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Avian gastric yeast (macrorhabdosis) in cockatiel, budgerigar and grey parrot: a focus on the clinical signs, molecular detection and phylogenetic evaluation

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Abstract

Macrorhabdus ornithogaster is a microorganism that causes nonspecific and general clinical symptoms and to this day, diagnosis and also treatment have been yet hard. The present study was conducted to survey the prevalence of macrorhabdosis and to characterize *M. ornithogaster* phylogenetically in Psittaciformes suspected of macrorhabdosis from January 2018 to May 2019 in Ahvaz, Iran. For this purpose, fecal samples were collected from Psittaciformes with signs of the disease. Wet mounts were prepared from fecal samples and examined carefully using a light microscope. Samples from parrots with gastrointestinal symptoms of the disease were chosen for molecular diagnosis of the organism and DNA was extracted from these samples. For detection of *M. ornithogaster*, primer sets (BIG1, Sm4) and (AGY1, Sm4) which target the 18S rDNA gene were selected and Semi-nested polymerase chain reaction (Semi-nested PCR) was performed. The PCR method confirmed the presence of *M. ornithogaster* in 14.00% of the samples. Purified PCR products were sequenced for more accurate confirmation and according to the gene sequence all sequences were owned by *M. ornithogaster*. The results disclosed a 96.03% - 100% identity when compared to other sequences of *M. ornithogaster* which had previously been deposited in the GenBank® from Germany and the USA. The results of this study proved the circulation of *M. ornithogaster* between cockatiel, budgerigar and grey parrot. The prevalence of *macrorhabdosis* was higher in cockatiel compared to budgerigar and grey parrot. As far as the authors know, this was the first record of macrorhabdosis in African grey parrots.

Introduction

Macrorhabdus ornithogaster- avian gastric yeast-proliferates at the isthmus of the proventriculus and ventriculus in birds.[1] It is the only member of the genus *Macrorhabdus*, Order Saccharomycetales, Class Saccharomycetes, Division Ascomycota, Kingdom Fungi.[2] The *M. ornithogaster* (Megabacterium) disease has many synonyms as megabacteriosis, macrorhabdosis,[3] or proventricular/ventricular disease (PVD).[4] It is Gram-positive, however, just the cytoplasm is stained with Gram staining.[5] In scratching the mucous membranes and stool of infected birds, it is a large 'cigar-shaped' organism. Sometimes, at one end, a Y-shaped branch is seen. *M. ornithogaster* has been described in various species of birds comprising ostriches,[6] Passeriformes (canary, toucan and zebra finch), Psittaciformes (budgerigars), Columbiformes (domestic pigeon and ruddy ground-dove), Galliformes (industrial broiler, turkey, guinea-fowl, free-range chicken and chukar partridge).[7] *Macrorhabdus*-like agents were also reported in mammals like dogs, cats and laboratory mice.[8,9] Clinical signs include weight reduction, debility and polyphagia, although, the bird often grinds its food and then allows it to fall out of the mouth. In acute forms, birds may regurgitate food and bloodstains may form around the beak. Undigested seeds may be excreted in the stool and in some cases melena may be seen.[10-12] The course of the disease is generally long, however, in parrots and budgerigars, it has been reported that proventricular hemorrhage or rupture may cause sudden death. In budgerigars, other diseases such as trichomoniasis, enteritis, heavy metal toxicosis, crop or ventriculus candidiasis, bacterial ventriculitis and neoplasia of the stomach can cause similar symptoms. Diagnosis of *M. ornithogaster* infection before death may be hard because

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the clinical symptoms can be nonspecific and unclear. Due to inconsistent shedding of the organism in stool, tracing *M. ornithogaster* in live birds is hard.[13] A routine approach for tracing *M. ornithogaster* before death in birds is a microscopic stool exam. Stool samples collected for several consecutive days are tested microscopically.[13] Stool exam with Gram's stains is an alternative diagnostic testing method.[13,14] Most diseased birds excrete large amounts of the organism, however, asymptomatic birds may excrete the organism inconsistently. Isolation of the organism by culture technique is not a common diagnostic test approach, however, an optimal culture protocol was developed in 2007.[5] Efforts to grow *M. ornithogaster* on traditional bacterial and fungal media have largely been unsuccessful. Gerlach reported isolating this organism once on MRS medium, a medium used to isolate *Lactobacillus spp.*, and other investigators have reported that it could be grown in liquid media containing minimum essential media, 20.00% fetal bovine serum (FBS) and 5.00% sucrose.[14] Because it cannot be readily and consistently cultured, *M. ornithogaster* has been characterized only genetically.[12] Also, isolated *M. ornithogaster* from different bird species were not different morphologically and it is recommended that they be characterized by molecular techniques.[13] Therefore, currently, the most credible method for the antemortem tracing of *M. ornithogaster* in bird species is the polymerase chain reaction (PCR) method.[15] Appropriate handling of *macrorhabdosis*, involves serious and rapid treatment of *macrorhabdosis*. Thus, any lag in antifungal treatment may result in a severe illness or even death. As a result, fast, accurate, affordable and timely diagnosis is important. Considering that many Psittaciformes in Ahvaz region is referred to the veterinary hospital with the mentioned symptoms, and considering the occurrence of diseases with similar clinical manifestations in Psittaciformes and the importance of differential and rapid diagnosis of the disease to apply appropriate treatment, this study was performed to identify the *M. ornithogaster* in Psittaciformes to determine the prevalence of macrorhabdosis in Psittaciformes in Ahvaz city and to characterize *M. ornithogaster* phylogenetically.

Materials and Methods

Sampling. The study was conducted from January 2018 to May 2019 in Ahvaz. One hundred pieces of different species of birds of the order Psittaciformes (African grey parrot, budgerigar, eclectus parrot, love bird, cockatiel, rose-ringed parakeet and amazon parrot) referred to the department of avian medicine, Ahvaz, Iran, were examined (Table 1). Clinical symptoms were written down by the owner's history and external physical examination. Based on the history presented by the owners and the clinical examination, the birds had clinical symptoms like lethargy, weight reduction, anorexia, grinding and throwing food out of the mouth, regurgitation, bloody vomiting, vomiting, diarrhea, undigested seeds in the dropping and melena (Table 2). Using sterile wooden spatulas, stools were gathered from the bed of cages of each bird and then stored in sterile vials separately and were immediately transported to the laboratory for further processing. This study was approved by the Shahid Chamran University of Ahvaz Ethical Commission for Animal Experiments under verification number ee /97.24.3.70429/scu.ac.ir.

Microscopic examination. The diagnosis was done by microscopic (Olympus, Tokyo, Japan) assessment of the fecal samples. In all 100 cases, the stool exam was done in live infected birds and fecal samples were spread onto glass slides before light microscopic observations and a wet mount was examined under 10× and 40× magnification. The number of microscopic positive samples by species is shown in Table 1.

DNA extraction. Fifty samples from 50 Psittaciformes with gastrointestinal signs were chosen for molecular diagnosis of the organism and DNA was extracted from these samples. Using sterile distilled water, a suspension of 10.00% of the fecal samples was prepared in 1.50 mL microtubes and centrifuged at 800 *g* for 15 sec to settle the larger pieces of stools. To new sterile microtubes, 100 µL of the supernatant was transmitted and centrifuged for 10 min at 5,000 *g* to form a precipitate at the bottom of the microtube. The supernatant was discarded and work was followed based on the manufacturer's procedure (CinnaGene, Tehran, Iran) with a little change as follows: 100 µL of prelysis buffer, 20.00 µL Ribotinase and 20.00 µL of lysozyme solution were poured into the tubes and they were incubated at 55.00 ˚C for 18 hr. Then, 100 µL of each sample was transferred to new sterile 1.50 mL microtubes, 400 µL of lysis buffer was poured and vortexed for 20 sec at maximum rapidity, afterward 300 µL precipitation solutions were added and vortexed for 5 sec at maximum rapidity. To a spin column with a collection tube, the solution was transferred. At 13,000 rpm and for 1 min, tubes were centrifuged. The spin-column was put in a new collection tube and 400 µL wash buffer (I) was poured into the tubes. Afterward, they were centrifuged for one min at 13,000 rpm. Then, the spin column was located in a new collection tube and wash buffer (Π) was poured into it and it was centrifuged for one min at 13,000 rpm (wash buffer (Π) step was repeated). The column was intently carried to a new 1.50 mL tube. Afterward, 50.00 µL of 65.00 ˚C preheated elution buffer was poured into the center of the column and then it was incubated at room temperature for 5 min. This solution was centrifuged at 13,000 rpm for one min. The filtrate was stored at – 70.00 ˚C and used as the template in PCR.

Table 1. The number of fecal samples and microscopic positive samples by species.

Common name	Scientific name	No. of fecal samples	Microscopic positive samples
Cockatiel	*Nymphicus hollandicus*	37	9
budgerigar	*Melopsitacus undulates*	21	5
Grey parrot	*Psittacus erithacus*	21	3
Love bird	*Agapornis roseicollis*	9	-
Rose-ringed parakeet	*Psittacula krameri*	8	-
Eclectus parrot	*Eclectus roratus*	3	-
Amazon parrot	*Amazona*	1	-
Total		100	17

PCR. Based on the technique of Tomaszewski *et al*. for molecular tracing of *M. ornithogaster*, Semi-nested PCR was done using two sets of primers which respectively produced products with 919 and 374 base pairs.[1] Primers were planned to amplify and sequence the 18S rDNA. In this study, two different PCR methods were used to amplify fragments of the 18S rDNA gene from fecal samples. The primary PCR was performed using first stage primers (the forward primer BIG1 (F-AGTGAAACTGCGAATGGCTC) (F1), and reverse primer Sm4 (R-CTTCGATCCCCTAACTTTCGTTC) described by Tomaszewski *et al*.[1] It created the product with 919 base pairs (Fig. 1A). The PCR product was evaluated by Semi-Nested PCR using the forward primer AGY1 (F-GGACTTATATTACTAGTCAG ATGG) (F2) and reverse primer Sm4 (R-CTTCGATCCCCTAACTTTCGTTC) described by Tomaszewski *et al*.[1] It created the product with 374 base pairs (Fig. 1B). Using a DNA thermos cycler (Eppendorf, Hamburg, Germany), amplicons were amplified as follow: Initial denaturation at 95.00 ˚C for 5 min followed by 32 amplification cycles (95.00 ˚C for 30 sec, 55.50 ˚C for 30 sec, and 72.00 ˚C for 1 min) and a final extension cycle (72.00 ˚C for 5 min). For primary and secondary amplification, a total volume of 25.00 µL of reaction mixture containing 1.00 µL of primer pair (10.00 µM) and 2.50 µL of template DNA, 8.00 µL of distilled water and 12.50 µL of 2X master mix (with 1.50 mM $MgCl_2$, 0.20 mM of dATP, dCTP, dGTP and dTTP; Amplicon, Odense, Denmark) were used.

By electrophoresis of 8.00 µL of product in 1.50% (w/v) agarose gel with Tris-acetate-EDTA (TAE; 100 mM Tris HCl and 40.00 mM EDTA) containing 3.00 µL safe stain (Sinaclon) primary and secondary PCR products were detected and observed by transillumination under UV (Uvidoc HD6; UVItec Ltd., London, UK). The size of the amplified products was appraised through comparison with a DNA ladder of 50 bp and 100 bp (Sinaclon).

Sequence analysis and phylogenetic analysis. With forward (F2) and reverse primer (R) by bioneer sequence service (Bioneer, Daejeon, South Korea) two Semi-Nested PCR products were sequenced. This nucleotide was stored in the GenBank® database under the accession numbers MW076832 and MW077919. After converting the sequences to FASTA format, they were aligned and the species were recognized by searching databases using the online system of local alignment tools (BLAST) on the National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov). ClustalW method using MEGA Software (version 10.0; Biodesign Institute, Tempe, USA) and BioEdit (version 7.0.5; Ibis Therapeutics, Carlsbad, USA) were used to compare our sequence with other related sequences in NCBI. A phylogenetic tree was created according to the nucleotide sequences of the 18S rDNA gene and the relevant areas of the other *M. ornithogaster* by the neighbor-joining algorithm with MEGA software.

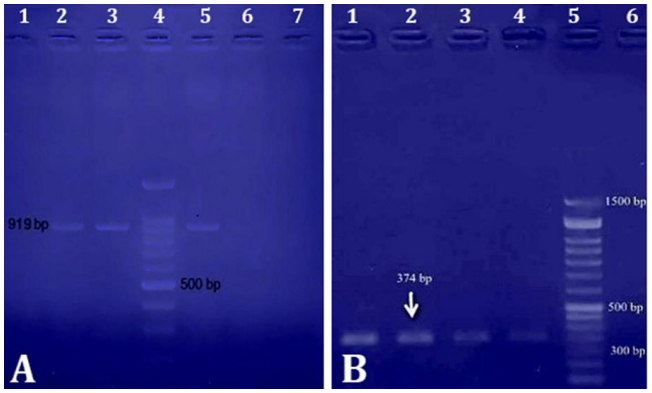

Fig. 1. A) Primary PCR for tracing *M. ornithogaster* using 18S rDNA outer primer set and examination of products by agarose gel electrophoresis. Lane 1: Negative control (water), Lane 2: Positive control, Lanes 3, 5: PCR product with a size of 919 bp of *M. ornithogaster* as positive samples, Lane 4: Lad: 100-bp molecular marker (Sinaclon, Tehran, Iran), Lanes 6, 7: Negative samples. **B)** Semi-nested PCR for tracing *M. ornithogaster* using 18S rDNA inner primer set and examination of products by agarose gel electrophoresis. Lane 1: Positive control, Lanes 2-4: Semi-nested PCR product with a size of 374 bp of *M. ornithogaster* as positive samples, Lane 5: Lad: 50-bp molecular marker (Sinaclon), Lane 6: Negative control (water).

Results

Microscopic examination. Out of 100 fecal samples collected from Psittaciformes, 17 samples were positive by microscopic examination (Fig. 2; Table 1).

PCR and molecular identification. Out of these 50 samples, seven samples (14.00%) were positive by PCR test (Table 2) and *M. ornithogaster* prevalence was higher in cockatiel compared to other Psittaciformes.

Table 2. Molecularly positive fecal samples, the most common clinical and gastrointestinal signs in birds.

Common name	No. of samples*	Positive samples (%)	Most common clinical signs
Cockatiel	21	4 (19.04%)	Grinding and throwing food out of the mouth, regurgitation, vomiting: 4/21 (19.04 %) Diarrhea: 2/21 (9.52%) Melena, undigested seeds in the feces: 1/21 (4.76%) Bloody vomiting: 1/21 (4.76%) Weight loss, lethargy, anorexia
Budgerigar	12	2 (16.66%)	Grinding and throwing food out of the mouth, regurgitation, vomiting: 2/12 (16.66%) Diarrhea: 2/12 (16.66%) Weight loss, lethargy, anorexia
Grey parrot	9	1 (11.11%)	Grinding and throwing food out of the mouth, regurgitation, vomiting: 1/9 (11.11%) Diarrhea: 1/9 (11.11%) Weight loss, lethargy, anorexia
Love bird	4	0 (0.00)	Regurgitation, lethargy, diarrhea
Rose-ringed parakeet	1	0 (0.00)	Regurgitation, lethargy, anorexia, vomiting
Eclectus parrot	2	0 (0.00)	Lethargy, anorexia, vomiting
Amazon parrot	1	0 (0.00)	Regurgitation, lethargy, anorexia
Total	50	7/50(14.00%)	-

* Number of fecal samples evaluated by PCR from birds with gastrointestinal signs.
The gastrointestinal clinical signs in birds with molecularly positive samples included grinding and throwing food out of the mouth, regurgitation, vomiting, diarrhea, melena, undigested seeds in the feces and bloody vomiting.

The initial complaint by bird owners was gastro-intestinal symptoms. The clinical symptoms seen by owners or identified on the external physical inspection are shown in Table 2. According to Table 2, all four cockatiels (19.04%, 4/21 cockatiels positive by PCR method had symptoms such as regurgitation, grinding and throwing food out of the mouth and vomiting. In two cockatiels (9.52%, 2/21) in addition to regurgitation, grinding and throwing food out of the mouth, vomiting and diarrhea were also observed. In one of the cocktails (4.76%, 1/21) all the symptoms (regurgitation, grinding, throwing food out of the mouth, vomiting, diarrhea, melena, undigested seeds in the dropping and bloody vomiting) were observed. Two budgerigars (16.66%, 2/12) positive by PCR method had symptoms such as regurgitation, grinding, throwing food out of the mouth, vomiting and diarrhea. One grey parrot (11.11%, 1/9) was positive by PCR method which had symptoms such as regurgitation, grinding and throwing food out of the mouth, vomiting and diarrhea. In budgerigars and grey parrots, clinical signs such as melena, undigested seeds in the dropping and bloody vomiting were not observed.

It should be noted that all these birds positive by PCR method, were microscopically positive. As noted in Table 2, most birds presented with signs such as lethargy, anorexia and weight loss were nonspecific in clinical disease symptoms.

Gene sequencing of *M. ornithogaster*. Phylogenetic analysis of 18S rDNA sequences showed that two sequences of the present study belonged to *M. ornithogaster*. All sequences obtained in this study were sent to GenBank® under access numbers MW076832 and MW077919. By the neighbor-joining method and the Jukes-Cantor distance model, phylogenetic trees were built (Fig. 3). Bootstrap support was evaluated with 1,000 duplicate analyses. The results disclosed a 96.03% to 100% identity when compared to other sequences *M. ornithogaster* (KX426589.1, KX426588.1, KX426586.1, AF350243.1, and DQ231141.1) which were previously deposited in GenBank® from Germany and the USA.

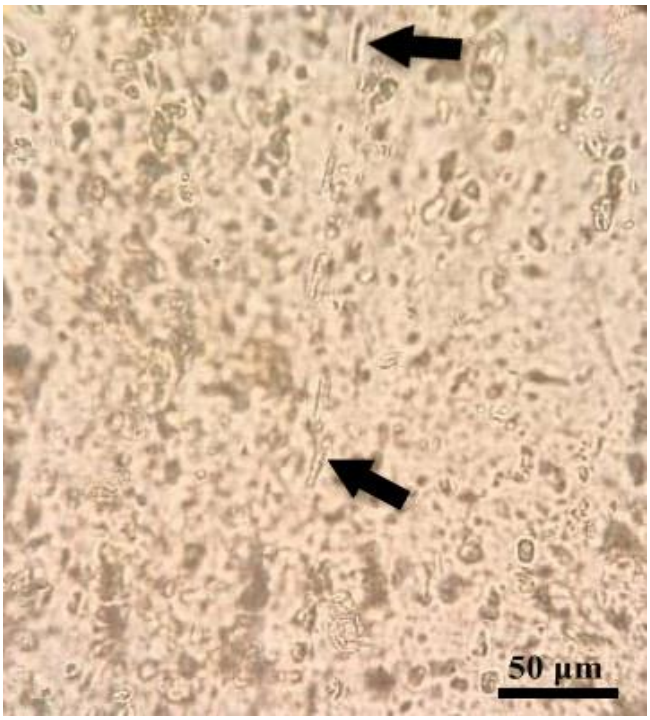

Fig. 2. Microscopic examination of a positive sample. The 'cigar-shaped' organism and a single Y-shaped branch are shown with arrows.

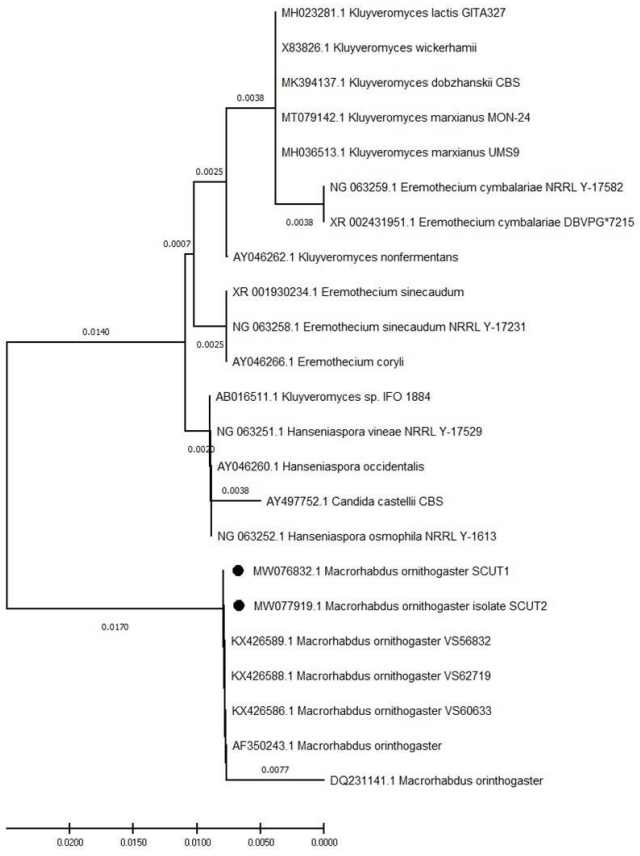

Fig. 3. Comparison of the phylogenetic position of the present *M. ornithogaster* based on 18S rDNA sequences (MW076832 and MW077919) with other related sequences in the GenBank® database by the neighbor-joining algorithm (MEGA10 Software).

Discussion

The budgerigar, lovebirds and cockatiels are often infected with *M. ornithogaster*.[12,14] In the present study, among the ornamental birds studied (Psittaciformes), cockatiels showed the highest yeast contamination, and macrorhabdosis was not detected in love birds. Paula *et al*. reported that the prevalence of *M. ornithogaster* among cockatiels, budgerigars and lovebirds was 73.68% (14/19), 40.90% (9/22) and 50.00% (2/4), respectively, in Uberaba, state of Minas Gerais.[16] In disagreement with Paula *et al.*, in the present study, the prevalence of *M. ornithogaster* among cockatiels, budgerigars and lovebirds was 19.04 % (4/21), 16.66% (2/12), and 0.00% (0/4), respectively, which was lower than the prevalence in the study of Paula *et al*.[16] The reasons for such conflicting results are unclear, however, several causes may be involved including the general condition of the bird, differences in sample size, management methods, the geographic dissemination of *M. ornithogaster* or laboratory methods for detecting yeast.

Also, intermittent shedding of *M. ornithogaster* in some samples of clinically sick birds cannot be ruled out which can lead to minimizing the prevalence of this yeast in the study community. Post-mortem inspection of the gastro-intestinal system may show other cases of *M. ornithogaster* infection in birds that do not excrete yeast cells,[17] however, no birds died during the present study and this was not possible in our study. In agreement with Paula *et al*., in the present study, *M. ornithogaster* prevalence was higher in cockatiel compared to other psittaciformes.[16] In the study of Powers *et al.,* out of 1,006 budgerigars, 177 were known histologically as infected with *M. ornithogaster* at post-mortem inspection.[18] Based on archival data, budgerigars were more probable to develop macrorhabdiosis than other bird species.[18] These observations were inconsistent with the results of the present study. In the present study among the ornamental birds studied (Psittaciformes), cockatiels showed higher yeast contamination than budgerigars. Following reports of deaths attributed to *M. ornithogaster*, Baron *et al*. collected fecal samples from budgerigars in New South Wales, Australia, and reported that 29.00% (16/54) of birds were positive for *M. ornithogaster*.[19] In the present study, we reported a lower prevalence of *M. ornithogaster* in budgerigar's stools (16.66% [2/12]), which contradicted the figures reported in the study by Baron *et al*. The reasons for such conflicting results were unclear, however, several items might be involved including differences in sample populations.

In the study of Fulton and Mani, in a Michigan zoo, 32 budgerigars died. Formalin-fixed tissues or complete birds were sent to the laboratory for diagnosis. *M. ornithogaster* was diagnosed at 21.88% (7/32) histologically.[20] The infection rate in the present study (16.66% [2/12]) was in contrary with the figures reported in the study by Fulton and Mani.[20] In the present study, histologic examination of the proventriculus was not possible as no birds did die during the present study. The reasons for such conflicting results were unclear, however, several causes might be involved including the general condition of the bird, differences in sample size, management methods, the geographic distribution of *M. ornithogaster* or laboratory methods used to detect yeast. The present study did not prove macrorhabdosis in love birds, rose-ringed parakeets, eclectus parrots and amazon parrots. These birds had clinical symptoms like regurgitation, anorexia, lethargy, vomiting and diarrhea, however, they were negative by microscopic and PCR method because the clinical symptoms of *M. ornithogaster* infection are not specific and can be seen in other diseases including giardiasis, trichomoniasis, other fungal and bacterial crop and proventriculus infections, worm infections of the gastrointestinal system, *Bornavirus* infection, foreign bodies, and heavy metal toxicosis. So far, limited studies have been performed on this disease in Psittaciformes in Iran. Kheirandish and Salehi surveyed budgerigars

suspected of macrorhabdosis in Kerman, Iran.[21] They stated that a certain diagnosis of macrorhabdosis was frequently proved by a fresh scrub of the proventricular mucus and histopathology. Madani *et al*. reported macrorhabdosis in a breeding budgerigar flock based on cytologic and histologic diagnosis in Tehran, Iran.[22] In their study, acute macrorhabdosis resulted in high mortality (more than 50.00%) in fledglings. The findings of this study were consistent with the findings of Kheirandish and Salehi[21] and Madani *et al*.[22] regarding the detection of infection in budgerigars, however, in the present study, histologic examination of the proventriculus was not possible as no birds died during the present study.

In the present study, among birds positive by microscopic examination and PCR method, regurgitation, grinding and throwing food out of the mouth and vomiting were observed in 19.04% of cockatiels, 16.66% of budgerigars and 11.11% of African grey parrot. Diarrhea was observed in 9.50% of cockatiels, 16.66% of budgerigars and 11.11% of African grey parrots. In budgerigars and grey parrots, clinical signs such as melena, undigested seeds in the dropping and bloody vomiting were not observed. As mentioned earlier, most birds presented with signs such as lethargy, anorexia and weight reduction which are nonspecific clinical disease symptoms. Therefore, it seems that gastrointestinal symptoms such as regurgitation, grinding and throwing food out of the mouth, vomiting and diarrhea are common in birds with macrorhabdosis in the southern region of Iran. Similar symptoms have been reported by different researchers, however, depending on the bird species, the symptoms may be somewhat different.[23] In a study by Ozmen *et al*. in budgerigars with macrorhabdosis, the symptoms included cachexia, diarrhea and hemorrhage of the proventriculus.[24] In the present study, 16.66% of budgerigars positive by microscopic examination and PCR method had symptoms such as regurgitation, grinding and throwing food out of the mouth, vomiting, diarrhea, lethargy, anorexia, weight loss, however, no birds died during the present study. Based on our findings in budgerigars, it seems that a chronic form of macrorhabdosis was common in budgerigars in the southern region of Iran.

Anorexia, weight reduction, anemia and melena are commonly found in cockatiels and sometimes in other species.[17,25] In agreement with these results, in the present study, cockatiels positive by the PCR method had symptoms such as anorexia, weight loss and melena. In a study by Poleschinski *et al*., symptoms such as regurgitation, diarrhea, and undigested seeds in the dropping in psittacine birds were reported that was consistent with the finding of the present study, however, they reported some respiratory problems such as dyspnea which was inconsistent with the findings of the present study.[26]

Sullivan *et al*. compared two methods: Fecal Gram's stain (FGS) and PCR for the diagnosis of *M. ornithogaster*.[15] They examined fecal samples and cloacal swab samples in a captive flock of budgerigars. 57.00% were positive by PCR and 24.00% were positive by FGS. All FGS-examined budgerigars were positive on PCR. Based on the findings of Sullivan *et al*., PCR of the cloacal swabs was more likely to detect *M. ornithogaster* than FGS in budgerigars.[15] Poleschinski *et al*. reported *M. ornithogaster* infection at 53.80% by microscopic examination and 46.20% by PCR examination of the stools [26] which was in accordance with the findings of the present study. In the present study, positive cases by PCR (seven positive samples) were less than the microscopic method (17 positive samples). In the present study, out of 100 fecal samples collected from Psittaciformes, 17 samples were positive by microscopic examination. All 17 suspected microscopic samples as well as those from Psittaciformes with the gastrointestinal symptoms of the disease, up to 50 samples, were chosen for molecular diagnosis of the organism. Out of these 50 samples, seven samples (14.00%) were positive by the PCR method. Out of the 17 samples positive by microscopic examination only seven samples were molecularly positive, and ten samples were negative by PCR method. According to the results of Poleschinski *et al*.,[26] and the present study, there was a possibility of false positives with the microscopic method.

Generally, the difference in the type of samples might cause a difference between the results of the present study and the studies mentioned. In some of the above studies, fresh scratches of ventricular or proventricular mucosa were tested, however, in the present study, since no birds did die, only fecal samples from suspected birds were examined. In this study, semi-nested PCR tests confirmed the presence of *M. ornithogaster* in 14.00% of the samples. Two PCR products were sequenced and according to the gene sequences, all of the sequences were owned by *M. ornithogaster*. The results revealed a 96.03 - 100% identity when compared with other sequences *M. ornithogaster* (KX426589.1, KX426588.1, KX426586.1, AF350243.1 and DQ231141.1) which were formerly placed in GenBank® from Germany and the USA. The present study showed that macrorhabdosis occured in African grey parrots, budgerigars and cockatiels in the southern region of Iran and should be considered by veterinarians and bird breeders and the disease should be considered in the differential diagnosis. According to the present study, the PCR method was suggested as an accurate method for diagnosing the disease. The most popular clinical symptoms in positive birds were signs such as regurgitation, grinding and throwing food out of the mouth, vomiting and diarrhea. Therefore, in birds with these symptoms, especially vomiting and diarrhea resistant to antibiotic treatments, macrorhabdosis should be spotted as one of the probable factors to take the essential and proper preventive and curative measures. Because the pathogen is yeast, birds with macrorhabdosis show very poor

responses to antibiotics and antifungal treatment is usually recommended. To the best knowledge of the authors, this was the first report of macrorhabdosis in African grey parrots.

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Conflict of interest

The authors declare there is no conflict of interest.

References

1. Tomaszewski EK, Logan KS, Snowden KF, et al. Phylogenetic analysis identifies the 'megabacterium' of birds as a novel anamorphic ascomycetous yeast, *Macrorhabdus ornithogaster* gen. nov., sp. nov. Int J Syst Evol Microbiol 2003; 53(Pt 4): 1201-1205.
2. Tomaszewski EK, Snowden KF, Phalen DN. The Whipple paradox: Megabacteria exposed as fungi. In: Proceedings of the annual conference of the association of avian veterinarians. Orlando, USA 2001; 99-100.
3. Baker JR. Clinical and pathological aspects of 'going light' in exhibition budgerigars (Melopsittacus undulatus). Vet Rec 1985; 116(15): 406-408.
4. Filippich LJ, Parker MG. Megabacteria and proventricular/ventricular disease in psittacines and passerines. In: Proceedings: Annual conference of the association of avian veterinarians. Nevada, USA 1994; 287-293.
5. Hannafusa Y, Bradley A, Tomaszewski EE, et al. Growth and metabolic characterization of Macrorhabdus ornithogaster. J Vet Diagn Invest 2007; 19(3): 256-265.
6. Amer MM, Mekky HM. Avian gastric yeast (AGY) infection (macrorhabdiosis or megabacteriosis). Bulg J Vet Med 2020; 23(4): 397-410.
7. Martins NRS, Horta AC, Siqueira AM, et al. *Macrorhabdus ornithogaster* in ostrich, rhea, canary, zebra finch, free range chicken, turkey, guinea-fowl, columbina pigeon, toucan, chukar partridge and experimental infection in chicken, Japanese quail and mice. Arq Bras Med Vet Zootec 2006; 58(3): 291-298.
8. Cooke SW. Role of Megabacteria in mammals. Vet Rec 2000; 146(15): 444.
9. Rossi G. Possibility of infecting mammals with megabacteria isolated from birds. Vet Rec 2000; 147(13): 371-372.
10. Baker JR. Megabacteriosis in exhibition budgerigars. Vet Rec 1992; 131(1): 12-14.
11. Fillippich LJ, Boyle DA, Webb R, et al. Megabacteria in birds in Australia. Aust Vet Pract 1993; 23: 71-76.
12. Phalen D. Diagnosis and management of *Macrorhabdus ornithogaster* (formerly megabacteria). Vet Clin North Am Exot Anim Pract 2005; 8(2): 299-306.
13. Phalen DN. Update on the diagnosis and management of Macrorhabdus ornithogaster (formerly megabacteria) in avian patients. Vet Clin North Am Exot Anim Pract 2014; 17(2): 203-210.
14. Gerlach H. Megabacteriosis. Semin Avian Exotic Pet Med 2001; 10(1): 12-19.
15. Sullivan PJ, Ramsay EC, Greenacre CB, et al. Comparison of two methods for determining prevalence of *Macrorhabdus ornithogaster* in a flock of captive budgerigars (*Melopsittacus undulatus*). J Avian Med Surg 2017; 31(2): 128-131.
16. Paula IH de, Linhares FP, Kanayama CY, et al. Megabacteria (*Macrorhabdus ornithogaster*) in psittacids kept in commercial establishments in the municipality of Uberaba - MG. PUBVET 2018; 12(2): 1-4.
17. Van Herck H, Duijser T, Zwart P, et al. A bacterial proventriculitis in canaries (Serinus canaria). Avian Pathol 1984; 13(3): 561-572.
18. Powers LV, Mitchell MA, Garner MM. Macrorhabdus ornithogaster infection and spontaneous proventricular adenocarcinoma in budgerigars (*Melopsittacus undulatus*). Vet Pathol 2019; 56(3): 486-493.
19. Baron HR, Stevenson BC, Phalen DN. Inconsistent efficacy of water-soluble amphotericin B for the treatment of Macrorhabdus ornithogaster in a budgerigar (*Melopsittacus undulatus*) aviary. Aust Vet J 2020; 98(7): 333-337.
20. Fulton RM, Mani R. Avian gastric yeast (*Macrorhabdus ornithogaster*) and *Mycobacterium genavense* infections in a zoo budgerigar (*Melopsittacus undulatus*) flock. Avian Dis 2020; 64(4): 561-564.
21. Kheirandish R, Salehi M. Megabacteriosis in budgerigars: diagnosis and treatment. Comp Clin Pathol 2011; 20: 501-505.
22. Madani SA, Ghorbani A, Arabkhazaeli F. Successful treatment of macrorhabdosis in budgerigars (*Melopsittacus undulatus*) using sodium benzoate. J Mycol Res 2014; 1(1): 21-27.
23. Moore RP, Snowden KF, Phalen, DN. A method of preventing transmission of so-called “Megabacteria” in budgerigars (*Melopsittacus undulatus*). J Avian Med Surg 2001; 15(4): 283-287.
24. Ozmen O, Aydoğan A, Haligur M, et al. The pathology of Macrorhabdus ornithogaster and Eimeria dunsingi (Farr, 1960) infections in budgerigars (*Melopsittacus undulatus*). Isr J Vet Med 2013; 68(4): 218-224.
25. Dorrestein GM, Zwart P, Buitelaar MN. Problems arising from disease during the periods of breeding and rearing canaries and other aviary birds [Dutch]. Tijdschr Diergeneeskd 1980; 105(13): 535-543.
26. Poleschinski JM, Straub JU, Schmidt V. Comparison of two treatment modalities and PCR to assess treatment effectiveness in Macrorhabdosis. J Avian Med Surg 2019; 33(3): 245-250.