DOI: 10.1002/vms3.602

CASE REPORT

WILEY

Systemic mucormycosis caused by *Rhizopus microsporus* in a captive bottlenose dolphin

Lingling Chang[1] | **Yanping Qi**[1] | **Yamian Wang**[2] | **Chen-Hsuan Liu**[3] | **Songbiao Chen**[1] | **Bichen Miao**[1] | **Dewen Tong**[1]

[1] College of Veterinary Medicine, Northwest A&F University, Yangling, Shaanxi, China

[2] Xi'an Qujiang Polar Ocean Park, Xi'an, Shaanxi, China

[3] Graduate Institute of Molecular and Comparative Pathobiology, School of Veterinary Medicine, National Taiwan University, Taipei, Taiwan

Correspondence
Prof Dewen Tong, College of Veterinary Medicine, Northwest A&F University, Yangling, Shaanxi, China.
Email: dwtong@nwsuaf.edu.cn

Lingling Chang and Yanping Qi contributed equally to this work.

Abstract

A 6-year-old female bottlenose dolphin (*Tursiops truncatus*) kept in dolphinarium died after a 3.5-month period of lethargy and inappetence despite antibiotics and supportive care. At necropsy, gross findings included diffuse varying-sized nodules in the lungs and scattered nodules throughout the heart, spleen, mesenteric and hilar lymph node and kidney. Microscopically, the lesions were characterised by disseminated fungal pyogranulomas with numerous intralesional Mucor-like fungi. The fungi structures were demonstrated by Periodic acid-Schiff and Gomori methenamine silver stain. Molecular analyses of the fungi were *Rhizopus microsporus* by PCR sequencing 18S ribosomal RNA gene. Ziehl–Neelsen stain failed to show acid-fast bacterial infection. Based on pathological and molecular examination, systemic granulomatous mucormycosis was diagnosed. To our knowledge, this is the first reported case of systemic mucormycosis caused by *Rhizopus microsporus* in bottlenose dolphin.

KEYWORDS
bottlenose dolphin, mucormycosis, pathology, *Rhizopus microsporus*

Mucormycosis is a saprophytic opportunistic infection in humans and animals caused by fungi in the order *Mucorales* (former class *Zygomycetes*) (Hoffmann et al., 2013). Within the order, the most often identified species belong to the genera *Rhizopus*, *Mucor*, *Lichtheimia* (formerly *Absidia*), *Mortierella* and *Rhizomucor* (Gomes et al., 2011; Seyedmousavi et al., 2018), which are ubiquitous in the environment, such as soil, air, dust and food stuffs (Hoffmann et al., 2013). Mucormycosis is rarely observed in marine mammals (dolphin, seal and whale) (Abdo et al., 2012a, b;Barnett et al., 2011; Dhib et al., 2019). Mucorales mainly cause diseases in debilitated animals with immune suppression. The common observed lesions of mucormycosis can occur in different organs, especially the skin, lung and gastrointestinal tract (Seyedmousavi et al., 2018).The mucormycosis lesions may lead to systemic spread to multiple organs and often have fatal outcome (Barnett et al., 2011; Ribes et al., 2000). The present report describes a case of systemic mucormycosis in a captive bottlenose dolphin (*Tursiops truncatus*) caused by *Rhizopus microsporus*.

A 6-year-old female bottlenose dolphin has been maintained in Xi'an Qujiang Polar Ocean Park, Xi'an, for 3 years dating from May 2017. On 13 May 2020, the animal suddenly exhibited signs of anorexia. Gastroscopy examination revealed a large amount of foreign body filled in the oesophagus lumen, and the oesophageal mucosa was mechanically damaged. Blood analysis showed increased count of white blood cell (WBC;11.97 $10^3/\mu$l, reference range 5.0–9.0 $10^3/\mu$l), neutrophils (8.73 $10^3/\mu$l, reference range 3.23–4.85 $10^3/\mu$l) and eosinophils (1.68 $10^3/\mu$l, reference range 0.53–1.02 $10^3/\mu$l) and increased levels of globulin(38.9 g/l, reference range 13–25 g/l)and blood urea nitrogen(BUN; 23.96 mmol/l, reference range 15–20 mmol/l). On 14 May 2020, a total of 1.89 kg foreign body in the oesophageal lumen was removed, mainly wall skin, and a small amount of wire and stone. Antibiotics of Cefdinir

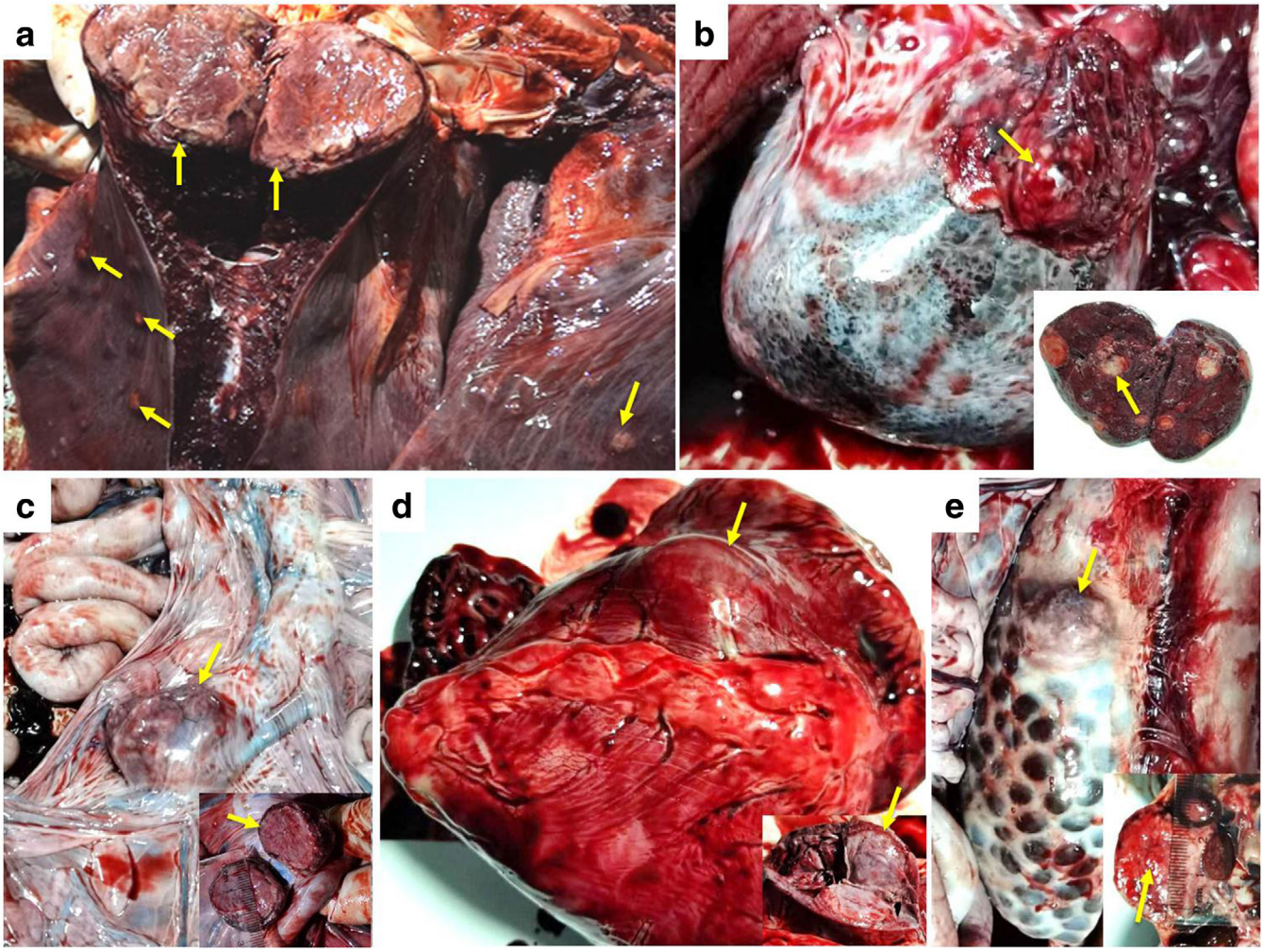

FIGURE 1 (a) There were multiple nodules (arrow) in the lungs. The large nodule, 16.5 cm in diameter, showed flesh pink on cut surface (arrow). (b) One nodule 3 cm in diameter on the surface of the spleen (arrow). Inset showed multiple nodules 0.5–3 cm in diameter on cut surface (arrow). (c) One reddish nodule 4.5 cm in diameter in the mesenteric lymph nodes (arrow). Inset showed flesh red on cut surface (arrow). (d) One reddish nodule 4.5 cm in diameter on the surface of the right ventricular in the heart (arrow). Inset showed flesh red on cut surface (arrow). (e) One dark-red nodule 3 cm in diameter on the surface of the right kidney (arrow). Inset showed flesh red on cut surface (arrow)

(per os, for 10 days) and Cefoperazone sodium (iv drip, for 2 days) were administered. Over the next 2 months, the dolphin exhibited alternant periods of anorexia and transient improvement. Follow-up blood samples revealed persistent increasing levels of WBC (20.09 $10^3/\mu$l, reference range 5.0–9.0 $10^3/\mu$l), eosinophils (4.16 $10^3/\mu$l, reference range 0.53–1.02 $10^3/\mu$l), lactic dehydrogenase (LDH; 1060 U/l, reference range 350–500 U/l) and globulin (57.5 g/l, reference range 13–25 g/l). Antibiotic treatment was continued with levofloxacin, cefdinir and amoxicillin for 1 month and followed by hydrochloride. On August 2020, 1 month before death, the animal state dramatically worsened. Blood examinations revealed the counts of WBC, neutrophils and eosinophils were within reference intervals. Marked increase of fibrinogen (5.26 g/l, reference range 1.7–2.8 g/l), LDH (1843 U/l, reference range 350–500 U/l), globulin (54 g/l, reference range 13–25 g/l) and BUN (28.09 mmol/l, reference range 15–20 mmol/l) was presented. On August 17, 111 days after the initial clinical signs, blowhole swab culture grew *Echerichia coli*, *Aspergillus fumigatus* and *P. aeruginosa*. Administration of corticosteroids (Dexamethasone), antifungal (Itraconazole) and antibiotics (Cefoxitin and Amikacin) was begun. Despite antibiotics and corticosteroids treatment, the dolphin developed respiratory failure and died on 31 August 2011.

A complete necropsy was performed immediately on the bottlenose dolphin. The dolphin was in general bodily condition (201 kg in body weight) and measured 260 cm in length. There were diffuse, well-demarcated, firm nodules up to 5 mm in diameter throughout the lung surface (Figure 1a). On cut surface, multiple 1–2 mm nodules were seen. In addition, large nodules measuring 10–16.5 cm in diameter were found in the ventral side the right lung, with flesh pink on cut surface (Figure 1a), and one large nodule exhibited cavitations in the centre. The hilar lymph node was notably enlarged and necrotic. In the spleen, multiple nodules of 0.5–3 cm in diameter were seen on the surface (Figure 1b). There were three dark-red nodules up to 4.5 cm in diameter in the mesenteric lymph nodes (Figure 1c). In the heart, there was one dark-red nodule, 4.5 cm × 3 cm, on the surface of the right

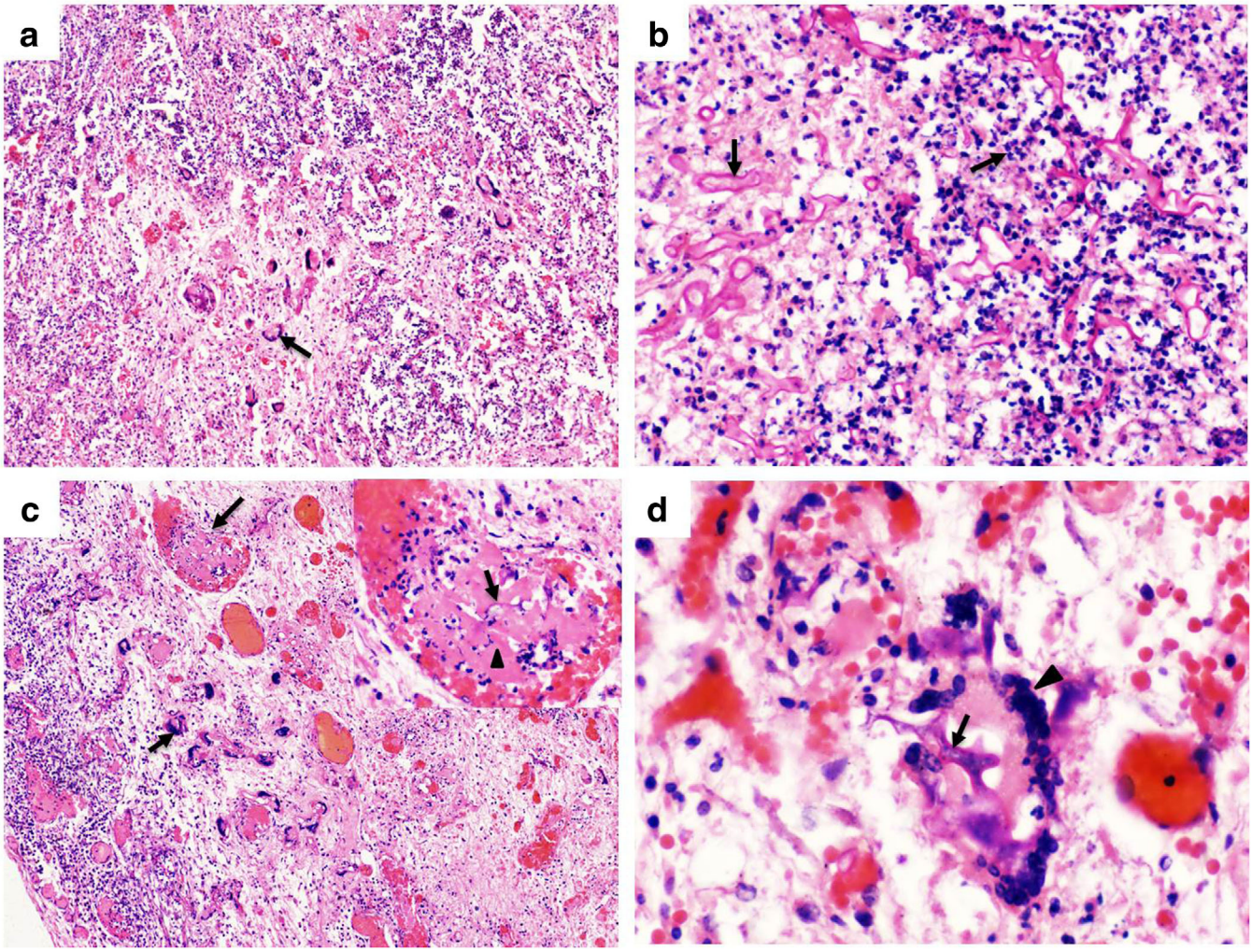

FIGURE 2 (a) Lung: pyogranulomatous (arrow) and neutrophilic inflammatory infiltrate effacing pneumonic architecture mixed with pleomorphic fungal structures (arrow). Multiple multinucleated giant cells scattered in the foci area (arrow). H&E, ×100. (b) Lung: many Mucor-like hyphae are surrounded with large amounts of neutrophil (arrow). H&E, ×400. (c) Mesenteric lymph node: extensive coagulation necrosis and significantly congestion and microthrombus (arrowhead) with marked granulomatous inflammation (arrow). Mucor-like hyphae are seen in inflammatory foci, especially in the cytoplasm of multinucleated giant cells (arrows). H&E, ×100. (d) Mesenteric lymph node: hyphae were located within blood vessels (arrows). H&E, ×400

ventricular base (Figure 1d). The right and left atria and ventricles were markedly dilated with a large amount of noncoagulated blood. The right and left ventricle walls were moderately thinned and discoloured and were soft. There was one nodule on the surface of the right and left kidney, measuring 3 cm and 2 cm in diameter, respectively (Figure 1e). The liver showed a slight swelling, and the cut surface showed dark reddish-brown in colour. There was one thrombus, 60 cm × 0.4–1.0 cm, in the lumen of the portal vein.

All tissues with gross lesions were fixed in 10% neutral buffered formalin for routine histopathology examination. Tissue sections were stained with haematoxylin and eosin (H&E), where selected sections were also stained with Grocott's methenamine silver (GMS), periodic acid-Schiff (PAS) and Ziehl–Neelsen (ZN) stains.

Microscopically, the lung nodules showed widespread necrosis with a marked pyogranulomatous and neutrophilic infiltrate (Figure 2a). Moreover, there were intralesional, perivascular and intravascular, highly pleomorphic, sparsely septate fungal hyphae (Figure 2b), which were characteristic features of Mucorales fungi. There was focally extensive effacement of normal hilar lymph node architecture by the same inflammatory infiltrate and microthromsis (Figure 2c) with accompanying fungal hyphae (Figure 2d). Similarly, the nodules in heart, kidney, mesenteric lymph node and spleen showed marked necrosis, vasculitis and pyogranulomatous inflammatory infiltrate with accompanying fungal hyphae. The liver showed a mild, chronic hepatitis with mild fatty degeneration. A mild, chronic gastritis and enteritis were identified, with no evidence of fungal hyphae.

GMS and PAS stains revealed large number of fungal hyphae in the nodules of the lung, lymphoid and kidney (Figure 3a, c). These hyphae were 6–8 μm wide, irregularly branched (usually right angles), with poorly evident septa and thick, nonparallel walls that often appeared collapsed (Figure 3b). Focal bulbous dilations at the extremities were

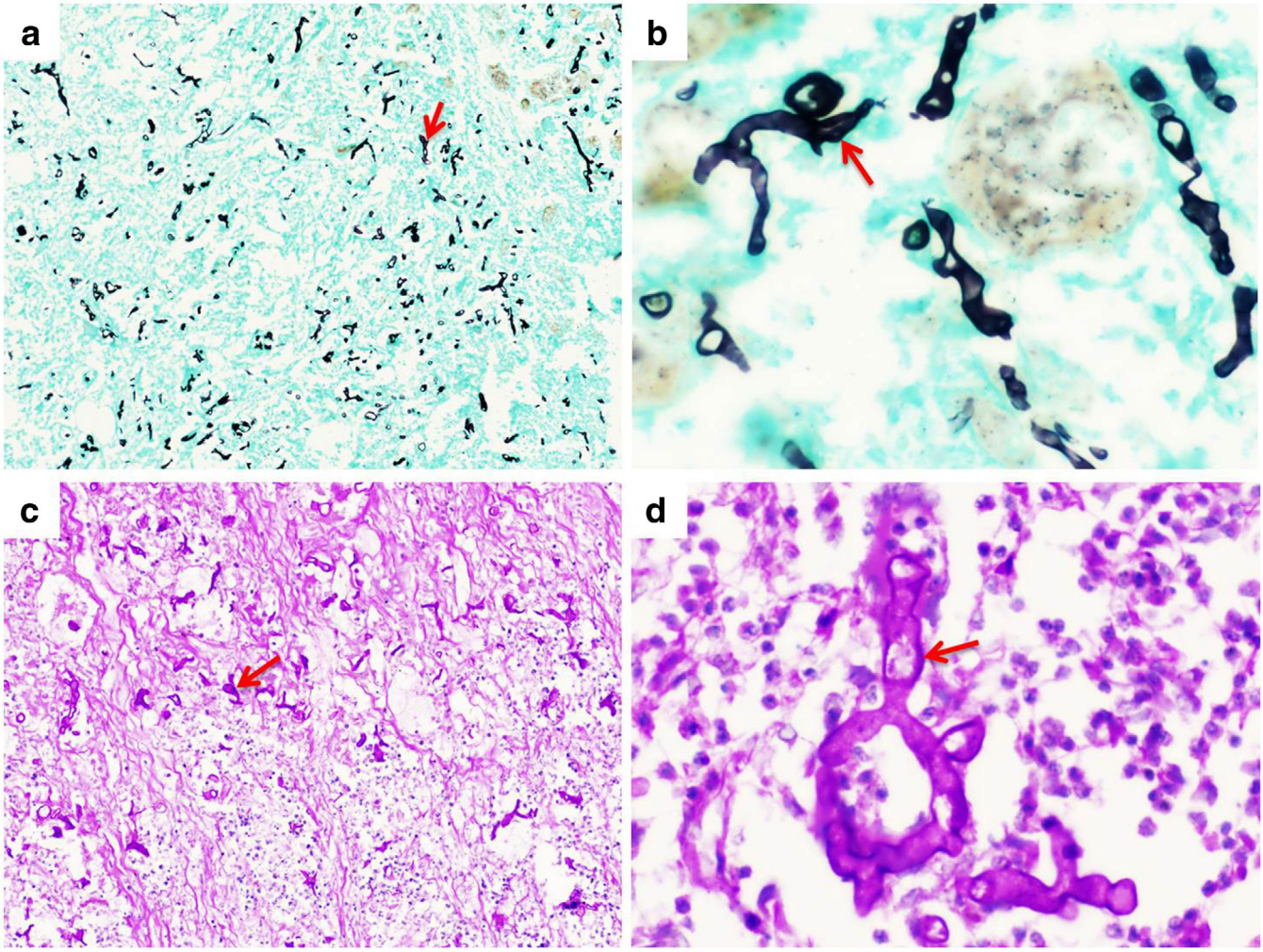

FIGURE 3 (a) GMS stains showing a large number of fungal hyphae in kidney (arrows). GMS stains, ×100. (b) Mucor-like hyphae were 6–8 um in diameter, rectangular branching, sparsely septate, broad thick-walled with focal bulbous dilations (arrows). GMS stains, ×400. (c) PAS stains showing fungal hyphae in mesenteric lymph node (arrows). PAS stains, ×100. (d) Mucor-like hyphae (arrows). PAS stains, ×400

frequently observed. When transversally sectioned, they were round to oval (Figure 3a, b). Ziehl–Neelsen stains were negative on all tissue sections (data not shown).

The fungal organisms were further confirmed by PCR and sequence analysis. The fresh tissue samples of lung, heart, stomach, intestine and kidney were selected for DNA extraction and PCR amplification. The primers ZM1 (5′-ATTACCATGAGCAAATCAGA-3′) and ZM2 (5′-TCCGTCAATTCCTTTAAGTTTC-3′) were used targeting the 18S ribosomal DNA of Mucorales and amplify a product of ~400 bp in length (Hammond et al., 2011; Rickerts et al., 2006). Sequences obtained were subjected to Blast Identity Search (NCBI) to analyze their identity with the reference sequences in Genbank. The results showed that the samples of lung, heart and kidney were PCR positive. Sequencing displayed 99% of homology with *Rhizopus microsporus*. The obtained sequence has been deposited in the GenBank under accession number: MW031863.

Isolation and identification of bacteria were performed at the First Affiliated Hospital of Xi'an Jiaotong University. Culture for aerobic and anaerobic microorganisms yielded *P. aeruginosa* from the lungs, and *Echerichia coli* from heart, stomach and uterus.

Cases of mucormycosis in dolphin are very scarce. In this study, dolphin mucormycosis infection caused by *R. microsporus* was based on histopathological findings and identification of molecular techniques. Generally, diagnosis of mucormycosis can be achieved by the demonstration of characteristic hyphae on tissue sections. Further, molecular techniques are helpful for fungal identification at the species level of Zygomycetes (Schwarz et al., 2006). Dolphin mucormycosis has been documented and the species of *Apophysomyces elegans* (Palmero et al., 2014; Robeck & Dalton, 2002; Walters et al., 2009) (Delaney et al., 2013), *Saksenaea vasiformis* (Palmero et al., 2014), *Rhizopus arrhizus* (Barnett et al., 2011), *Cunninghamella bertholletiae* (Bragulat et al., 2017) and *Lichtheimia ramosa* (Dhib et al., 2019) have been identified. In this case, histopathological examination showed irregular, nonseptate (or rarely septate hyphae) and right-angled branching hyphae consistent with mucorales fungi, further identified as *Rhizopus microsporus* by PCR sequencing 18S rRNA gene. *R. microspores* had a worldwide

distribution and are found in soil, air and decaying matter and growing well on substrates such as fruits, cereals and bread. Reports of *Rhizopus microsporus* infection are common in humans (Kimura et al., 2012; Xess et al., 2018). A few cases of *Rhizopus microsporus* infection have also been reported in domestic and wild animals (Muir & Raidal, 2012; Slaviero et al., 2020; Sosa et al., 2013).

Clinical haematology examination revealed a high WBC count nearly all over the course of the dolphin disease. Leukocytosis is a common finding in infectious diseases in bottlenose dolphins. Notably, marked increase levels of LDH, globulin and fibrinogen concentrations observed in this dolphin are consistent with previous reports (Abdo et al., 2012a,b; Dhib et al., 2019). It has been reported that higher fibrinogen concentrations could facilitate fungal infection, and it can simply be an indicator of mucormycosis (Robeck & Dalton, 2002).

Predisposing factors favouring mucormycosis infections in marine mammal that are immunocompromised or otherwise debilitated due to metabolic disorders. Although pre-existing immunosuppressive diseases were uncertain in this bottlenose dolphin, a large number of foreign objects found in the oesophageal lumen could have been a factor leading to immune suppression. The animal keeper told us that it was uncommon for dolphin to ingest foreign objects. The compromised immune status of this dolphin could have resulted in *Rhizopus microsporus* and opportunistic bacterial infection. The *P. aeruginosa* and *Echerichia coli* were isolated in this case. In marine mammals, *P. aeruginosa* could have been a disruptor of the body's normal immune system which contributed to *Mucor* infection (Abdo et al., 2012a, b; Dhib et al., 2019).

Due to the angioinvasive property of *Mucorales*, haematogenous spread to multiple organs is often reported (Seyedmousavi et al., 2018). This bottlenose dolphin may have become infected with *Rhizopus microsporus* from a contaminated aquatic environment; the fungus enters the body through the respiratory tract and can then disseminate to distant sites. Systemic mucormycosis is rarely reported in dolphin (Robeck & Dalton, 2002). To our knowledge, this is the first reported case of systemic mucormycosis caused by *Rhizopus microsporus* in a captive bottlenose dolphin.

Systemic mucormycosis can originate from any of the primary sites of infection, particularly the skin, lung, nasal sinus and digestive tract (Abdo et al., 2012a, b). In the present case, the obvious pathological finding was severe diffuse fungal pyogranulomas in the lungs, and scattered fungal pyogranulomas in the heart, kidney, mesenteric lymph node and spleen. Our pathological findings suggested that the systemic mucormycosis in this bottlenose dolphin was likely to have originated from the lungs. Notably, multiple pyogranulomatous lesions are macroscopically indistinguishable from tuberculosis (Ortega et al., 2010), especially when it is located in lungs. Ziehl–Neelsen stains were negative on tissue section, thus ruling out acid-fast bacteria such as Mycobacterium and Nocardia.

The present case emphasises the pathogenic potential of *Rhizopus microsporus* mucormycosis in a captive bottlenose dolphin, as well as the potential for infection with opportunistic microflora, particularly *P. aeroginosa* in an immunocompromised bottlenose dolphin.

ACKNOWLEDGEMENTS

We are grateful to the staff of the Xi'an Qujiang Polar Ocean Park, Xi'an, Shaanxi, for helping in samples collection and laboratory detection.

ETHICAL STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. All relevant guidelines for the use of animals in scientific studies were followed. The study did not include any experimentation on animals or humans, and samples were taken from natural died animal.

CONFLICT OF INTEREST

All authors declared no competing interests.

AUTHOR CONTRIBUTIONS

Lingling Chang: Formal analysis; methodology; writing-original draft. **Yanping Qi**: Formal analysis; investigation, writing-review & editing. **Yamian Wang**: Formal analysis; resources. **Chen-Hsuan Liu**: Investigation; writing-review & editing. **Songbiao Chen**: Investigation; writing-review & editing. **Bichen Miao**: Investigation; writing-review & editing. **Dewen Tong**: Conceptualisation; writing-review & editing.

DATA AVAILABLE STATEMENT

The data that support the findings of this study are openly available in GenBank at https://www.ncbi.nlm.nih.gov/nuccore/MW031863, reference number MW031863.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1002/vms3.602

ORCID

Dewen Tong https://orcid.org/0000-0003-2069-8306

REFERENCES

Abdo, W., Kakizoe, Y., Ryono, M., Dover, S. R., Fukushi, H., Okuda, H., Kano, R., Shibahara, T., Okada, E., Sakai, H., & Yanai, T. (2012a). Pulmonary zygomycosis with *Cunninghamella bertholletiae* in a killer whale (*Orcinus orca*). *Journal of Comparative Pathology*, *147*, 94–99.

Abdo, W., Kawachi, T., Sakai, H., Fukushi, H., Kano, R., Shibahara, T., Shirouzu, H., Kakizoe, Y., Tuji, H., & Yanai, T. (2012b). Disseminated mycosis in a killer whale (*Orcinus orca*). *Journal of Veterinary Diagnostic Investigation*, *24*, 211–218.

Barnett, J. E. F., Davison, N. J., Thornton, S. M., Riley, P., Cooper, T., & Wessels, M. E. (2011). Systemic mucormycosis in a hooded seal (*Cystophora cristata*). *Journal of Zoo and Wildlife Medicine*, *42*, 338–341.

Bragulat, M. R., Castella, G., Isidoro-Ayza, M., Domingo, M., & Cabanes, F. J. (2017). Characterization and phylogenetic analysis of a *Cunninghamella bertholletiae* isolate from a bottlenose dolphin (*Tursiops truncatus*). *Revista Iberoamericana de Micología*, *34*, 215–219.

Delaney, M. A., Terio, K. A., Colegrove, K. M., Briggs, M. B., & Kinsel, M. J. (2013). Occlusive fungal Tracheitis in 4 captive bottlenose dolphins (*Tursiops truncatus*). *Veterinary Pathology*, *50*, 172–176.

Dhib, I., Yaacoub, A., Chouaieb, H., Jaim, A., Ben Said, M., Letscher, V., & Fathallah, A. (2019). *Lichtheimia ramosa* mucormycosis in a bottlenose dolphin. *Austin Journal of Veterinary Science & Animal Husbandry*, *6*, 1064.

Gomes, M. Z. R., Lewis, R. E., & Kontoyiannis, D. P. (2011). Mucormycosis caused by unusual mucormycetes, non-rhizopus, -mucor, and -lichtheimia species. *Clinical Microbiology Reviews*, *24*, 411–+.

Hammond, S. P., Bialek, R., Milner, D. A., Petschnigg, E. M., Baden, L. R., & Marty, F. M. (2011). Molecular methods to improve diagnosis and identification of mucormycosis. *Journal of Clinical Microbiology*, *49*, 2151–2153.

Hoffmann, K., Pawlowska, J., Walther, G., Wrzosek, M., de Hoog, G. S., Benny, G. L., Kirk, P. M., & Voigt, K. (2013). The family structure of the Mucorales: A synoptic revision based on comprehensive multigene-genealogies. *Persoonia*, *30*, 57–76.

Kimura, M., Nishimura, K., Enoki, E., Chikugo, T., & Maenishi, O. (2012). Chlamydospores of *Rhizopus microsporus* var. rhizopodiformis in tissue of pulmonary mucormycosis. *Mycopathologia*, *174*, 441–450.

Muir, M., & Raidal, S. R. (2012). Necrotising ventriculitis due to combined infection with *Rhizopus microsporus* var. chinensis and *Candida krusei* in an eclectus parrot (*Eclectus roratus*). *Australian Veterinary Journal*, *90*, 277–280.

Ortega, J., Uzal, F. A., Walker, R., Kinde, H., Diab, S. S., Shahriar, F., Pamma, R., Eigenheer, A., & Read, D. H. (2010). Zygomycotic lymphadenitis in slaughtered feedlot cattle. *Veterinary Pathology*, *47*, 108–115.

Schwarz, P., Bretagne, S., Gantier, J. C., Garcia-hermoso, D., Lortholary, O., Dromer, F., & Dannaoui, E. (2006). Molecular identifiation of zygomycetes from culture and experimentally infected tissues. *Journal of Clinical Microbiology*, *44*, 340–349.

Palmero, F., Parraga, D. G., Navado, E. M., & García-Hartmann, M. (2014). *Zygomycosis in bottlenose dolphins (Tursiops truncatus) caused by Rhizopus oryzae*. Presented at the Annual Symposium of the European Association for Aquatic Mammals. Tenerife.

Ribes, J. A., Vanover-Sams, C. L., & Baker, D. J. (2000). Zygomycetes in human disease. *Clinical Microbiology Reviews*, *13*, 236–+.

Rickerts, V., Just-Nubling, G., Konrad, F., Kern, J., Lambrecht, E., Bohme, A., Jacobi, V., & Bialek, R. (2006). Diagnosis of invasive aspergillosis and mucormycosis in immunocompromised patients by seminested PCR assay of tissue samples. *European Journal of Clinical Microbiology*, *25*, 8–13.

Robeck, T. R., & Dalton, L. M. (2002). *Saksenaea vasiformis* and *Apophysomyces elegans* zygomycotic infections in bottlenose dolphins (*Tursiops truncatus*), a killer whale (*Orcinus orca*), and pacific white-sided dolphins (*Lagenorhynchus obliquidens*). *Journal of Zoo and Wildlife Medicine*, *33*, 356–366.

Seyedmousavi, S., Bosco, S. D. G., de Hoog, S., Ebel, F., Elad, D., Gomes, R. R., Jacobsen, I. D., Jensen, H. E., Martel, A., Mignon, B., Pasmans, F., Pieckova, E., Rodrigues, A. M., Singh, K., Vicente, V. A., Wibbelt, G., Wiederhold, N. P., & Guillot, J. (2018). Fungal infections in animals: A patchwork of different situations. *Medical Mycology*, *56*, E4–E4.

Slaviero, M., Vargas, T. P., Bianchi, M. V., Ehlers, L. P., Spanamberg, A., Ferreiro, L., Araújo, R., & Pavarini, S. P. (2020). *Rhizopus microsporus* segmental enteritis in a cow. *Medical Mycology Case Reports*, *28*, 20–22.

Sosa, M., Gamble, K. C., Delaski, K., & Righton, A. (2013). Clinical challenge: Systemic *Rhizopus microsporus* infection with renal cavitation in a grey seal (*Halichoerus grypus*). *Journal of Zoo and Wildlife Medicine*, *44*, 1134–1138.

Walters, C., Townsend Jr., F. I., Staggs, L., Dalton, L., & Osborn, S. (2009). Posaconazole for the treatment of *Zygomycosis* in Cetaceans. Speaker Speech Iaaam.

Xess, I., Pandey, M., Singh, G., Agarwal, R., Kumar, R., & Jyotsna, V. P. (2018). Emerging infections due to *Rhizopus microsporus* in India: Series of eight cases from a single centre. *Medical Mycology*, *56*, S93–S93.

How to cite this article: Chang, L., Qi, Y., Wang, Y., Liu, C.-H., Chen, S., Miao, B., & Tong, D. (2021). Systemic mucormycosis caused by *Rhizopus microsporus* in a captive bottlenose dolphin. *Veterinary Medicine and Science*, *7*, 2404–2409. https://doi.org/10.1002/vms3.602