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Review article

Avian macrorhabdosis *(Macrorhabdus ornithogaster)* causing proventriculitis: Epidemiology, diagnosis, and control

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Abstract

Macrorhabdosis is a chronic gastric debilitating disease causing loses in most bird's species. Therefore, this article was designed to outline avian macrorhabdosis disease regarding the incidence, susceptibility, transmission, clinical picture, diagnosis, and control. Macrorhabdosis "formerly megabacteriosis" is a general designation for an important clinically or sub-clinically infectious disease of wild and in-captive companion, psittacin, and passerine birds world wild. Macrorhabdosis is caused by *Macrorhabdus ornithogaster (M. ornithogaster)* which mainly colonizes the proventriculus close to the proventicular-gizzard isthmus. This organism was previously categorized as bacteria, but later it classified as fungus. Infection occurs through ingestion of contaminated feed and water by the droppings or the regurgitation of acute infected or chronic carrier birds. Infected birds *M. ornithogaster* may appear asymptomatic without signs or show anorexia, depletion, vomiting, diarrhea, and finally death. The lesions mostly constricted to the proventriculus or proventricular/ventricular ismuth as a form of enlargement and thickening with loose koilin layer. Diagnosis of infection is mainly based on microscopic and histopthological examinations and rapid detection of *M. ornithogaster* using molecular techniques. Application of sanitary and quarantine measures is the must for prevention of infection. Treatment of macrorhabdosis is difficult, however, antifungal and protozoa drugs were satisfactory in certain cases.

Keywords: Avian macrorhabdosis, Megabacteriosis, Incidence, Diagnosis, Control

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INTRODUCTION

Macrorhabdosis or avian gastric yeast, caused by *Macrorhabdus ornithogaster* (*M. ornithogaster*) (formerly megabacterium), is a chronic wasting and progressively debilitating gastrointestinal disease characterized by anorexia, emaciation, and death (Queirós et al., 2011; Poleschinski et al., 2019). The previous synonyms of the disease were megabacteriosis (Huchzermeyer et al., 1993), megabacteria associated disease (Perry, 1993), going light syndrome (Baker, 1985), proventricular/ventricular disease (Filippich and Parker, 1994), wasting disease, budgie wasting disease, bacteria giganticus, and avian gastric yeast (Phalen et al., 2002). It may even play a part in proventricular dilation disease which has found to be viral in nature.

The name of *M. ornithogaster* is originated from the Greek words "macrorhabdus" (long rod) and "ornithogaster" (stomach of bird). This organism was firstly detected as a Gram-positive bacillus that were normal microflora in the proventriculus of Budgerigars (Scanlan and Graham, 1990). It was formerly not clear whether *M. ornithogaster* is bacteria or fungi (Jamesk, 1999). However, recent studies confirmed that the organism is a fungus (Tomaszewski et al., 2003; Hannafusa et al., 2007) after examination of wet mount using mini-Flotac system or by using histological technique. It is the only member of the genus *Macrorhabdus*, Order *Saccharomycetales*, Class *Saccharomycetes*, Division Ascomycota, and Kingdom Fungi (Tomaszewski et al., 2001).

The pathogen colonizes the digestive tract of small companion birds such as Canaries (*Insula canaria*), cockatiel (*Nymphicus hollandicus*), Budgerigars (*Melopsittacus undulatus*), African Gray Parrots (*Psittacus erithacus*), Green-cheeked Parakeets (*Pyrrhura molinae*), Finches (*Fringillidae*), and lovebirds (*Agapornis*) (Tomaszewski et al., 2003; Kafrashi and Babazadeh, 2022; Talazadeh et al., 2023). Transmission occurs via the horizontal route and infected birds are the main source of infection (Lanzarot et al., 2013). The clinical signs of infected birds are regurgitation, grinding and throwing food out of the mouth, vomiting, and diarrhea with the presence of undigested seeds in the droppings (Fulton and Mani, 2020; Talazadeh et al., 2023).

Regarding the eradication measures of macrorhabdosis, affected birds are mostly resistant to treatments (Baron et al., 2020), therefore, essential and proper preventive measures should be taken in consideration.

Therefore, this article was designed to describe the knowledge about avian macrorhabdosis disease regarding the incidence, susceptibility, transmission, clinical picture, diagnosis, and control.

INCIDENCE AND DISTRIBUTION

Macrorhabdosis was reported in as early as 1982 (Dorrestein et al., 1980; Henderson et al., 1988). Moreover, the disease is thought to have been introduced into Australia and other countries through imported Budgerigars in the early 1990s and caused abundant losses (Scanlan and Graham, 1990; Filippich et al., 1993; Baker, 1996; Baker, 1997), but the incidence was declined markedly. It is difficult to determine where the disease was originated, but it seems to be a continuing problem in the United Kingdom, Australia, United States, Germany, Japan, and probably other countries as well (Table 1).

Table 1 Incidences of *M. ornithogaster* in various avian species in different countries (2004-2023)

Country	Findings	Reference
USA	Ninety percentages of love birds gave active yeast in their slides, but only 9 (0.09%) cases had non-active yeast.	Antinoff et al. (2004)
	Examined droppings of captive budgerigars revealed 57% positive samples using PCR compared to 24% using Grams staining.	Sullivan et al. (2017)
	Out of 1,006 budgerigars, 177 were histologically confirmed positive.	Powers et al. (2019)
	Formalin-fixed tissues sections of 32 budgerigars showed presence of the pathogen in 21.88% (7/32)	Fulton and Mani (2020)
Japan	The highest infection rate was in cockatiels (42/50), while the lowest one was in parrots (1/5).	Hannafusa et al. (2007)
Germany	A total of 1.137 birds in 16 orders of Psittaci-, Passed-, Anseri-, Galli- and Columbiformes were examined and the organism was found on the glandular stomach surface of 2 diseased feral pigeons.	Hanka et al. (2010)
Peru	A total of 99 out of 150 exotic small psittacines and passerines were positive (66%) with signs of weight loss, regurgitation, gasping, and lethargy.	Luján Vega et al. (2016)
New South Wales (Australia)	A percentage of 29% (16/54) of budgerigar's fecal samples were positive.	Baron et al. (2020)
Iran	Almost half of 145 cockatiels, gray parrots, lovebirds, green cheeks, and budgerigars with lethargy, weight loss, regurgitation, and gasping showed active or non-active yeast in their slides. Some birds (25/150) were re-infected 2 months post-treatment.	Kafrashi and Babazadeh (2022)
	A total 17 out of 100 fecal samples collected from cockatiel, budgerigar, and grey parrot were microscopically positive while, it was detected in 7/50 (14%) of fecal samples using molecular techniques.	Talazadeh et al. (2023)

SUSCEPTIBILITY

Factor affecting susceptibility

It is important to mention that immuno-suppression seems to be the most risk factor increasing the susceptibility to *M. ornithogaster* infection (Behnke and Fletcher, 2011). Moreover, infections with *M. ornithogaster* are usually accompanied by other conditions such as parasitism (Henderson et al., 1988; Martins et al., 2006; Ozmen et al., 2013) and bacterial infections (Babazadeh et al., 2015). It has been suggested that the disease prevalence was significantly greater in chicks from *M. ornithogaster* positive parents than negative ones, even when reared by *M. ornithogaster*-negative parents (Filippich and Hendrikz, 1998).

Host species

Macrorhabdosis is an important infectious disease of psittaciformes such as Budgerigars (Scanlan and Graham, 1990; Baker, 1992; Filippich and Perry, 1993; Tonelli, 1993; Filippich and Hendrikz, 1998; Moore et al., 2001; Kheirandish and Salehi, 2011; Madani et al., 2014; Püstow and Krautwald-Junghanns, 2017; Sullivan et al., 2017; Powers et al., 2019; Baron et al., 2020; Fulton and Mani, 2020), Canaries (Van Herck et al., 1984; Schulze and Heidrich, 2001; Scullion and Scullion, 2004; Marlier et al., 2006; Martins et al., 2006; Lanzarot et al., 2013), Gold and Zebra Finches (Gerlach, 2001; Snyder et al., 2013), and Grey Parrot (Talazadeh et al., 2023), and other lovebirds (Werther et al., 2000; Gonzalez, 2007). Moreover, Japanese quails, partridges, rhea, turkeys, pigeons, ruddy ground-doves, and domestic ducks could be infected with *M. ornithogaster* (Schulze and Heidrich, 2001; Martins et al., 2006; Jansson et al., 2008; Hanka et al., 2010).

Cases of *M. ornithogaster* infections were also noticed in 10-day to 12- week-old ostrich chicks (Huchzermeyer et al., 1993; Marinho et al., 2004). Acute infections were noticed in passeriformes such as Toucan and Zebra Finch (Martins et al. 2006), while chronic disease was seen in chickens, turkeys, and Guinea fowls. Domestic chickens showed infection with *M. ornithogaster* in different continents such as Europe, North and South America, and Australia (Mutlu et al., 1997; Schulze and Heidrich, 2001; Phalen and Moore, 2003; Martins et al., 2006; Phalen et al., 2007; Hanka et al., 2010; Behnke and Fletcher, 2011). It is likely to mention that *Macrorhabdus*-like agents were detected in mammals such as dogs, cats, and laboratory mice (Cooke, 2000; Rossi, 2000).

INFECTION AND TRANSMISSION

Sources of infection

Infections with *M. ornithogaster* resulting from ingestion of contaminated feed and water with droppings of diseased birds or even sub-clinically chronic carriers shedders (Kheirandish and Salehi, 2011; Lanzarot et al., 2013). The droppings of relapsed birds act as a potential source for shedding of the infection (Antinoff et al., 2004). There is a strong possibility for reinfection of recovered birds by contaminated feed. In the study of Kafrashi and Babazadeh (2022), some birds (25/150) showed signs of *M. ornithogaster* infection after 2 months which may be due to incomplete elimination of the microorganism from the gastric tract of the treated birds. Stressors including shipping, overcrowding, and mixing of different birds species are predisposing causes of infection.

Modes of transmission

Experimentally infected white-leghorn cockerels with *M. ornithogaster* shed the pathogen to close mate contact non-infected birds (Phalen and Moore, 2003). Recovered birds transmit infection to the other birds and the surrounding environment. Free living wild birds help in the disease spread (Schulze and Heidrich, 2001). Besides, lacking of the standard biosecurity measures helps in increasing the possibility of the disease transmission among different avian species (Martins et al., 2006).

SIGNS AND LESIONS

M. ornithogaster usually colonizes the isthmus between the proventriculus and gizzard (Van Herck et al, 1984; Scanlan and Graham, 1990). The signs of *macrorhabdosis* may be somewhat different, depending on the affected bird species (Moore et al., 2001), and the severity may range from high with mortality to mild or asymptomatic.

Acute signs

In acute *M. ornithogaster* infections, infected birds seemed to be in a normal condition, however, severe depression, fluffing up, and lethargy might suddenly occur (Jamesk, 1999; Marlier et al., 2006). Some cases showed sudden death with empty crop in few days. The droppings of the infected birds may be dark green to brown/black, occasionally reddish-tinged, with very little white color urates, and sometimes tarry while being small in size. Acute macrorhabdosis with a mortality rate of more than 50% was observed in a breeding budgerigar flock in Iran (Madani et al., 2014). An experimental infection of white-leghorn cockerels with *M. ornithogaster* induced a decrease in the growth parameters without signs (Phalen and Moore, 2003).

Chronic signs

Infected birds with chronic *M. ornithogaster* exhibited progressive lethargy, cachexia, depletion, severe weight loss, and emaciation over a number of weeks or months. The birds in this stage either die or recover after relapsed weeks or months. Regurgitation and the droppings contain undigested seeds or pellets were also observed in the chronic form (Helgar, 2001; Kunkle, 2003). Juvenile ostrich with macrorhabdosis showed weight loss, slow growth, anaemia, diarrhea with soiled vent, and finally death with mortality rates of 40% to 80% (Huchzermeyer et al., 1993), while Budgerigars exhibited cachexia and diarrhea (Ozmen et al., 2013). On occasion, Cockatiels and other species may vomited blood and displayed anemia and melena (Dorrestein et al., 1980; Van Herck et al., 1984). Un-common respiratory signs have been also reported in *M. ornithogaster* infection and the birds showed gasping, dyspnea, stretched necks up in the air or mouth-gag repeatedly, appearing to have trouble either swallowing or regurgitating (Poleschinski et al., 2019).

Lesions

Atrophy of the breast muscles, prominent keel bone on the chest, abdominal swelling, dilation of the proventriculus with the presence of thick white mucus, thickening and enlargement of the proventriculus and gizzard wall, and loose koilin layer have been observed in *M. ornithogaster* infected birds. Histologically, inflammation, haemorrhages, erosion, and ulceration of the proventriculus and the proventricular-gizzard junction have been also detected in psittacine birds (Baker, 1992; Werther et al., 2000; Helgar, 2001; Schulze and Heidrich, 2001; Marlier et al., 2006; Martins et al., 2006).

Pathogenesis

Macrorhabdosis induces a negative impact on the digestion process, thus diseased birds show loss of body weight and cachexia despite a normal appetite or excessive feeding. Infection with *M. ornithogaster* is also associated with an increase in the pH of the proventriculus (from 2.7 to 7.3) which resulted in the destruction of the covering koilin layer (Van Herck et al., 1984; Jamesk, 1999; Werther et al., 2000; Kunkle, 2003).

DIAGNOSIS

The diagnosis of macrorhabdosis is based on the case history, necropsy, microscopic demonstration of *M. ornithogaster* in droppings or proventriculus smears and histopathology sections, and using some molecular techniques such as polymerase chain reaction (PCR).

Microscopic examination

Cloacal swabs were collected for the direct detection of the microorganism (Lanzarot et al., 2013). A very large or long "rod-shaped" and a Y-shaped branched end Gram-positive *M. ornithogaster* could be seen in Grams or Giemsa-stained-smears or wet slides from the gastric mucosa or droppings of infected birds (Antinoff et al., 2004; Martins et al., 2006; Hannafusa et al., 2007). Positive smears show organisms with an approximate size of 1.5-3 µm in diameter and 20-90 µm in length (Dorrestein et al., 1980). However, microscopic yeast count (mini-Flotac system) is considered as a more quantitative examination method than the Grams staining or wet mount method (Borrelli et al., 2015). The possibility of false positive results is possible during the microscopic method (Poleschinski et al., 2019; Talazadeh et al., 2023).

Histopathologic examination

Sections of the proventriculus could be stained with haemotoxylin and eosin, silver stain, and periodic acid-Schiff for histopathological examination and detection of *M. ornithogaster* (Scanlan and Graham, 1990; Filippich et al., 1993; Marlier et al., 2006; Martins et al., 2006; Madani et al., 2014). *M. ornithogaster* are usually seen on the proventriculus surface mucosa or in the crypts of the mucosa, especially at the proventricular–ventricular junction (Van Herck et al., 1984; Martins et al., 2006). Moderate to severe lymphoplasmacytic and heterophilic inflammation of the proventriculus and gizzard, mucosal necrosis with disruption of the mucosal architecture, absence of glandular structure of the proventriculus, degeneration of the koilin layer and infiltration of lymphocytes and plasma cells in mucosal, submucosal, and muscular layers of the gizzard, as well as submucosal fibrosis were also seen (Van Herck et al., 1984; Baker, 1992; Hungerford et al., 1998; Gerlach, 2001; Schulze and Heidrich, 2001; Kunkle, 2003; Martins et al., 2006).

Radiography

Radiographs may reveal proventricular dilatation as well as an hourglass-like constriction between the proventriculus and ventriculus. Thickening of the proventricular wall can also be seen with contrast (barium sulphate) (Werther et al., 2000; Antinoff et al., 2004).

Polymerase chain reaction

PCR testing of the 18S ribosomal RNA gene of *M. ornithogaster* was performed (Kanno and Matsumoto, 2023). Despite direct fecal examination being a commonly used diagnostic method, the PCR is anticipated to offer a higher detection rate (Kanno and Matsumoto, 2023). However, the actual diagnostic accuracy of the PCR for *M. ornithogaster* remains unknown. This test is relatively underutilized due to its expense, outsourcing requirements, and time-consuming nature, as fecal examination alone can often provide a definitive diagnosis. Despite there is no available information on the diagnostic accuracy of the Macrorhabdus PCR test, it is unclear how PCR testing should be appropriately utilized in clinical practice. Presence of *M. ornithogaster* below the detection limit of a fecal examination, resulting in a false-negative. Moreover, there are multiple forms of *M. ornithogaster*, which can result in false negatives if they cannot be found by an inexperienced observer. Moreover, different structures of fungi may be erroneously recognized as *M. ornithogaster*, resulting in false positives. Therefore, a combination with the PCR test should be used to reduce the overall number of missed tests, rather than relying on one method. If the PCR test is positive despite negative direct fecal examination results, sequencing tests should be conducted. It has been reported that PCR is more accurate than fecal Grams staining for the diagnosis of *M. ornithogaster* in captive budgerigars (Phalen, 2014; Sullivan et al., 2017; Poleschinski et al., 2019). Recently, *M. ornithogaster* has been diagnosed in 14% of cloacal swabs of Cockatiel, Budgerigar, and Grey Parrot using semi-nested PCR test (Talazadeh et al., 2023).

Differential diagnosis

Signs of *M. ornithogaster* infection are not specific and can be confused with others such as giardiasis, trichomoniasis, worm infections, fungal and bacterial gastro-intestinal infections, bornavirus infection, foreign bodies, and heavy metal intoxications. The differential diagnosis is relied mainly on the characteristic microscopic detection of *M. ornithogaster*.

PREVENTION AND CONTROL

Prevention

Good management and hygiene such as proper cleaning and disinfection of the cages, nest box, and feeding utensils, and providing high quality feed are important to prevent macrorhabdosis (Amer and Mekky, 2020). Captive and wild psittacine birds should not be mixed. Regular monitoring and screening of the newly introduced birds especially those from wildlife are essential. Application of hygienic standards in incubators by keeping clean eggs is important (Moore et al., 2001). Application of protective measures during handling with diseased bird is also critical.

Treatment

Treatment of macrorhabdosis is often difficult due to the usual subclinical infections. However, anti-fungal drugs including nystatin and amphotericin B are effective against *M. ornithogaster* infection (Hungerford et al., 1998; Jamesk, 1999; Kunkle, 2003; Kheirandish and Salehi, 2011; Baron et al., 2020). Amphotericin B at a dose of 100 mg/kg body weight (Bwt) could

be given orally twice a day for 30 days (Hannafusa et al., 2007; Phalen, 2014). Treatment of infected Zebra Finches with amphotericin B in combination of correction of the light cycle returned bird's mortality to normal and improved the general health conditions (Snyder et al., 2013). However, 4 weeks administration of oral amphotericin B (100 mg/kg Bwt) was unsatisfactory for the infected budgerigars (Püstow and Krautwald-Junghanns, 2017). The authors explained this result by the stress of birds handling during the drug administration along with the log course of treatment. In Australia, an old study of Filippich and Perry (1993) also showed that *M. ornithogaster* strains were resistant to amphotericin B. Nystatin (10 mg/kg Bwt) is also efficient for the treatment in Goldfinches, not Budgerigars (Moore et al., 2001; Scullion and Scullion, 2004). Kheirandish and Salehi (2011) found complete reduction of mortality following nystatin treatment of infected Budgerigars. Similarly, moderate reduction of budgerigars mortality was reported after 4 weeks feed treatment with nystatin, 3 weeks water treatment with vinegar, and 4 weeks water treatment with sodium benzoate (500 mg/L of the drinking water) (Madani et al., 2014).

Metronidazole in a dose of 30 mg/kg Bwt is also suggested. Fluconazole (100mg/kg Bwt) could be promising for the treatment of *M. ornithogaster*. However, the mentioned dose of fluconazole may be highly toxic to Budgerigars (Filippich and Hendrikz, 1998).

It has been found that iodine compounds, ketoconazole, terbinafine, lufenuron, and itraconazole were not effective against *M. ornithogaster* in companion birds (Filippich et al., 1993; Phalen et al., 2002; Phalen, 2005). Besides, the polyenes macrolides have been successfully used to the treatment of such infection (Silva et al., 2022).

As *M. ornithogaster* grows only in an alkaline environment, thus increasing the gastric acidity by acidification of water with organic acids is important. Addition of some acids such as apple vinegar (10 drops/1.5 L of drinking water), acetic acid, and citric acid or grapefruit juice to drinking water can reduce the infection (Hannafusa et al., 2007; Phalen, 2014). Addition of some feed additives such as *Lactobacillus acidophilus* can be helpful to increase the gastric acidity (Jamesk, 1999). Vitamins such as vitamin B complex in a dose of 10 drops in 1.5 L of drinking water is a supportive treatment. Periodical checkup of birds every 3-months by Grams staining of the droppings is recommended.

CONCLUSIONS

As macrorhabdosis is an important clinical or sub-clinical disease of wild and in captive companion, psittacin, and passerine birds, thus promote using of strong quarantine measures and reducing the stress conditions can help in reduction of the disease development. Moreover, periodic diagnostic checkup procedures should be applied for captive birds. More research work about the education of the disease nature, the best treatments protocols, and the vaccine development are required.

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AUTHOR CONTRIBUTIONS

WAA: Collected the literature, drafted and revised the manuscript, and approved the final manuscript.

CONFLICT OF INTEREST

The author has not declared any conflict of interest.

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