ORIGINAL ARTICLE

Veterinary Research Forum. 2021; 12 (3) 361 - 367
doi: 10.30466/vrf.2019.108417.2571
Journal Homepage: vrf.iranjournals.ir

Veterinary Research Forum

A study on the oral and cloacal bacterial flora of Mugger crocodiles (*Crocodylus palustris*) in the Negour protected area, Iran

Amin Gholamhosseini*, Hassan Sharifiyazdi, Mostafa Rakhshaninejad, Siyavash Soltanian, Reza Salighehzadeh*, Hesamodin Kordestani

Department of Clinical Sciences, School of Veterinary Medicine, Shiraz University, Shiraz, Iran.

Article Info

Article history:

Received: 23 May 2019
Accepted: 29 July 2019
Available online: 15 September 2021

Keywords:

Bacterial flora
Crocodylus palustris
Iran
Polymerase chain reaction

Abstract

Mugger crocodile is the only crocodile existing in Iran. The present study was aimed to investigate the bacterial flora in oral and cloacal cavities of wild Mugger crocodiles in Negour protected area, Iran. The isolation and molecular characterization of oral and cloacal bacterial flora were performed in 22 Mugger crocodiles captured in Negour protected area, Iran. Ten bacterial species from all oral samples and six bacterial species from all cloacal samples were recovered. The most commonly isolated bacteria in oral samples were *Burkholderia contaminans* and *Lactococcus garvieae*, respectively; whereas, in cloacal samples, it was *Lactococcus lactis*. It is likely that the isolated bacteria would pose a threat to both crocodiles and humans health. It can threaten crocodiles during stressful conditions; while, humans would be susceptible if they are bitten by crocodiles, consume their meat or spend time near their natural environment. This study provides useful information about bacterial diversity which could help to select the most appropriate anti-bacterial when dealing with infections caused by crocodiles.

Introduction

Crocodilians are notorious for having the strongest bite among all the known animals[1] and their bite can leave the victim seriously injured. The severity of the lesions left could range from simple punctures or tearing skin wounds to fractures, amputations and death.[2] The reports of the hostile encounter between humans and crocodiles have been documented in many countries though some regions of the world have reported fewer attacks than others.[3] The prospect is that the number of these conflicts between humans and crocodiles would see an upsurge for two main reasons including the increasing damage made to crocodiles habitat by humans and an increase in the number of crocodiles.[4]

Those who survive from attacks of crocodiles are prone to infectious wounds compounded by bacteria as well as physical injuries.[2] A number of studies have reported that the oral cavity of wild and captured crocodiles contains multiple bacteria and fungi.[5] Despite the fact that wound infections could be healed to some extent by several existing antibiotics, sometimes, the sepsis could prove to be fatal.[6] The resistance of crocodilians to illnesses and their capacity to recover from injuries seem to be great. However, some studies have reported the presence of symptoms in crocodilians indicative of infections caused by bacteria, which could be deadly if transmitted to humans.[7]

The south-eastern part of Iran, in Sistan and Baluchestan province, near the border with Pakistan, is the western most range for *Crocodylus palustris* and the only distribution area for this species in Iran. The main part of the distribution range is an area of 3,800 km^2 and, due to its importance as a crocodile habitat; it was designated as a Gandou Protected Area, with Gandou being the local name for the crocodiles.[8] Some parts of the area have been designated as Ramsar sites. The Mugger crocodile is listed as vulnerable in the International Union for Conservation

***Correspondence:**

Amin Gholamhosseini. DVM, PhD
Department of Clinical Sciences, School of Veterinary Medicine, Shiraz University, Shiraz, Iran
E-mail: Amingholamhosseini@shirazu.ac.ir
Reza Salighehzadeh. PhD
Department of Clinical Sciences, School of Veterinary Medicine, Shiraz University, Shiraz, Iran
E-mail: r.salighehzadeh@shirazu.ac.ir

of Nature red list of threatened species and the main threatening factors for this species are habitat destruction and fragmentation.[8]

The Mugger crocodile (*C. palustris* Lesson, 1831) is a medium-sized crocodile (maximum length up to 5.00 m) and has the widest muzzle among the classified members of the genus *Crocodylus*. It has been primarily seen in Indian subcontinent, particularly in India, Pakistan, Sri Lanka and Iran.[9] The species has adapted well to reservoirs, irrigation canals, man-made ponds and different types of water environments including rivers, lakes and swamps. It is a hole-nesting species and like other hole-nesters, it lays egg during the dry seasons of the year. Sexual maturity for females happens when they approximately reach a length of 1.80-2.00 m. By this time, they could lay 25-30 eggs.[9]

Despite the increase in the number of studies devoted to crocodiles over the past decade across the world, none have examined the effect of bacterial flora on the crocodiles of the region mentioned above, *i.e.*, Negour. The present study was aimed to investigate the bacterial flora in oral and cloacal cavities of wild Mugger crocodiles in Negour protected area. It is hoped that the results of the study would prove to be helpful in assessing the health risks that humans are subjected to upon their contact with crocodiles.

Materials and Methods

Animals and study area. This study was conducted on the Mugger crocodile (*C. palustris*) captured from two places inside Negour protected area (25°53'40''N-61°29'60''E and 25°50'12''N-61°30'48''E), in south-east of Iran near Chabahar county in Sistan and Baluchestan province, which has been recognized as the most suitable habitat for Mugger crocodiles in Iran.[10] This reserve has been relatively well-conserved for populations of Mugger crocodile with individuals of various sizes and with a male-dominated ratio. Also, interviews were carried out with local people and with the Department of Environment Guards at the same time.

Sample collection. Depending on the sizes of the crocodiles, they were captured either by hand or by a technique called break-away snare in November 2017 in Negour protected area. The captured crocodiles were then examined for the detection of any potential signs of disease and their sex using cloacal examination to determine the latter. To collect the samples, a sterile culture swab was passed through each crocodile oral cavity and cloaca. Then, swabs were cultured in tryptic soy broth (Merck, Darmstadt, Germany) transport mediums, which are ideal for aerobic and anaerobic bacteria and then sent for further analyses to the Aquatic Animal Health and Diseases Laboratory, School of Veterinary Medicine, Shiraz University, Shiraz, Iran. This experiment was performed in accordance with the Iranian animal ethics framework under the supervision of the Iranian Society for the Prevention of Cruelty to Animals and Shiraz University Research Council (IACUC No. 4687/63).

Bacterial analyses. Bacterial analyses were carried out using various bacterial growth media including brain heart infusion (Merck) agar, MacConkey agar (Merck) and de Man, Rogosa and Sharpe agar (MRS; Merck). In order to inoculate the whole plates, the quadrant streak method was utilized.[11] The plates were then incubated at 37.00 ˚C for 24 hr. The incubation of plates of MRS agar took place in a carbon dioxide atmosphere of 5.00%; while, the BHI and MacConkey plates were incubated in aerobic conditions.[12] Twenty-four hr after the incubation, a second inoculation was performed if the purification of the strains seemed necessary. For the next 24 hr, the same procedures, as described for the first inoculations, were followed for the plates.

DNA preparation and polymerase chain reaction (PCR) assay. The boiling method for the extraction of genomic DNAs of bacterial isolates was used.[13] Identification of all isolates was performed based on direct 16S rDNA sequencing using universal primers. The 16S rDNA gene was amplified using conventional PCR with universal primers fD1 and rP2 (Table 1), producing an amplicon of approximately 1,500 bp.[14] Each PCR reaction mix (50.00 µL total) included 4.00 µL of DNA sample (40.00 ng), 2.00 µL of each primer (20.00 pmol), 25.00 µL of 2X Master Mix PCR mixture (Ampliqon, Odense, Denmark) and 17.00 µL of distilled water. The DNA amplification was performed in a thermal cycler (MJ Mini; BioRad, Hercules, USA) in compliance with the conditions as follows: A 5 min initial denaturation at 95.00 ˚C, followed by 37 cycles of denaturation at 94.00 ˚C for 1 min, annealing at 59.00 ˚C for 1 min, extension at 72.00 ˚C for 60 sec and final extension at 72.00 ˚C for 5 min. After the PCR, 5.00 µL of PCR products were subjected to electrophoresis in 1.50% (w/v) agarose gel prepared with 1X Tris-acetate-ethylenediaminetetraacetic acid buffer (Dena Zist Asia, Mashhad, Iran) and run at 100 V for 60 min. The PCR product bands were then stained with RedSafe® (iNtRON Biotechnology, Gyeonggi-do, South Korea) and visualized by an ultraviolet transilluminator (SR1X3; Nasl Omid Pajohesh, Tehran, Iran). For the determination of the sizes of PCR products, they were compared with the migration of a 100 bp molecular weight ladder (K-Plus DNA Ladder, Delhi, India).

16S rDNA gene sequencing for bacterial identification. The PCR products of 16S rDNA of the 16 isolates were sequenced directly. A 3730 DNA Analyzer was used to sequence the PCR products (Applied Bio-systems, Foster City, USA). In order to create a constant sequencing of the inserted DNA, forward and reverse data were applied. Previously accessible sequences in National Center for Biotechnology Information (NCBI), made

Table 1. Characteristics of primers used for diagnosis of bacteria based on 16S rDNA molecular marker.

Primer	Primer sequences 5'-3'	Target gene	Amplified product size
rP2	ACGGCTACCTTGTTACGACTT	16S rDNA	1500 bp
fD1	AGAGTTTGATCCTGGCTCAG	16S rDNA	1500 bp

available through Basic Local Alignment Search Tool (BLAST), were utilized for a deeper comparison of the continuous sequences. Multiple-sequence alignment analysis was performed using the MEGA software (version 6.0; Biodesign Institute, Tempe, USA) via FASTA algorithms. The accession numbers of bacteria registered in the Genbank are presented in Table 2.

Results

Twenty-two Mugger crocodiles were captured (six sub-adults and 16 adults). Sixteen Mugger crocodiles were males (four sub-adults and 12 adults) and six were females (three sub-adults and three adults). Oral samples were obtained from 21 Mugger crocodiles; while, cloacal samples were obtained from all captured Mugger crocodiles.

In the PCR assay, DNA samples extracted from bacterial samples gave the expected fragment size of 1,500 for the 16S rDNA (Fig. 1). The partial fragments of amplified 16S rDNA from 16 representative bacteria were successfully sequenced. Subsequently, following BLAST-N analysis, the bacterial species were identified based on the 16S rDNA sequence similarity. From the Mugger crocodiles, 16 bacterial isolates were isolated, of which four (*Lactococcus lactis*) were identified in both oral and cloacal cavities, 10 (*Burkholderia cepacia*, *Burkholderia contaminans*, *Citrobacter sedlakii*, *Enterococcus durans*, *Escherichia coli*, *Lactococcus garvieae*, *L. lactis* and *Weissella paramesenteroides*) were identified only in oral cavity and six (*Burkholderia cenocepacia*, *L. lactis*, *Leuconostoc mesenteroides* and *Leuconostoc pseudo-mesenteroides*) were identified only in the cloacal cavity.

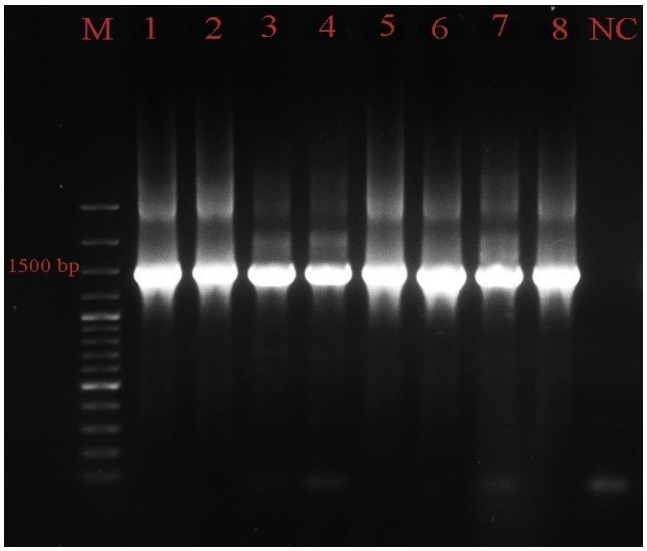

Fig. 1. Electrophoretic analysis (1.50% agarose gel) of PCR-amplified 16S rDNA fragments from several bacterial isolates. M: Gene ladder (100 bp); Lanes 1 to 8: Positive samples; NC: Negative control.

Ten species of bacteria were isolated from oral samples of Mugger crocodile, of which 10 were determined to the species level. Of these bacteria, five were Gram-positive and five were Gram-negative. From oral samples, *B. contaminans* and *L. garvieae* had the greatest recurrence of isolates (Table 2). Accession numbers MH295792 to MH295796 and MH295803 to MH295807 were assigned for the bacterial characterization of *L. lactis*, *E. durans*, *B. contaminans*, *B. cepacia* and *W. paramesenteroides* and *L. garvieae*, *L. garvieae*, *B. contaminans*, *E. coli* and *C. sedlakii*, respectively (Table 2).

Table 2. Species of bacteria identified in the oral and cloacal cavities of wild Mugger crocodile (*Crocodylus palustris*).

Isolate source	The closest species based on 16S rDNA	Similarity (%)	Gram/Type	Accession number
Oral cavity	*Burkholderia cepacia*	99.00	-/AN	MH295795
Oral cavity	*Burkholderia contaminans*	98.00	-/AN	MH295794 MH295805
Oral cavity	*Citrobacter sedlakii*	99.00	-/A	MH295807
Oral cavity	*Enterococcus durans*	98.00-99.00	+/AN	MH295793
Oral cavity	*Escherichia coli*	98.00-99.00	-/A	MH295806
Oral cavity	*Lactococcus garvieae*	99.00	+/A	MH295803 MH295804
Oral cavity	*Lactococcus lactis*	99.00	+/AN	MH295792
Oral cavity	*Weissella paramesenteroides*	99.00	+/AN	MH295796
Cloacal cavity	*Burkholderia cenocepacia*	99.00	-/AN	MH295798
Cloacal cavity	*Lactococcus lactis*	96.00-99.00	+/AN	MH295797 MH295801 MH295802
Cloacal cavity	*Leuconostoc mesenteroides*	99.00	+/AN	MH295799
Cloacal cavity	*Leuconostoc pseudomesenteroides*	99.00	+/AN	MH295800

A: Aerobic; AN: Anaerobic.

Six species of bacteria were found from cloacal samples of Mugger crocodiles, of which 6 were identified to the species level. Five of these bacteria were Gram-positive and 1 was Gram-negative. The bacterium found most frequently in cloacal samples was *L. lactis* (Table 2). Accession numbers MH295797 to MH295802 were assigned for the bacterial characterization of *L. lactis, B. cenocepacia, L. mesenteroides, L. pseudomesenteroides, L. lactis* and *L. lactis,* respectively (Table 2).

Discussion

Few studies have attempted to investigate the presence of bacterial flora in cloacal and oral cavities of wild crocodilians. To the best of the authors' knowledge, only 6 crocodilian species have been monitored in their natural habitat including *Crocodylus acutus*,[12] *C. moreletii*,[12] *Alligator mississippiensis*,[15] *C. niloticus*,[16] *C. johnstoni*,[16] and *C. porosus.*[17] As far as we know, the present study is the first attempt to investigate the presence of bacterial flora in the oral and cloacal cavities of *C. palustris* living in the wild environment.

The genera *Aerococcus, Aeromonas, Arcanobacterium, Citrobacter, Corynebacterium, Enterococcus, Escherichia, Fusobacterium, Klebsiella, Kluyvera, Listeria, Moraxella, Obesumbacterium, Pantoea, Pasteurella, Proteus, Pseudomonas, Rhodococcus, Salmonella, Serratia, Shigella, Staphylococcus* and *Streptococcus* have already been found to be present in the oral cavity of crocodilians.[5,12,17]

As far as we know, our study examined for the first time the existence of the genera *Lactococcus, Burkholderia* and *Weissella* in the oral cavity of crocodiles in the wild environment. Moreover, it is the first of its kind to record the presence of the following species in the oral cavity of crocodilians: *C. sedlakii, B. cepacia, B. contaminans, E. coli, L. lactis, L. garvieae,* and *W. paramesenteroides*.

The following bacteria have already been found in the cloaca of crocodilians: genera *Aeromonas, Alcaligenes, Citrobacter, Edwarsiella, Enterobacter, Enterococcus, Escherichia, Hafnia, Klebsiella, Pantoea, Proteus, Rhodococcus, Salmonella, Serratia,* and *Yersinia*.[12,15-18] However, *B. cenocepacia, L. lactis, L. mesenteroides* and *L. pseudomesenteroides* appear to be new species identified for the cloacal flora of crocodilians.

Crocodilians have been reported to be highly immune to disease with a considerable capacity for recovery, mainly due to their improved level of serum anti-microbial activity.[19] Huchzermeyer has made clear that crocodiles are vulnerable to few bacteria that could infect them with specific diseases and even fewer were specific to crocodiles.[20] However, there is a great number of bacteria which could cause non-specific blood-poisoning. Considering that all crocodiles selected and examined for the present study were evidently healthy, we supposed that all bacteria species identified in this research were part of the normal flora of *C. palustris*. That said, a number of the isolated bacteria could be regarded as the pathogens of crocodilians when stress may have an adverse effect on the host, particularly when held captive.[20] *Aeromonas hydrophila, Arcanobacterium pyogenes, Citrobacter freundii, Corynebacterium* sp., *Edwardsiella tarda, E. coli, Klebsiella* sp., *Pantoea agglomerans, Pasteurella multocida, Proteus* sp., *Pseudomonas aeruginosa, Serratia marcescens, Staphylococcus aureus, Staphylococcus* sp., and *Streptococcus* sp.,[12,20] are among the bacteria species which are carried by *C. acutus* and/or *C. moreletii* in the Yucatan Peninsula and isolated from cases of blood poisoning in crocodiles. *Streptococcus agalactiae* has also been found to be an active pathogen causing necrotizing fasciitis in the young cases of *C. porosus* held captive.[21]

Huchzermeyer has reported that many of *Salmonella* serovars were isolated from crocodiles; they were seen as a part of the normal intestinal tract flora of crocodilians.[20] However, those that experienced serious conditions under stress were highly vulnerable to *Salmonella*, causing enteritis and blood poisoning in them.[20] The species of *Salmonella* were not isolated in the present study.

Huchzermeyer has also concluded that cloacal flora could play a role in infecting eggshell, eggshell membrane and yolk during the process eggs are laid.[20] The findings of a study done by Peucker *et al.,*[22] on the presence of bacteria in shell and yolk of farmed Australian freshwater crocodiles, *C. johnstoni,* eggs, showed that among the examined bacteria, 13 were identified in *C. acutus* and/or *C. moreletii* including *A. hydrophila, Alcaligenes faecalis, C. freundii, E. tarda, Enterobacter cloacae, E. coli, Klebsiella* sp., *P. agglomerans, Proteus vulgaris, P. aeruginosa, P. fluorescens, Salmonella arizonae* and *S. marcescens*.[12] Others have also reported the presence of *E. cloacae, Citrobacter* sp., *Proteus* sp., and *P. aeruginosa* in lesions of infected eggs.[23] Thomas *et al.*, have attempted to isolate a number of *Salmonella* serotypes from *C. porosus* eggshell or yolk.[24] The infection caused by bacteria in eggs is among the factors that can bring about egg mortality as well as yolk sac infections and omphalitis in hatchlings, which can lead to their death.[20] In such condition, the bacterial infection of eggs could endanger crocodiles conservation; thus, an examination of the bacteria present on the eggshell of *C. palustris* in Negour Protected Area appeared to be necessary. In this study, no infectious bacteria were isolated from the cloacal cavity.

Bacteria species which crocodiles carry are transmittable to humans. There are three possible ways that bacteria could be transmitted from crocodiles to humans including crocodile meat consumption,[25-26] being bitten by crocodiles[5-6] and involvement in the activities happening around crocodile waters.[15] When a crocodile bites someone, the wounds are generally infected by the bacteria present in the oral cavity of the animal.[3,6] Some bacteria have been found to cause wound infection in

attacks done by crocodilian including *A. hydrophila, Burkholderia pseudomallei, Pseudomonas* spp., *Serratia* spp., *Citrobacter diversus, Enterococcus* spp., *Clostridium* spp., and *P. agglomerans*.[27] In addition, among the bacteria found in the oral cavity of crocodiles in the present study, *E. durans* and *E. coli* have been identified in infected wounds caused by animal or human bites.[28]

Research have shown that when crocodilians are farmed and their meat is later harvested to be used by humans, their meat often carries bacteria which most likely could contaminate the meat during slaughter and dressing procedures.[24,25] Microbiological analyses of the meat harvested from a number of species of farmed crocodilian have confirmed that some bacteria genera such as *Salmonella, Staphylococcus, Flavobacterium, Pseudomonas, Acinobacter, Enterobacter, Moraxella, Micrococcus, Streptococcus* and *Escherichia,* could actually have contaminated the meat.[24] Moreover, some reports from the Amazonas have confirmed that after consuming meat from black caiman, *Melanosuchus niger,* an infection caused by *Bacillus cereus* could be found.[25] In another case in South Africa, a man who consumed infected crocodile meat, contaminated by *Salmonella enterica* subspecies diarizonae, was further diagnosed with.[29] In general, instances of salmonellosis in humans often occurred when they directly or indirectly had been in contact with reptiles.[30] In Mexico, despite the measures taken to protect crocodiles and the prohibition of their hunt since 1970, still there are cases of opportunistic killings, occasionally happening near where humans have settled,[31] and the meat consumed of these illegally-hunted animals could cause infection. The *E. coli* identified in the present study has also been regarded as an important pathogen that can grow on contaminated food causing acute infections in the intestine.[32] In Iran, crocodile meat is not consumed and there is no concern.

Moreover, in this study, a number of the bacteria was found in the cloacal cavity of crocodiles that could be considered potential pathogens, capable of affecting the quality of water and human health. In their studies on the bacteria from the cloaca of the American alligator, *A. mississippiensis*, *C. acutus* and *C. moreletii* as well as the bacteria present on the water samples taken from their natural environment, Charruau *et al.*[12] and Johnston *et al.*[15] have found that the florae present in the cloaca of alligator were similar to those present in the water they lived in. From this understanding, Johnston *et al*. came to the conclusion that alligators could potentially contaminate their water habitat with bacteria via the release of feces.[15] This finding has also been supported by other studies that have found that the bacterial florae in crocodilians are similar to those present in their aquatic habitat.[33] Other researchers have reported that when crocodiles are held captive in a water tank, the water container is found to be contaminated with bacteria present in the crocodilian excrement.[20,33] On this account, we could expect that the type of bacteria present in *C. palustris* studied for this research, can also be present in its corresponding habitat. A number of these bacteria is opportunistic pathogens and humans involved in activities in water and mud should bear in mind that these pathogens can cause wound or serious infections in their intestines.[34]

As a result, some of the bacteria identified in the oral and cloacal cavities in *C. palustris* studied in our research could be potential pathogenic agents for crocodiles, humans (or domestic animals injured or wounded by crocodile bites) and its environment. Currently, to fight infections caused by bacteria, humans depend on a large group of antibiotics. A number of studies has focused on injuries and wounds caused by crocodiles and come up with a broad range of preventive antibiotics that could be used to heal the wounds created by crocodile bites.[2,3,6] Although it is true that a great number of bacteria could be beaten by antibiotics, there are some species which seem to be not susceptible to some antibiotics and show resistance to them.[35] Also, bacteria are very rapid in changing and evolving in response to the circumstances and have a strong tendency to become antibiotic-resistant only after particular antibiotic has been used frequently to fight them.[35] Thus, considering the bacteria found in crocodiles in Negour Protected Area in this study, it is recommended that the focus of the future studies be the investigation of those bacteria sensitivity and resistance to increase the chance of a soon recovery for those suffering from wounds happened during attacks by crocodiles. The findings from such studies could provide useful information about how to treat both infections in crocodiles themselves and in humans and the domestic animals wounded by crocodiles.

This study offers some useful and significant information about bacterial flora carried by *C. palustris* in Negour Protected Area, Iran. The examinations of these bacteria are of great importance in two aspects: First, they could potentially endanger the health of crocodiles, particularly in conditions involving stress and secondly, they may seriously put the health of humans at risk in three possible ways including attacks by crocodiles and wound infection, crocodile meat consumption or simply by engaging in activities happening in the vicinity of crocodiles natural environment. There are a number of factors that if left unaddressed could actually increase the potential threat posed by bacterial contamination transmitted by crocodiles to humans. This bacterial contamination could occur more frequently if humans continue to destruct crocodiles natural environment, leading to an increase in the interactions between humans and crocodiles. The information offered in this study about a variety of different bacteria could help other researchers to choose and develop anti-bacterial products appropriate for the treatment of bacterial infection caused by bacteria present in bites and wounds left by crocodiles.

Acknowledgments

This study was supported by School of Veterinary Medicine, Shiraz University, Shiraz, Iran.

Conflict of interest

The authors declare that there is no conflict of interest.

References

1. Erickson GM, Lappin AK, Vliet KA. The ontogeny of bite-force performance in American alligator (*Alligator mississippiensis*). J Zool 2003; 260: 317-327.
2. Langley RL. Adverse encounters with alligators in the United States: an update. Wilderness Environ Med 2010; 21(2): 156-163.
3. Wamisho BL, Bates J, Tompkins M, et al. Ward round - Crocodile bites in Malawi: microbiology and surgical management. Malawi Med J 2009; 21(1): 29-31.
4. Vyas R. Mugger (*Crocodylus palustris*) population in and around Vadodara city, Gujarat State, India. Russ J Herpetol 2010; 17(1): 43-50.
5. Silva JSA, Mota RA, Pinheiro Júnior JW, et al. Aerobic bacterial microflora of Broad-snouted caiman (*Caiman latirostris*) oral cavity and cloaca, originating from Parque Zoológico Arruda Câmara, Paraíba, Brazil. Braz J Microbiol 2009; 40(1): 194-198.
6. Caldicott DGE, Croser D, Manolis C, et al. Crocodile attack in Australia: an analysis of its incidence and review of the pathology and management of crocodilian attacks in general. Wilderness Environ Med 2005; 16(3): 143-159.
7. Garcia ME, Lanzarot P, Costas E, et al. Isolation of *Serratia fonticola* from skin lesions in a Nile Crocodile (*Crocodylus niloticus*) with an associated septicaemia. Vet J 2008; 176(2): 254-256.
8. Campos JC, Mobaraki A, Abtin E, et al. Preliminary assessment of genetic diversity and population connectivity of the Mugger Crocodile in Iran. Amphib-reptil 2018; 39(1): 126-131.
9. Da Silva A, Lenin J. Mugger Crocodile *Crocodylus palustris*. In: Manolis SC, Stevenson C (Eds). Crocodiles. Status survey and conservation action plan. 3rd ed. Darwin, Australia: Crocodile Specialist Group 2010; 94-9811.
10. Mobaraki A, Abtin E, Kami HG, et al. Reproductive biology of the Mugger Crocodile, *Crocodylus palustris*, in Iran (Reptilia: Crocodylidae). Zool Middle East 2013; 59(3): 207-213.
11. Markey B, Leonard F, Archambault M, et al. Clinical veterinary microbiology. 2nd ed. Maryland Heights, USA: Mosby 2013; 215-286.
12. Charruau P, Pérez-Flores J, Pérez-Juárez JG, et al. Oral and cloacal microflora of wild crocodiles *Crocodylus acutus* and *C. moreletii* in the Mexican Caribbean. Dis Aquat Organ 2012; 98(1): 27-39.
13. Holmes DS, Quigley M. A rapid boiling method for the preparation of bacterial plasmids. Anal Biochem 1981; 114(1): 193-197.
14. Marín M, Garcia-Lechuz JM, Alonso P, et al. Role of universal 16S rRNA gene PCR and sequencing in the diagnosis of prosthetic joint infection. J Clin Microbiol 2011; 50(3): 583-589.
15. Johnston MA, Porter DE, Scott GI, et al. Isolation of faecal coliform bacteria from the American alligator (*Alligator mississippiensis*). J Appl Microbiol 2010; 108(3): 965-973.
16. Lovely CJ, Leslie AJ. Normal intestinal flora of wild Nile crocodiles (*Crocodylus niloticus*) in the Okavango Delta, Botswana. J S Afr Vet Assoc 2008; 79(2): 67-70.
17. Anderson JR. Characterisation of the oral and cloacal flora of wild and farmed crocodiles (*Crocodylus porosus* and *C. johnstoni*) in northern Australia. Thesis. James Cook University of North Queensland. Townsville, Australia 1999.
18. Madsen M, Hangartner P, West K, et al. Recovery rates, serotypes, and antimicrobial susceptibility patterns of salmonellae isolated from cloacal swabs of wild Nile crocodiles (*Crocodylus niloticus*) in Zimbabwe. J Zoo Wildl Med 1988; 29(1): 31-34.
19. Merchant ME, Leger N, Jerkins E, et al. Broad spectrum antimicrobial activity of leukocyte extracts from the American alligator (*Alligator mississippiensis*). Vet Immunol Immunopathol 2006; 110(3-4): 221-228.
20. Huchzermeyer FW. Crocodiles: biology, husbandry and diseases. 1st ed. Wallingford, UK: CABI Publishing 2003; 139-157.
21. Bishop EJ, Shilton C, Benedict S, et al. Necrotizing fasciitis in captive juvenile *Crocodylus porosus* caused by *Streptococcus agalactiae*: an outbreak and review of the animal and human literature. Epidemiol Infect 2007; 135(8): 1248-1255.
22. Peucker SKJ, Davis BM, Van Barneveld RJ. Crocodile farming research: hatching to harvest. Kingston, Australia: Rural Industries Research and Development Corporation 2005; 1-34.
23. Schumacher J, Cardeilhac PT. Mycotic infections of egg membranes in the American alligator (*Alligator mississippiensis)*. In proceedings: International Association for Aquatic Animal Medicine. Virginia, USA 1990; 138-140.
24. Thomas AD, Forbes-Faulkner JC, Speare R, et al. Salmonelliasis in wildlife from Queensland. J Wildl Dis 2001; 37(2): 229-238.
25. Suárez MC, Pérez L, Murcia LM, et al. Food poisoning due to consumption of black cayman meat (*Melanosuchus niger*) in the Nazareth indigenous school, Amazonas, April 1997 [Spanish]. Biomédica 2000; 20: 42-48.

26. Magnino S, Colin P, Dei-Cas E, et al. Biological risks associated with consumption of reptile products. Int J Food Microbiol 2009; 134(3): 163-175.
27. Mekisic AP, Wardill JR. Crocodile attacks in the Northern Territory of Australia. Med J Aust 1992; 157(11-12): 751-754.
28. Brook I. Management of human and animal bite wound infection: an overview. Curr Infect Dis Rep 2009; 11(5): 389-395.
29. Narayana S, Metz LI, Kowalski TJ. African tick bite fever and crocodile meat–associated salmonellosis coinfection in a returning traveler. Gundersen Lutheran Med J 2008; 5: 17-18.
30. Mermin J, Hutwagner L, Vugia D, et al. Reptiles, amphibians, and human *Salmonella* infection: a population-based, case-control study. Clin Infect Dis 2004; 38(Suppl 3): S253-S261.
31. Cedeño-Vázquez JR, Ross JP, Calmé S. Population status and distribution of *Crocodylus acutus* and *C. moreletii* in southeastern Quintana Roo, Mexico. Herpetolo Nat Hist 2006; 10(1): 53-66
32. Schlech III WF, Acheson D. Foodborne listeriosis. Clin Infect Dis 2000; 31(3): 770-775.
33. Madsen M. Enumeration of salmonellae in crocodile pond water by direct plate counts and by the mpn technique. Water Res 1994; 28(9): 2035-2037.
34. Noonburg GE. Management of extremity trauma and related infections occurring in the aquatic environment. J Am Acad Orthop Surg 2005; 13(4): 243-253.
35. Bergstrom CT, Feldgarden M. The ecology and evolution of antibiotic-resistant bacteria. In: Stearns SC, Koella JC (Eds). Evolution in health and disease. 2nd ed. Oxford, UK: Oxford University Press 2008; 125-138.