of Avian Medicine and Surgery*, **31**, 128–131.
- Tomaszewski, E., K. Snowden & D. Phalen, 2001. The Whipple paradox: Megabacteria exposed as fungi. In: *Proceedings of the Annual Conference of the Association of Avian Veterinarians*, Orlando, Bedford, pp. 99–100.
- Tomaszewski, E. K., K. S. Logan, K. F. Snowden, C. P. Kurtzman & D. N. Phalen, 2003. Phylogenetic analysis identifies the ‘megabacterium’ of birds as a novel anamorphic ascomycetous yeast, *Macrorhabdus ornithogaster* gen. nov., sp. nov. *International Journal of Systematic and Evolutionary Microbiology*, **53**, 1201–1205.
- Van Herck, H., T. Duijser, P. Zwart, G. M. Dorrestein, M. Buitelaar & M. H. Van Der Hage, 1984. A bacterial proventriculitis in canaries (*Serinus canaria*). *Avian Pathology*, **13**, 561–572.
- Werther, K., R. P. Schocken-Iturrino, C. E. S. Verona & L. S. S. Barros, 2000. Megabacteriosis occurrence in budgerigars, canaries and lovebirds in Ribeirao Preto region – Sao Paulo state – Brazil. *Revista Brasileira de Ciência Avícola*, **2**, 183–187.

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