Fusarium solani *hyalohyphomycosis in loggerhead sea turtles (*Caretta caretta*): a diagnostic and therapeutical challenge*

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Summary
Fusarium spp. are pathogens plants, animals and humans, isolated from soil, plants and water systems. They are distributed worldwide and include saprotrophic, biotrophic-pathogenic or endophytic fungi, or producers of mycotoxins (fumonisins). Human isolates are becoming the leading mycosis affecting immunocompromised patients and frequently involved in mycoses of aquatic mammals and reptiles, included sea turtles or their eggs. Here reported are three severe cases of unusual localizations of *Fusarium* in loggerhead sea turtle (*Caretta caretta*) and their diagnostic, therapeutic and clinical output. In the clinical practice, correct genus-level identification of *Fusarium* species is critically important to enable correct treatment as *in vitro* antifungal susceptibility testing is mandatory for each *Fusarium*-like isolate. For this reason, susceptibility testing can significantly help the practitioner in choosing the most appropriate therapeutic protocol.

Introduction

Mycoses in the hyalohyphomycosis group are heterogeneous, defined by the presence of hyaline hyphae in tissues. The number of organisms causing hyalohyphomycosis is increasing and the most clinically important genera are *Fusarium* spp., *Scedosporium* spp., *Acremonium* spp., *Scopulariopsis* spp., *Purpureocillium* and *Paecilomyces* spp. (Tortorano *et al.* 2014). *Fusarium* (order *Hypocreales*) is a large genus with at least 200 species divided into 20 complexes, and many members are commonly found in the environment, where they are isolated from soil, plants and water systems, distributed worldwide and encompassing saprotrophic, biotrophic-pathogenic or endophytic fungi. Agents of any type of fusariosis are mainly found in three species complexes: *F. solani* complex, *F. oxysporum* complex and *F. fujikuroi* complex. *Fusarium* spp. are notorious as pathogen to plants, animals and humans and as a producer of secondary metabolites causing toxicoses (fumonisin mycotoxins, toxic and/or carcinogenic to human and domesticated animals) or invasive disease (Brown and Proctor 2013, Nuñez Otaño *et al.* 2014, Wellehan and Divers 2019). Human isolates mostly cause a broad spectrum of opportunistic superficial infections in immunocompetent individuals (i.e. onychomycosis or keratitis), but in recent years they have been increasingly associated with invasive and disseminated infections, predominantly in severely immunocompromised patients, diabetics, cancer patients taking cytotoxic drugs, and burn patients, becoming the leading mycosis affecting immunocompromised patients, and the second most common cause of filamentous fungi infections after aspergillosis, with high morbidity and mortality rates (Montali *et al.* 1981, Brown and Proctor 2013, Al-Hatmi *et al.* 2018, Leu *et al.* 1995, Alastruey-Izquierdo *et al.* 2008).

Fusarium spp. are frequently isolated from marine mammals: *Fusarium solani* in captive California sea lions (*Zalophus californianus*) and grey seals (*Halichoerus grypus*) (Montali *et al.* 1981), white-sided dolphin (*Lagenarhynchus acutus*), pigmy sperm whale (*Kogia breviceps*) and harbour deals (*Phoca*

vitulina) (Frasca *et al.* 1996) or other marine organisms: American horseshoe crabs *Limulus polyphemus* (Tuxbury *et al.* 2014), wild narrow-clawed crayfish *Astacus leptodactylus* (Salighehzadeh *et al.* 2019), black gill disease in cultured Kuruma prawn *Penaeus japonicas* (Bian and Egusa 1981) and giant tiger prawn *P. monodon* (Khoa *et al.* 2004), captive lined seahorses *Hippocampus erectus* (Salter *et al.* 2012). Fusarial infections in freshwater and marine fish include deep mycoses, ocular and skin lesions, and fatal ulceration and necrohemorrhagic dermatitis (Yanong 2003, Noga 2010).

Fusarium spp. has been identified as causative agent of cutaneous hyalohyphomycosis in captive loggerhead sea turtles, where the lesions were described as superficial, white and scaly or ulcerative (Austwick *et al.* 1981, Cabañes *et al.* 1997), and in a stranded Kemp's ridley sea turtle with pulmonary hyalohyphomycosis (Orós *et al.* 2004), where fungal pneumonia was associated with cold stunning (Knotek and Divers 2019). *Fusarium solani* has also been found in sea turtle eggs, associated with mass mortalities (up to 83.3%) in natural and relocated nests of the sea turtle *Caretta caretta* (Sarmiento-Ramìrez *et al.* 2014a). Colonization of eggs with *Fusarium* is considered among the main causes of globally declining turtle populations (*Chelonia mydas, Caretta caretta, Eretmochelys imbricata, Lepidochelys olivacea, Dermochelys coriacea*, and *Natator depressus*) (Sarmiento-Ramìrez *et al.* 2014b).

Treatment of fusariomycosis is very challenging. *Fusarium* spp. shows a remarkably high degree of intrinsic multidrug resistance to a wide spectrum of commonly used antifungals azoles, echinocandins and polyenes. The triazole group of antifungals works by inhibiting the fungal lanosterol 14α-demethylase in the ergosterol biosynthesis pathway. The polyenes include amphotericin B, which binds to ergosterol thereby forming pores and damaging the cell membrane. Finally, the echinocandins, namely caspofungin, micafungin and anidulafungin, affect cell wall synthesis by inhibiting 1,3-β-D-glucan synthase. *Fusarium* species share their high degree of intrinsic resistance to most currently used antifungal agents with *Acremonium* and *Trichoderma*, which belong to the same order *Hypocreales*, and with *Microascus, Scopulariopsis, Scedosporium* and *Lomentospora* in the adjacent order *Microascales*. Intrinsic multiresistance of these fungi is unique among the *Ascomycota* and suggests that this property was acquired in a common ancestor of the two orders (Al-Hatmi *et al.* 2018).

For practical purposes in the clinical practice, correct genus-level identification of *Fusarium* species is a critically important action to enable correct treatment and infection control.

Since the distribution of *Fusarium* species varies with geographic region (Wang *et al.* 2011) and different species have different drug susceptibility patterns (Nucci and Anaissie 2007), accurate species assignment is important for epidemiological studies and to guide clinical management. *F. solani* and *F. verticillioides* are usually resistant to azoles and exhibit higher amphotericin B minimal inhibitory concentration (MICs) than other *Fusarium* spp. (Nucci and Anaissie 2007, Wang *et al.* 2011). In contrast, *F. oxysporum* may be susceptible to voriconazole and posaconazole. (Nucci and Anaissie 2007, Tanaka *et al.* 2012).

Under this respect, *in vitro* antifungal susceptibility testing is mandatory for each *Fusarium*-like isolate causing a confirmed systemic infection (Lass-Flörl *et al.* 2010, Summerbell 2002, Johnson 2008, Perkhofer 2010). Itraconazole and other azole drugs may need to be replaced, supplemented with amphotericin B, or discontinued as useless against the pathogen. Empirically, a combination of terbinafine with either posaconazole or voriconazole may be used while identification, speciation, and sensitivity results are pending, but intrinsic resistance interferes with antifungal therapy, clearly challenging treatment owing to the limited therapeutic options, and finally resulting in high mortality rates in patient (Brown and Proctor 2013, Sharma *et al.* 2017, Al-Hatmi *et al.* 2018, Mader 2019). Whenever feasible, infected tissue should be excised surgically (Wellehan and Divers 2019).

Case history, clinical signs, pathological findings, and diagnosis

Case 1

A juvenile cold stunned loggerhead sea turtle (*Caretta caretta*) stranded in February on the coast of Adriatic Sea near to San Foca (Lecce, Italy), with average sea temperature of 9 °C. The animal was referred to 'Torre Guaceto' Marine Turtle Rescue Centre (Carovigno, Italy), and immediately moved to the Department of Veterinary Medicine of Bari (Italy) for diagnostics and treatment because of severe signs ascribable to cold stunning (hypothermia).

At admission the turtle weighted 5 kg, and the straight carapace length resulted 33 cm. The animal was lethargic and severely emaciated. Blood samples were collected in heparinized tubes from cervical sinus for complete haematological and plasma chemistry analyses. Afterward, X-ray examination was performed in order to check for foreign objects and/or pneumonia because of abnormal buoyancy. Both resulted negative. After determination of glycemia (resulted within the ranges), fluids were intracoelomically administered

(1 part dextrose 5%, 1 part saline, 1 part electrolytic rehydrating solution, 1 part sterile water) at 1% b.w., then the body temperature was increased 3 °C/day until it reached 24 °C. Antimicrobial and antifungal therapy was initiated when the turtle's temperature reached 19 °C to 20 °C (Norton and Walsh 2012).

The turtle presented several head, shell and plastron traumatic injuries, probably resulting from repeated dashes against rocks. The shell showed several disseminated superficial and ulcerous lesions, involving keratinized scales and the underlying bone plates, and one large lesion in the column region that continued on the right and left side of carapace, with necrosis of column bones, carapacial bone plates and ribs too (Figure 1a). Also, the head and plastron showed deep lesions with loss of tissue and exposition of bone superficies.

Lesions were sampled using a sterile lancet collecting material from the advancing edges of the lesions, after disinfection of the surface with iodopovidone and local anaesthesia induced by injection of a solution of 2% lidocaine hydrochloride; the bioptic samples were used for microbial and mycological culturing, and for histologic examination.

Standard prophylactic antimicrobial and antimycotic treatment was started (Mader 2019, Manire *et al.* 2003, Paré and Jacobson 2007, McArthur *et al.* 2004), with marbofloxacin (2 mg/kg, IM q 24 h; Lai *et al.* 2009) and itraconazole (5 mg/kg, PO, q 24 h administered with assisted feeding; Mader 2019). Topical iodopovidone ointment was applied on shell and plastron lesions.

The samples for bacteriological testing were cultured on tryptic soy blood agar and Mac Conkey agar, incubated for 24 h at 37 °C. On the basis of biochemical reaction and morphological characteristics, two different isolates were noticed, then identified with the biochemical system API 20 NE. *Vibrio fluvialis* and *Aeromonas hydrofila* were isolated from lesions and tested for susceptibility to several antibiotic drugs (ampicillin, ceftazidime, cephalothin, cefuroxime, doxycycline, norfloxacin, ciprofloxacin, enrofloxacin, marbofloxacin). The isolates resulted susceptible to ceftazidime, norfloxacin, ciprofloxacin, enrofloxacin, marbofloxacin and resistant to ampicillin, cephalothin, cefuroxime, doxycycline. On the basis of that result, marbofloxacin was continued for 60 days, and suspended after bacteriological cultures resulted negative.

The samples for mycological examination were stained with calcofluor white and examined with fluorescent microscope. Few single septate nonbranching hyphae bright greenish stained were noticed, so the presence of a fungal infections was supposed. Thus, samples were cultured onto Sabouraud agar chloramphenicol 0.05% and

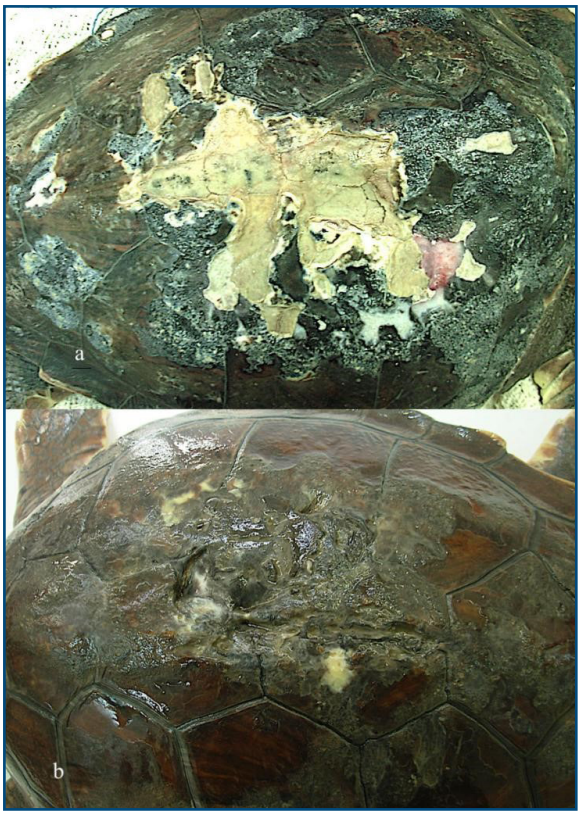

Figure 1. a. *Carapacial lesions of the loggerhead sea turtle at admission;* **b.** *at release (case 1).*

incubated at 25 °C in air for 14 days. Whitish gray cottony colonies suggestive of *Fusarium* spp. were isolated from all samples. Successive subcultures performed on potato dextrose agar in the dark showed sickle-shaped multiseptated macroconidia, and one- to two-celled microconidia formed from unbranched phialides, conidiophores, and chlamydospores typical of *Fusarium solani* (De Hoog 1995) (Figure 2). This evidence, its reported *in vitro* and *in vivo* resistance to most of the available antifungal drugs, together with the lack of clinical outcome of the current therapy, suggested to perform susceptibility test on the isolate. Voriconazole, posaconazole, itraconazole, ketoconazole, fluconazole and amphotericin B were tested. Isolates of *F. solani* resulted resistant to all the antimycotic drugs tested, so itraconazole was discontinued, and only topical iodopovidone ointment was administered.

Tissue samples submitted to the Unit of Pathology for histology were fixed in 10% neutral buffered formalin for routinely process, embedded in paraffin, sectioned at 5 microns, stained with hematoxylin and eosin (HE) and examined by light microscopy. Histological analysis revealed necrosis and the presence of inflammatory cells (heterophils, monocytes, and lymphocytes) at the level of the basement membrane and in the dermis, together

with multifocal necrosis and severe haemorrhages, with presence of moderately basophilic hyphae; consequently an elective stain with stain periodic acid-Schiff (PAS) was decided. PAS staining revealed hyphae morphologically similar in all samples, with thin, generally parallel walls and variably spaced septa, frequent acute and right angle branching septate hyaline hyphae (Figure 3), with optional sporulation, and occasional invasion of blood vessels

During the period of hospitalization, the conditions of the turtle worsened. After two months of autonomous feeding with an average daily assumption of 200 g anchovies, the turtle became progressively anorectic due to the progressive avulsion of upper and lower ramphoteca, so a permanent oesophagostomy tube was applied under general anaesthesia (Figure 4) (Di Bello *et al.* 2011). Prophylactic marbofloxacin (2 mg/kg PO, q 24 h, Marìn *et al.* 2009) was administered for as long as the tube was maintained, together with the food (2-3% of b.w. of homogenized fish supplemented with vitamins). The permanent oesophagostomy tube was well tolerated by the turtle, which began voluntary feeding in one month, after the complete avulsion of ramphoteca, which leaved the maxillary and mandibular bones exposed and partially eroded on the right side (Figure 5a). The tube was left in place for further 7 days, then it was removed after a light sedation with medetomidine (0.05 mg/kg, IM) reversed with atipamezole (0.25 mg/kg, IM) in order to apply stitches to close oesophagostomy. The recovery was uneventful.

Fungal testing, performed every month, resulted negative after 4 months since the admission, then all the lesions progressively healed, including the beak, which started to show signs of new growth of the corneal part. Complete beak repair took further 4 months (Figure 5b), and the carapace lesions repaired with scar tissue to cover the bone plates (Figure 1b). At that time, the turtle was back to 'Torre Guaceto' Marine Turtle Rescue Centre for summer release.

Case 2

A juvenile cold stunned loggerhead sea turtle (*Caretta caretta*) stranded in August on the coast of Adriatic Sea near to Jesolo (Venice, Italy), with an unusually low average sea temperature of 15 °C. The animal was referred to 'Torre Guaceto' Marine Turtle Rescue Centre, and immediately after moved to the Department of Veterinary Medicine of Bari for diagnostics and treatment because of extensive shell lesion (probably boat impact) limited to corneal scales.

At admission the turtle weighted 1.2 kg, and the straight carapace length resulted 25 cm. The

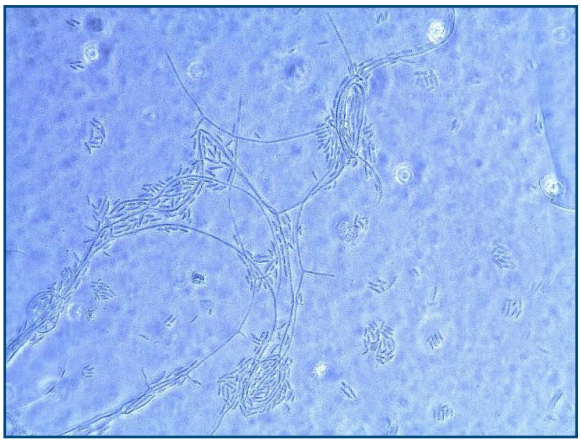

Figure 2. *Culture on potato dextrose agar showing sickle-shaped multiseptated macroconidia and one/two celled microconidia formed from unbranched phialides, conidiophore and chlamydospores.*

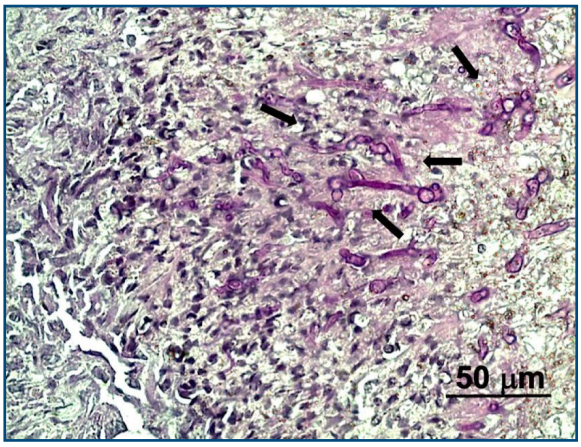

Figure 3. *Micrograph of necrotic tissue with* Fusarium solani *hyphae invasion (arrows).* Periodic Acid Schiff stain, 40X (case 1).

animal was lethargic and severely emaciated. The standard procedure for the treatment of cold stunning was started (see above). Samples of the lesions were obtained for microbial and mycological culturing, and histologic examination (see above), together with blood samples for haematological and biochemical exams (Tables I and II). Standard prophylactic antimicrobial and antimycotic treatment was started (see above).

Once recovered from hypothermia, the young turtles started feeding voluntarily, and never stopped.

Prevotella rettseri, Serratia marcescens, Pseudomonas aeruginosa, Proteus spp. were isolated from lesions and tested for susceptibility (see above); all of them resulted susceptible to fluoroquinolones. On the basis of that result, marbofloxacin was continued for 50 days, then suspended after bacteriological cultures resulted negative.

In about a month the lesion extended as area and depth until it involved the underlying bone planes,

Figure 4. *Permanent oesophagostomy tube in place (case 1).*

which remained uncovered after the corneal scales detached (Figure 6). Mycological culturing isolated *Fusarium solani* from all samples. Also, in this case susceptibility test on the isolate was performed, and *F. solani* resulted resistant to all the antimycotic drugs tested, so itraconazole was discontinued, and only topical iodopovidone ointment was administered.

Histological analysis revealed necrosis and a general situation similar to case 1, as in both subjects the colonization interested corneal structures.

Fungal testing, performed every month, resulted negative after 5 months since the admission, and the lesion gradually repaired with scar tissue.

At that time, the turtle was back to 'Torre Guaceto' Marine Turtle Rescue Centre for summer release.

Case 3

A juvenile cold stunned loggerhead sea turtle (*Caretta caretta*) stranded in March on the coast of Adriatic Sea near to Lecce (Italy), with average sea

Table I. *Haematological results.*

	Turtle 1	Turtle 2	Turtle 3	Reference values*		
				Median	Min	Max
HCT (%):[17]	18.00	24.00	23.00	28	17	45
		RBC				
RBC (x10^6/µL):[17]	2	2.66	2.54	1.87	0.3	6
		WBC				
WBC (x10^3/µL):[17]	3.20	4.30	5.80	5.9	2	18.9
Heterophils (%):[18]	64	73	79	75.8	51.61	88.6
Lymphocytes (%):[18]	30	22	16	18.41	4.4	30.92
Monocytes (%):[18]	0	1	1	1.2	0	5.3
Eosinophils (%):[18]	6	4	4	4.5	0	29.4
Basophils (%):[18]	0	0	0	0	0	0
		PLT				
Estimated	adequate	adequate	adequate			

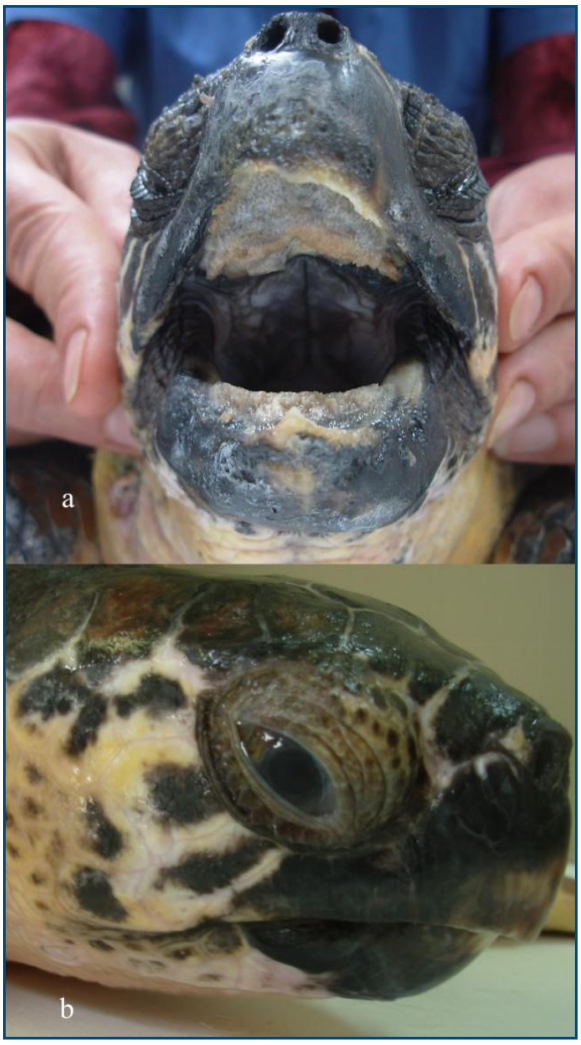

Figure 5. a. *Eroded maxillary and mandibulary bones after complete avulsion of upper and lower ramphoteca;* **b.** *complete repair of beak 4 months after ramphoteca avulsion (case 1).*

Table II. *Plasma chemistry results.*

	Turtle 1	Turtle 2	Turtle 3	Reference values*		
				Median	Min	Max
AST (IU/L):[17]	460	653	389	194	< 10	844
ALT (IU/L):[17]	14	35	18	24	< 10	258
ALP (IU/L):[17]	45	71	64	67	51	562
CPK(IU/L):[20]	188	538	874	534	3	1,899
Total bilirubin (mg/dL):[17]	0.21	0.32	0.01	0.2	0.2	0.5
Glucose (mg/dL):[17]	79	98	77	129	19.8	291.9
Total cholesterol (mg/dL):[17]	63	73	52	139	50.2	397.7
Uric acid (mg/dL):[20]	0.8	0.9	1.9	2.4	0.7	4.2
Creatinin (mg/dL):[17]	0.32	0.45	0.22	0.4	0.3	0.8
Total protein (g/dL):[17]	2.60	4.3	1.9	2.4	2	11
Albumin (g/dL):[17]	1.2	1.3	0.8	1.1	1	1.4
Calcium (mg/dL):[17]	6.8	7.2	8.1	8	2.8	12.4
Phosforum (mg/dL):[17]	4.6	4.5	5.1	6.4	4.1	7.9
Ionized Na (mEq/L):[20]	146	151	134	156	135	175
Ionized K (mEq/L):[20]	3.7	5.8	3.2	5.1	3.3	13.9
Ionized Cl (mEq/L):[20]	112	125	109	130	107	158

Figure 6. *Shell damages after corneal scales detachment (case 2).*

temperature of 11 °C. The animal was referred to 'Torre Guaceto' Marine Turtle Rescue Centre, and immediately moved to the Department of Veterinary Medicine of Bari for diagnostics and treatment because of severe signs ascribable to cold stunning.

At admission the turtle weighted 8.7 kg, and the straight carapace length resulted 45 cm. The animal was lethargic and emaciated. The standard procedure for the treatment of cold stunning and prophylactic antimicrobial and antimycotic treatment were started (see above). The severe emaciation was probably due to the extensive injury to the rostrum and the nasal cavities that the animal presented to admission, with complete absence of soft and cartilaginous tissues of the nose and of the rearward conchae (Figure 7). In addition to the usual hematologic, bacteriological and mycological procedures, X-ray and computed tomographic examination of the head were performed.

X-ray exam revealed extensive loss of bone tissues in the nasal cavity with absence of the nasal conchae. For the CT exam, images were acquired with the turtle placed in ventral recumbency under general anaesthesia (propofol 2 mg/kg IV; Mader 2019). Contiguous transverse slices were obtained from the basisphenoid to the external nares, showing severe osteolysis of the prefrontal bone, destruction of the cartilage of nasal septum, erosive changes of the dorsal and ventral conchae, and soft tissue opacification of the right dorsal nasal concha sinus and left ventral concha sinus.

The turtle rejected various proposed foods (anchovies, squid, cuttlefish, mussels) for several days, probably because of complete loss of the olfactory capacity, so the esophagogastric tube was applied. Prophylactic marbofloxacin (2 mg/kg PO, q 24 h; Marìn *et al.* 2009) was administrated.

Samples of the lesions were obtained for microbial and mycological culturing. Some of the bioptic samples were sent to the Pathology Unit of Department of Veterinary Medicine of Bari for histologic examination.

Aeromonas hydrofila was isolated from lesions and tested for susceptibility (see above), resulted susceptible to fluoroquinolones. On the basis of that result, marbofloxacin PO was continued for 40 days, and then suspended after bacteriological cultures resulted negative.

Mycological culturing isolated *Fusarium solani* from all samples. In this case too susceptibility test on the isolate was performed, and *F. solani* resulted resistant to all the antimycotic drugs tested, so itraconazole was discontinued, and only topical iodopovidone ointment was administered.

In case 3 the lesions were very advanced, with extensive destruction of soft and bone tissues, replaced by repair tissue colonized in depth by the fungal hyphae and with extensive microvasculitis caused by the invasion of the microcirculation. The extensive injuries did not recover, so the proposal of live food (crustaceans and fish) was attempted before considering euthanasia. The subject responded positively, by actively preying on living preys, so the tube was removed, and the turtle was back to 'Torre Guaceto' Marine Turtle Rescue Centre for summer release.

Discussion

Dermatomycoses occur regularly in reptiles and are largely underdiagnosed, as lesions are indistinguishable from those caused by bacterial infections at a gross examination, so they are often misdiagnosed as such. Mixed fungal and bacterial infections are also common, and it may be difficult to establish which of the two is the primary offender, so both microbiological and mycological diagnostics have always to be recommended (Paré and Jacobson 2007).

Figure 7. *Extensive injury to the rostrum and the nasal cavities (case 3).*

Mycoses seem particularly prevalent in sea turtles (Mader 2019, Manire *et al.* 2003, Paré and Jacobson 2007, Cabañes *et al.* 1997, Duguy *et al.* 1998), most commonly related to immunosuppressed status, traumatic lesions, captivity, and cold-stunning.

In particular, some authors (Cafarchia *et al.* 2019) have postulated that from an epidemiological point of view the origin of this opportunistic infection has to be considered related to the presence of *F. solani* in rescue center tanks and to immunosuppression due to the traumatic lesions suffered, surgical treatment applied, and other stressful conditions associated with transportation or rehabilitation of these marine turtles. The finding that animals admitted to the center for more than 20 days were more frequently colonized also suggested an association between *Fusarium*-like organisms and the skin lesions that occurred, given the presence of *F. solani* in the tank at the rescue centers, a recognized risk factor of infection in receptive adult turtles. This finding suggested that the environmental conditions and management at the rescue center might favor *Fusarium* spp. growth and might be the source of colonization. This latter hypothesis was supported by the fact that sand from the filter of the two cited centers was positive for *Fusarium* spp.

On the contrary, in the present cases the lesions were already present and invaded by the *Fusarium* spp. before admission to the center. In particular, analysis of the sands of the center's filtering plant (equipped with a skimmer and UV passage for the sterilization of the filtered water before returning to the tanks) did not reveal any contaminating pathogenic organisms, so the hypothesis posed by the cited authors cannot be accepted and should not be generalized.

Otherwise, sudden drops in seawater temperature often result in hypothermia in juveniles or sub-adult sea turtles. These cold-stunned turtles typically become weak, float, and strand, with different possible types of wounds and lesions, and their rehabilitation can become long and difficult because of multiple metabolic disorders and opportunistic infections due to immunosuppression. Severe mycotic infections can be common sequelae in these animals, and antifungal drugs are part of the standard pharmacological treatment of this syndrome (Mader 2019, Manire *et al.* 2003, Paré and Jacobson 2007). Fungi as *Fusarium* spp. in particular are often implicated, but other species of scarce or little-known pathogenicity, such as *Colletotrichum acutatum*, have caused disseminated mycoses in cold-stunned turtles (Manire *et al.* 2003), indicating how severely immunocompromised these animals may become. Nonetheless, fungal infections have also been reported in wild stranded sea turtles in Florida unrelated to cold stunning events (Paré and Jacobson 2007).

In the reported cases, no particular immunosuppressed status has been noticed in hematologic exams (Tables 1 and 2). In fact, all the parameters were within the normal ranges, if compared with bibliographic data on juvenile loggerhead sea turtles (Casal *et al.* 2007, Casal *et al.* 2009, Gelli *et al.* 2009) and comparable with data reported for stranded loggerheads (Deem *et al.* 2009). No particularly compromised scenery was depicted, so the severity of the *Fusarium solani* infection has to be ascribed to other potential factors that may facilitate fungal invasion: concomitant viral or bacterial infections, bacteria-infected traumatic lesions, large areas of devitalized skin, nutritional nitrogen imbalance and stressors associated with stranding (Frasca *et al.* 1996, Lass-Flörl 2010).

Unlike most fungi species, in which conidiation is stimulated by emergence of the fungus through the water-air interface, the conidiation of *Fusarium* spp. typically occurs both in submersion and upon exposure to air. This explains the rapid seeding of these infections to numerous capillary beds, especially in the skin, a process that gives rise to the widespread ecthyma gangrenosum-like lesions that characterize disseminated fusarial infections (Summerbell 2002). Angioinvasion, with vascular thrombosis and tissue infarction, explains the evolution of lesions to necrotic and bone-involving levels. Therefore, *Fusarium* spp. infection has not to be undervalued as it can generate extensive and ravaging lesions (Salter *et al.* 2012).

Due to the critical role of immune response in the outcome of fusariosis, the optimal treatment strategy for patients with severe *Fusarium* spp. infection remains unclear. Reversal of immunosuppression is recommended whenever possible. Early therapy of localized lesions (including surgical debridement) is important to prevent progression to a more aggressive or disseminated infection (Tortorano *et al.* 2014).

The definitive diagnosis requires isolation of *Fusarium* spp. from infected sites (culture, histology, molecular probes). The repeatedly positive cultures of biopsies from the lesions for *Fusarium solani*, along with evidence of tissue invasion with mycelial elements having the configuration of *Fusarium* spp., indicate that this fungus was acting as a pathogen in the three turtles.

In invasive infection *Fusarium solani* can also be isolated from blood cultures in up to 40-60% of human cases (Tortorano *et al.* 2014), but in the present cases blood was not used to attempt isolation.

Culture identification is important because of the histopathological similarities between *Fusarium* spp. and other hyalohyphomycetes. Although the genus *Fusarium* can be identified by culture by the

production of hyaline, crescent or banana-shaped multicellular macroconidia, species identification is difficult and may require molecular methods, although they should be used only to supplement conventional laboratory tests (Tortorano *et al.* 2014).

Since the distribution of *Fusarium* species varies with geographic region 51 and different species have different drug susceptibility patterns (Nucci and Anaissie 2007) accurate species assignment is important for epidemiological studies and to guide clinical management. Even though there is no correlation between *F. solani* species complex and antifungal susceptibility, *F. solani* and *F. verticillioides* are usually resistant to azoles and exhibit higher amphotericin B minimal inhibitory concentration (MICs) than other *Fusarium* spp. (Nucci and Anaissie 2007, Wang *et al.* 2011). In contrast, *F. oxysporum* may be susceptible to voriconazole and posaconazole (Nucci and Anaissie 2007, Tanaka *et al.* 2012).

Finally, the receptivity of man to fusariosis must not be underestimated, as *Fusarium* spp. can act as zoonotic agent for the operators or the visitors of sea turtle rescue centres. Opportunistic *Fusarium* species cause a broad spectrum of infections predominantly in immunocompromised individuals via direct inoculation and airborne uptake as the most common routes of infections. The clinical signs of fusariosis depend largely on the immune status of the host and the portal of entry, which include paranasal sinuses, lungs and skin, with neutropenia as one of the most important risk factors for acquiring disseminated disease (Tortorano *et al.* 2014); therefore the operators of the centres must be informed of the risk and equipped accordingly for the management of the infected subjects, while the visitors must not be admitted to the tanks of subjects with active fusariosis.

References

Al-Hatmi A.M.S., Bonifaz A., Ranque S., Sybren de Hoog G., Verweij P.E. & Meis J.F. 2018. Current antifungal treatment of fusariosis. *Int J Antimicrob Agents*, **51** (3), 326-332.

Alastruey-Izquierdo A., Cuenca-Estrella M., Monzón A., Cuenca-Estrella Mellado E. & Rodríguez-Tudela J.L. 2008. Antifungal susceptibility profile of clinical *Fusarium* spp. isolates identified by molecular methods. *J Antimicrob Chem*, **61**, 805-809.

Austwick P.K.C. & Keymer I.F. 1981. Fungi and actinomycetes. *In* Diseases of the Reptilia, Vol 1. (J.E. Cooper and O.F. Jackson, eds). Academic Press New York, 193-231.

Betiana N., Piña C.I., Bucsinsky A.M. & Arambarri A.M. 2014. Fungal diversity on broad-snouted caiman (*Caiman latirostris*) eggs, and their effects on hatchlings. *Herp J*, **24**, 217-222.

Bian B.Z. & Egusa S. 1981. Histopathology of black gill disease caused by *Fusarium solani* (Martius) infection in the Kuruma prawn, *Penaeus japonicus* Bate. *J Fish Dis*, **4**, 195-201.

Brown D.W. & Proctor R.H. 2013. *Fusarium*. Genomics, molecular and cellular biology. Calster Academic Press, Norfolk, UK.

Cabañes F.J., Alonso J.M., Castellá G., Alegre F., Domingo M. & Pontet S. 1997. *Cutaneous hyalohyphomycoses* caused by *Fusarium solani* in a loggerhead sea turtle. *J Clin Micobiol*, **35**, 3343-3345.

Cafarchia C., Paradies R., Figueredo L., Iatta R , Desantis S., Di Bello A.V.F., Zizzo N. & Diepeningen A.D. 2020. *Fusarium* spp. in loggerhead sea turtles (*Caretta caretta*): from colonization to infection. *Vet Pathol*, **57** (1) 139-146.

Casal A.B. & Orós J. 2007. Morphologic and cytochemical characteristics of blood cells of juvenile loggerhead sea turtles (*Caretta caretta*). *Res Vet Sci*, **82**, 158-165.

Casal A.B., Camacho M., Lopez-Jurado L.F., Juste C. & Oros H. 2009. Comparative study of hematologic and plasma biochemical variables in Eastern Atlantic juvenile and adult nesting loggerhead sea turtles (*Caretta caretta*). *Vet Clin Pathol*, **38**, 213-218.

Deem S.L., Norton T.M., Mitchell M., Segars A., Alleman A.R., Cray C., Poppenga R.H., Dodd M. & Karesh W.B. 2009. Comparison of blood values in foraging, nesting, and stranded loggerhead turtles (*Caretta caretta*) along the coast of Georgia, USA. *J Wildl Dis*, **45** (1), 41-56.

De Hoog G.S. & Guarr J. 1995. Atlas of Clinical Fungi. Utrecht: Centraalbureau voor Schimmelcultures.

Di Bello A.V.F., Valastro C., Freggi D., Lai O.R., Soloperto S. & Crescenzo G. 2011. The use of oesophagostomy tube for the force-feeding in sea turtles. Proc. 31st Annual Symposium on Sea Turtle Biology and Conservation, San Diego, 12-15 April 2011. NOAA Technical Memorandum NMFS-SEFSC-631, 187.

Duguy R., Moriniere P. & Le Milinaire C. 1998. Facteurs de mortalité observes chez le tortues marines dans le golfe de Gascogne. *Ocean Act*, **21**, 383-388.

Frasca S., Dunn J.L., Cooke J.C. & Buck J.D. 1996. Mycotic dermatitis in an Atlantic white-sided dolphin, a pygmy sperm whale, and two harbor seals. *J Am Vet Med Assoc*, **208**, 727-729.

Gelli D., Ferrari V., Zanella A., Arena P., Pozzi L., Nannarelli S., Vaccaro C., Bernardini D. & Romagnoli S. 2009. Establishing physiological blood parameters in the loggerhead sea turtle (*Caretta caretta*). *Eur J Wildl Res*, **55**, 59-63.

Johnson E.M. 2008. Issues in antifungal susceptibility testing. *J Antimicrob Chemother*, **61** (1), i13-i18.

Khoa L.V., Hatai K. & Aoki T. 2004. *Fusarium incarnatum* isolated from black tiger shrimp, *Penaeus monodon* Fabricius, with black gill disease cultured in Vietnam. *J Fish Dis*, 27, 507-515.

Knotek Z. & Divers S.J. 2019. Pulmonology. *In* Reptile and amphibian medicine and surgery. 3rd ed. (D.R. Mader, eds). Saunders Elsevier, St. Louis, 786-804.

Lai O.R., Marín P., Laricchiuta P., Marzano G., Crescenzo G. & Escudero E. 2009. Pharmacokinetics of marbofloxacin in loggerhead sea turtles (*Caretta caretta*) after single intravenous and intramuscular doses. *J Zoo Wildl Med*, **40** (3), 501-7.

Lass-Flörl C., Perkhofer S. & Mayr A. 2010. *In vitro* susceptibility testing in fungi: a global perspective on a variety of methods. *Mycoses*, **53**, 1-11.

Leu H.S., Lee A.Y.S. & Kuo T.T. 1995. Recurrence of *Fusarium solani* abscess formation in an otherwise healthy patient. *Infection*, **23**, 303-305.

Mader D.R. 2019. Reptile and amphibian medicine and surgery. 3rd ed. St. Louis, Saunders Elsevier.

Manire C.A., Rhinehart H.L., Pennick G.J., Sutton D.A., Hunter R.P. & Rinaldi M.G. 2003. Steady-state plasma concentration of itraconazole after oral administration in Kemp's ridley sea turtles, *Lepidochelys kempi. J Zoo Wildl Med*, **34**, 171-178.

Marín P., Lai O.R., Laricchiuta P., Marzano G., Di Bello A., Cárceles C.M. & Crescenzo G. 2009. Pharmacokinetics of marbofloxacin after a single oral dose to loggerhead sea turtles (*Caretta caretta*). *Res Vet Sci*, **87** (2), 284-6.

McArthur S. 2004. Feeding techniques and fluids. *In* Medicine and surgery of tortoises and turtles. (McArthur S., Wilkinson R., Meyer J., eds) Blackwell Publishing Ltd, Oxford, 257-264.

Montali R.J., Bush M., Strandberg J.D, Janssen D.L., Boness D.J. & Whitla J.C. 1981. Cyclic dermatitis associated with *Fusarium* sp infection in pinnipeds. *J Am Vet Med Assoc*, **179** (11), 1198-1202.

Noga E.J. 2010. Fish disease: diagnosis and treatment, 2nd ed. Wiley-Blackwell, Ames, IA.

Norton T.M. & Walsh M.T. 2012. Sea turtle rehabilitation. *In* Fowler's zoo and wild animal medicine, 7th ed. (Miller R.E. and Fowler M., eds). Saunders Elsevier, St. Louis, 239-246.

Nucci M. & Anaissie E. 2007. *Fusarium* infections in immunocompromised patients. *Clin Microbiol Rev*, **20** (4), 695-704.

Nuñez Otaño N.B., Piña C.I., Bucsinsky A.M. & Arambarri A.M. 2014. Fungal diversity on broad-snouted caiman (*Caiman latirostris*) eggs, and their effects on hatchlings. *Herpetological Journal*, **24**, 217-222.

Orós J., Delgado C., Fernández L. & Jensen H.E. 2004. Pulmonary hyalohyphomycosis caused by *Fusarium* spp. in a Kemp's ridley sea turtle (*Lepidochelys kempi*): an immunohistochemical study. *N Z Vet J*, **52**, 150-152.

Paré J.A. & Jacobson E.R. 2007. Mycotic diseases of reptiles. *In* Infectious diseases and pathology of reptiles. (Jacobson E.R., ed.). Boca Raton CRC Press Taylor & Francis Group, 527-543.

Perkhofer S., Mrazek C., Hartl L. & Lass-Flörl C. 2010. *In vitro* susceptibility testing in fungi: what is its role in clinical practice? *Curr Infect Dis Rep*, **12**, 401-408.

Salighehzadeh R., Sharifiyazdi H., Akhlaghi M., Khalafian M., Gholamhosseini A. & Soltanian S. 2019. Molecular and clinical evidence of *Aeromonas hydrophila* and *Fusarium solani* coinfection in narrow-clawed crayfish *Astacus leptodactylus*. *Dis Aquat Organ*, **132** (2), 135-141.

Salter C.E., O'Donnell K., Sutton D.A., Marancik D.P., Knowles S., Clauss T.M., Berliner A.L. & Camus A.C. 2012. Dermatitis and systemic mycosis in lined seahorses *Hippocampus erectus* associated with a marine-adapted *Fusarium solani* species complex pathogen. *Dis Aquat Organ*, **101** (1), 23-31.

Sarmiento-Ramírez J.M., Abella-Pérez E., Phillott A.D., Sim J., van West P., Martín M.P., Marco A. & Diéguez-Uribeondo J. 2014a. Global distribution of two fungal pathogens threatening endangered sea turtles. *PLOS ONE*, **9** (1), e85853.

Sarmiento-Ramìrez J.M., van der Voort M., Raaijmakers J.M. & Diéguez-Uribeondo J. 2014b. Unravelling the microbiome of eggs of the endangered sea turtle *Eretmochelys imbricate* identifies bacteria with activity against the emerging pathogen *Fusarium falciforme*. *PLoS One*, **9** (4), e95206.

Sharma C. & Anuradha C. 2017. Molecular bases of antifungal resistance in filamentous fungi. *Int J Antimicrob Agents*, **50** (5), 607-616.

Summerbell R. 2002. *Ascomycetes*. *In* Pathogenic fungi in humans and animals. 2nd ed. (Howard D.H., ed.). New York, Marcel Dekker, Inc., 237-488.

Tanaka M., Izawa T., Kuwamura M., Nakao T. Maezono Y., Ito S., Murata M., Murakami M., Sano A. & Yamate J. 2012. Deep granulomatous dermatitis of the fin caused by *Fusarium solani* in a false killer whale (*Pseudorca crassidens*). *J Vet Med Sci*, **74** (6),779-782.

Tortorano A.M., Richardson M., Roilides E., van Diepeningen A., Caira M., Munoz P., Johnson E., Meletiadis J., Pana Z.D., Lackner M., Verweij P., Freiberger T., Cornely O.A., Arikan-Akdagli S., Dannaoui E., Groll A.H., Lagrou K., Chakrabarti A., Lanternier F., Pagano L., Skiada A., Akova M., Arendrup M.C., Boekhout T., Chowdhary A., Cuenca-Estrella M., Guinea J., Guarro J., de Hoog S., Hope W., Kathuria S., Lortholary O., Meis J.F., Ullmann A.J., Petrikkos G. & Lass-Flörl C. 2014. European Society of Clinical Microbiology and Infectious Diseases Fungal Infection Study Group; European Confederation of Medical Mycology. ESCMID and ECMM joint guidelines on diagnosis and management of hyalohyphomycosis: *Fusarium* spp., *Scedosporium* spp. and others. *Clin Microbiol Infect*, **3**, 27-46.

Tuxbury K.A., Shaw G.C., Montali R.J., Clayton L.A., Kwiatkowski N.P., Dykstra M.J. & Mankowski J.L. 2014. *Fusarium solani* species complex associated with carapace lesions and branchitis in captive American horseshoe crabs *Limulus polyphemus*. *Dis Aquat Organ*, **109** (3), 223-230.

Wang H., Xiao M., Kong F., Chen S., Dou H., Sorrell T., Li R. & Xu Y. 2011. Accurate and practical identification of 20 *Fusarium* species by seven-locus sequence analysis and reverse line blot hybridization, and an *in vitro* antifungal susceptibility study. *J Clin Microbiol*, **49** (5), 1890-1898.

Wellehan F.F.X. & Divers S.J. 2019. Mycology. *In* Reptile and amphibian medicine and surgery. 3rd ed. (Mader D.R., ed). Saunders Elsevier, St. Louis, 270-280.

Yanong R.P.E. 2003. Fungal diseases of fish. *Vet Clin North Am Exot Anim Pract*, **6**, 377-400.