Bacteria and parasites in *Podarcis sicula* and *P. sicula klemmerii*

Ludovico Dipineto¹*, Pasquale Raia², Lorena Varriale¹, Luca Borrelli¹, Vittorio Botta¹, Carmela Serio², Michele Capasso¹ and Laura Rinaldi¹

**Abstract**

**Background:** New epidemiological data on bacterial and parasitic infections in 24 Italian wall lizards, namely *Podarcis sicula* (mainland population) and *P. sicula klemmerii* (insular population) in southern Italy were provided. To achieve this goal, samples were collected from individuals belonging to the two populations and analysed by microbiological and parasitological methods.

**Results:** A wide range of bacteria (e.g. *Pantoea* spp., *Citrobacter* spp., *Morganella* spp., *Pseudomonas*, *Enterobacter* spp., *Staphylococcus* spp. and *Escherichia coli*) and parasites (e.g. *Ophionyssus natricis*, coccidia, Dicrocoelidae) were detected in both *P. sicula* and *P. sicula klemmerii* individuals. Insular population presented similar bacterial and parasitic diversity to its mainland counterpart. Ampicillin was the antimicrobial with the highest resistance rate.

**Conclusion:** This study highlighted various bacteria and parasites, some of them potentially zoonotic. Further studies are needed to better understand the epidemiology and transmission routes of these pathogens along with their impact on the welfare and behaviour of Italian wall lizards.

**Keywords:** *Podarcis sicula*, Italian wall lizard, Bacteria, Parasites, FLOTAC

**Background**

The Italian wall lizard, *Podarcis sicula*, is one of the most common lizards in Italy [1]. The snout-vent length (SVL) is, on average, 15 to 25 cm long. The colour pattern is characterized by a green or brown back and whitish belly, although melanistic variants, with either shorter, or longer SVL, are known to occur in several islands in the Mediterranean sea. As a common, and easily managed study model, the Italian wall lizard was subjected to several studies, regarding aspects as disparate as phenotypic response to predation [2], feeding behaviour [3], ontogeny [4], adaptation to novel environments [5], biogeography [6, 7], and its role as a biological indicator [8].

*Podarcis sicula klemmerii* [9] is one of the subspecies belonging to *P. sicula*. It is confined to a small, 1 km² large islet, Liscia, off the western coast of Italy. As with many other insular populations of the Italian wall lizard, *P.s. klemmeri* is melanic, meaning the back appears tinged with blue, and the pale underside is bluish as well, rather than the usual white of continental populations.

Melanic variants have been investigated for the so-called Island syndrome [10], which is a suite of phenotypic character shifts in insular populations including, besides melanism, changed body size, feeding behaviour and ecology, patterns of aggressiveness, and life history traits [11–15]. The link between melanism and characters shifts on islands was found to be in the activity levels of melanocortin receptors, MCRs [13]. Melanocortins form a suite of five receptors activated pleiotropically by a single DNA locus, the proopiomelanocortin POMC gene, that happen to regulate feeding and sexual activity, immunocompetence and body colour [16–18]. Interestingly, Monti et al. [19] tested whether *P. s. klemmeri* individuals have different ectoparasite loads (i.e. the density of ticks and mites on the skin) as compared to the continental individuals. They found reduced load in insular individuals, consistently with their comparatively higher α-melanocyte-stimulating hormone (MSH) levels.

Herein, we deepen microbiological and parasitological investigations on *P. s. klemmeri*. Besides a few studies [20, 21], these aspects have not been scrutinized so far,
yet remain very important in the case of melanic insular lizards, whose immune system is expected to be depressed by great investment in reproduction, and life at high density [13, 19, 22]. This study was undertaken with the aim to evaluate the presence of potentially zoonotic bacteria and parasites in wild-caught insular individuals of *P. sicula klemmeri*, along with mainland individuals of *P. sicula*.

**Results**

A wide range of bacteria and parasites were detected in both *P. sicula* and *P. sicula klemmeri* individuals. Among the 24 analysed animals, 23 (95.9%) were positive to at least one bacterium and 19 (79.1%) were positive to at least one parasite.

Regarding microbiological analysis, *Pantoea* spp. was isolated in 4/24 (16.7%) oral swabs, *Citrobacter* spp. in 1/24 (4.2%), *Morganella morgani* in 1/24 (4.2%), *Pseudomonas aeruginosa* in 1/24 (4.2%) and Coagulase-Negative Staphylococci (NCS) in 7/24 (29.1%). For cloacal swabs, *Citrobacter* spp. was found in 10/24 (41.7%) animals of which 1/10 (10.0%) was identified as *Citrobacter koseri*, *Enterobacter* spp. was found in 8/24 (33.3%) animals of which 1/8 (12.5%) was identified as *Enterobacter aerogenes*, *Escherichia coli* was found in 3/24 (12.5%) animals of which 1/3 (33.3%) was serotyped as serogroup O 145, *Morganella morgani* was isolated in 2/24 (8.3%) individuals, *Shewanella* spp. in 1/24 (4.2%), *Providencia* spp. in 2/24 (8.3%), Coagulase-Negative Staphylococci (NCS) in 20/24 (83.3%) and *Pseudomonas* spp. in 10/24 (41.7%) individuals. Several bacteria were routinely isolated from the same animal.

With respect to antimicrobial susceptibility testing, *Citrobacter* spp. showed the highest resistance profile. Specifically, two out of ten strains of *Citrobacter* spp. were resistant to three or more drugs (“multidrug-resistant”) of which, one strain was resistant to ampicillin, doxycycline and streptomycin and the other one was resistant to ampicillin, amoxicillin-clavulanic acid, doxycycline and nitrofurantoin. The remaining strains were resistant to ampicillin, one was also resistant to doxycycline and one other was also resistant to amoxicillin-clavulanic acid, doxycycline and nitrofurantoin. The scotch test highlighted the presence of *Ophionyssus natricis* mite. During the faecal examination, eggs of pinworms (20/24; 83.3%), *O. natricis* (12/24; 50%), and Dicrocoelidae (6/24; 25%) as well as oocysts of coccidia (11/24; 45.8%) were detected. As with bacteria, different parasite species were simultaneously detected from the same animal. In addition, adult liver flukes (Dicrocoelidae) were also found during the necropsy. The parasitological and microbiological results related to each lizard are detailed in Table 1.

**Discussion**

Our results provide new epidemiological data on bacterial and parasitic infections in *P. sicula* and *P. sicula klemmeri*. These species were poorly investigated from a sanitary perspective so far. Bacterial and parasitic infections in reptiles have recently gained scientific relevance. However, the majority of studies were carried out on captive-bred individuals of Cryptodira (tortoises, [23]), Serpentes and Sauria (snakes and other lizards, [24]). Conversely, data on infections in wild lizards are scarce.

The results of our study showed the presence of endoparasites (coccidia, pinworms and liver flukes) and ectoparasites (*O. natricis*) in *P. sicula* and *P. sicula klemmeri*. With respect to the presence of pinworms, our findings are in line with those by Casanova et al. [20] who detected the presence of a nematode belonging to the Oxyuroidea superfamily in *P. sicula*. Nevertheless, our results showed the first epidemiological information on parasites infecting *P. sicula klemmeri*. Regarding the presence of *O. natricis*, there are no studies in literature that highlight its presence in *P. sicula* and *P. sicula klemmeri*, although the infestation by this mite has been described in various genus of Sauria as *Lacerta*, Podarcis and Darevskia. In addition, the carrier role of *O. natricis* in the transmission of a blood parasite belonging to the genus *Karyolysus* has been reported in lizards [25]. An interesting finding of our study was the detection at necropsy of liver flukes (Dicrocoelidae) in the continental lizards, although species identification was not performed.

Bacteriological results also added new epidemiological data in *P. sicula* due to detection of some potential zoonotic species as *P. aeruginosa* and *E. coli* O145. The bacterial isolation performed on oral swabs gave as result the presence of bacteria belonging to the genera *Pantoea*, *Pseudomonas*, *Morganella*, *Staphylococcus*, *Citrobacter*, *Shewanella* never detected before in *P. sicula* and *P. sicula klemmeri*. However, these bacteria are frequent in reptiles, along with other bacteria such as *Enterobacter* spp., *E. coli* and *Providencia* spp. [26–28]. Bacteria isolated from cloacal swabs were *Enterobacter* spp., *Citrobacter* spp., *Morganella* spp., *E. coli*, *Providencia* spp. *Shewanella* spp., and *Pseudomonas* spp., gram-negative bacteria frequently isolated in other reptiles [29] as well as *Staphylococcus* spp. The isolation of *E. coli* O145 in one *P. sicula klemmeri* is noteworthy due to the potential zoonotic role of this serogroup which is considered a shigatoxin-producing *E. coli*.

It is interesting to notice that the insular population presents similar bacterial diversity of its mainland counterpart, although the differences in sample size urge caution in interpreting these results. Noteworthy, 9 different bacterial genera were identified in *P. s. klemmeri* (up to six in a single individual), against 4 in mainland individuals (up to three in a single individual). A weakness of
this study was the failure to isolate bacteria in the oral cavity of some lizards due to the difficult to keep viable some strains during the isolation procedures. To our knowledge this is the first study to assess the antimicrobial resistance profiles of potentially zoonotic bacteria carried by *Podarcis* spp. It is difficult to speculate regarding the results of the antimicrobial resistance recovered in the present study. However, one possible mechanism by which lizards acquire antimicrobial resistant bacteria in their environment range may be directly

| Lizard ID | Parasites | Bacteria | Cloacal | Oral |
|-----------|-----------|----------|--------|------|
| LIC 1001  | Negative  | – – Positive | Enterobacter spp, Staphylococcus spp, Pseudomonas spp. | Pantoea spp, Staphylococcus spp. |
| LIC 1002  | Pinworms Cocacidae | 300 225 | Positive | Enterobacter aerogenes, Citrobacter spp, Staphylococcus spp. |
| LIC 1003  | Pinworms Cocacidae | 720 150 | Positive | Morganella morganii, Citrobacter spp, Staphylococcus spp. |
| LIC 1004  | Pinworms Cocacidae | 720 150 | Positive | Citrobacter spp, Shewanella spp, E. coli, Staphylococcus spp, Pseudomonas spp. |
| LIC 1005  | Pinworms Liver flukes | 2.880 1.440 | Positive | Citrobacter spp, Staphylococcus spp. |
| LIC 1006  | Pinworms Liver flukes | 2.880 1.440 | Positive | Morganella morganii, Citrobacter spp, Staphylococcus spp, Pantoaea, Staphylococcus spp. |
| LIC 1007  | Pinworms Cocacidae | 3.600 2.100 | Positive | Enterobacter spp, E. coli, Staphylococcus spp. |
| LIC 1008  | Negative | – – Positive | Enterobacter spp, Providencia spp, Staphylococcus spp. |
| LIC 1009  | Pinworms Cocacidae | 3.600 2.100 | Positive | E. coli O145, Staphylococcus spp. |
| LIC 1010  | Pinworms Liver flukes | 330 300 | Positive | Staphylococcus spp. |
| LIC 1011  | Pinworms Liver flukes | 3420 1.890 | Positive | Pseudomonas spp. |
| LIC 1012  | Pinworms Liver flukes | 3420 1.890 | Positive | Citrobacter spp, Providencia spp, Staphylococcus spp, Pseudomonas spp. |
| LIC 1013  | Pinworms Cocacidae | 870 690 | – | Enterobacter spp, Staphylococcus spp. |
| LIC 1014  | Pinworms Cocacidae | 870 690 | – | Citrobacter koseri, Staphylococcus spp, Pseudomonas spp. |
| LIC 1015  | Pinworms Liver flukes | 540 300 | – | Enterobacter spp, Staphylococcus spp. |
| LIC 1016  | Pinworms Liver flukes | 540 300 | – | Staphylococcus spp, Pseudomonas spp. |
| LIC 1017  | Pinworms Cocacidae | 540 300 | – | Pseudomonas spp. |
| LIC 1018  | Pinworms Cocacidae | 360 450 | – | Staphylococcus spp. |
| LIC 1019  | Pinworms Liver flukes | 360 450 | – | Staphylococcus spp, Pseudomonas spp. |
| LIC 1020  | Pinworms Cocacidae | 360 450 | – | Enterobacter spp, Staphylococcus spp. |
| PLIC 1001 | Pinworms Liver flukes | 420 375 | – | NOT IDENTIFIED |
| PLIC 1002 | Negative | – – – | Enterobacter spp, Citrobacter spp, Staphylococcus spp. |
| PLIC 1003 | Negative | – – – | Citrobacter spp, Staphylococcus spp. |
| PLIC 1004 | Negative | – – – | Citrobacter spp, Staphylococcus spp, Pseudomonas spp. |

*EPG/OPG = eggs/oocysts per gram of faeces*
through exposure to human or livestock waste, or indirectly through consumption of prey which may harbor resistant bacteria. The figure for parasites is even harder to interpret given the smaller sample size. Yet, we found coccidia, liver flukes and pinworms in the insular populations, and pinworms only (in just one individual) within continental lizards. While difference in collecting season, sample size, and the instance of necropsy in two individuals only suggest great caution, data seem to indicate an overall higher parasite/bacterial load in insular lizards. Assuming this is true, it remains to be elucidated whether this depends on population density on Licosa, or on less competent immune system in insular melanic individuals [12, 13].

Conclusion
In conclusion, the results of this study highlighted various bacteria and parasites, some of them pathogenic, able to infect the species P. sicula and the subspecies P. sicula klemmeri. Further studies are needed to better understand the epidemiology and transmission routes of these pathogens along with their impact on the welfare and behaviour of Italian wall lizards.

Methods
Study animals
From November 2015 to May 2016, we collected and examined, within 24 h from capture, a total of 24 individuals, 20 (14 males and 10 females) belonging to the subspecies P. sicula klemmeri, and 4 individuals belonging to the species P. sicula. The P. sicula klemmeri specimens were from the small islet of Licosa, whereas the P. sicula specimens from Punta Licosa mainland (Campania region of southern Italy). The study site, collection and housing methods were described in Raia et al. [12, 13]. Lizards captured on Licosa island were identified with the code "PLIC" and a progressive number (from LIC 1001 to 1020); the lizards from Licosa were described in Raia et al. [12, 13].

In order to perform the microbiological analysis, oral and cloacal swabs were individually collected by sterile cotton-tipped swabs and then inoculated in Phosphate Buffered Saline (PBS). For the parasitological analyses, different methods were used: faecal samples were collected from each lizard, scotch tape test was performed on the skin of each animal and, eventually, a necropsy with stereo microscope was carried out on two lizards found dead on the insular study site. After sampling, animals were released in the same area where they were captured. All experiments described were performed in accordance with the local and national guidelines governing animal experiments (86/609/CEE and its modifications).

Microbiological and parasitological analyses
Oral and cloacal swabs stored in PBS were inoculated in buffered peptone water (BPW), Campylobacter-selective enrichment broth (CSEB), cooked meat medium (CMM), modified tryptone soy broth (MTSB). The samples inoculated in BPW were incubated at both 25 °C and 37 °C for 24 h and then inoculated/streaked into Rappaport-Vassiliadis broth (RV), Columbia blood agar base (CBA; Oxoid, Milan, Italy), Pseudomonas cetrimide agar (PCA; Oxoid), MacConkey agar (MCA; Oxoid) and Baird-Parker agar (BPA; Oxoid). The samples inoculated in MTSB were incubated at both 25 °C and 37 °C for 24 h and then spread on sorbitol MacConkey agar (SMCA; Oxoid). The samples inoculated in CSEB were incubated in microaerophilic atmosphere at both 25 °C and 42 °C for 48 h and then streaked on Campylobacter blood-free selective agar (CBFA; Oxoid). Instead, the samples inoculated in CMM were incubated in anaerobic atmosphere at both 25 °C and 37 °C for 24 h, and then streaked on anaerobe basal agar (ABA; Oxoid). The remaining PBS were incubated at 4 °C for 14 days, spread on Yersinia selective agar base (cefsulodin-irgasan-novobiocin, CIN Agar; Oxoid) and incubated at 30 °C for additional 24–48 h. CBA, PCA, MCA, SMCA, CEOA and BPA were incubated at both 25 °C and 37 °C for 24–48 h, whereas RV was incubated at both 25 °C and 42 °C for 24–48 h and then streaked on xylose lysine deoxycholate agar (XLD) and brilliant green agar (BGA); CBFA were incubated in microaerophilic atmosphere at both 25 °C and 42 °C for 24–48 h, ABA plates were anaerobically incubated both at 25 °C and 37 °C for 48 h and checked daily for a further week before discarding. All the isolates were previously identified according to their morphological features, their growing need, Gram colouring, motility and pigments production test and through conventional biochemical and phenotypic test. Progressively, the isolates were identified through the biochemical systems API 20 E, API 20 NE (bioMerieux, Mercy-l’Etoile, France) and RapID ANA II, RapID NF PLUS, RapID STAPH PLUS (Oxoid). Escherichia coli strains were serogrouped with antisera poly- and monocpecific (Sifin) whereas, suspected Campylobacter strains were identified by PCR as reported by Gargiulo et al. [30].

In order to detect and count parasitic elements (eggs, larvae, cysts, oocysts), each faecal sample collected from the 24 lizards was analysed with the FLOTAC pellet technique [24, 31]. The analytic sensitivity of the FLOTAC pellet technique varied on the basis of the weight of each pellet μl [24] and expressed as eggs/oocysts per gram (EPG/OPG) of faeces. Two flotation solutions were used: sodium chloride (NaCl, specific gravity = 1200) and zinc sulfate (ZnSO4, specific gravity = 1350) to detect protozoa/nematoda and trematoda, respectively. The scotch tape test was performed on the skin of the lizards, specifically, at the level of skin folds, around the
eye, the cloaca and the eardrum recesses. The necropsy of the two lizard carcasses was performed under a stereomicroscope using the reptile necropsy protocol reported in Jacobson [32].

Antimicrobial susceptibility tests

_Pseudomonas aeruginosa_, Escherichia coli O145, _Citrobacter_ spp., _Enterobacter_ spp. isolates were submitted to antimicrobial susceptibility testing using the disc diffusion method according to Clinical Laboratory Standard Institute [33]. The antimicrobials tested were ampicillin (10 μg), ceftazidime (30 μg), ciprofloxacin (5 μg), enrofloxacin (5 μg), sulphamethoxazole-trimethoprim (25 μg), nalidixic acid (30 μg), amoxicillin-clavulanic acid (30 μg), doxycycline (30 μg), gentamicin (10 μg), streptomycin (10 μg), amikacin (30 μg), nitrofurantoin (30 μg), colistin sulphate (10 μg), piperacillin 100 (μg) and Cefpodoxime Combination Disc Kit (Oxoid). The inhibition zones were measured and scored as sensitive, intermediate susceptibility and resistant according to CLSI documents [34].

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Availability of data and materials

The datasets used and analyzed are available from the corresponding author upon reasonable request.

Authors' contributions

All authors read and approved the final manuscript. LD, LR and PR designed the study, analysed the data, edited and critically revised the manuscript; VB, CS, LV, LB and MC performed microbiological and parasitological analysis and wrote the first draft of the manuscript.

Ethics approval and consent to participate

All experiments described were performed in accordance with the local and national guidelines governing animal experiments (86/ 609/CEE and its modifications) and were approved by animal research ethics committee of the Italian Ministry of the Environment and Protection of Land and Sea (protocol number 0013996/PNM).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Author details

1Dipartimento di Medicina Veterinaria e Produzioni Animali, Università di Napoli Federico II, Naples, Italy. 2Dipartimento di Scienze della Terra, dell’Ambiente e delle Risorse, Università di Napoli Federico II, Naples, Italy.

Received: 1 August 2018 Accepted: 21 November 2018 Published online: 10 December 2018

References

1. Corti C, Lo Cascio P, Razzetti E. Herpetofauna of the Italian islands. In: Bernini F, Sindacco R, Dona G, Razzetti E, editors. Atlas of Italian amphibians and reptiles. Firenze: Edizioni Polistampa; 2006. p. 612–43.
2. Venuz B, Gibac I, Van Damme R. Differences in morphology, performance and behaviour between recently diverged populations of _Podarcis sicula_ mirror differences in predation pressure. Oikos. 2007;116:143–52.
3. Capula M, Aloise G. Extreme feeding behaviours in the Italian wall lizard. _Podarcis sicula_. Acta Herpetol. 2011;6:1–4.
4. Piras P, Salvi D, Ferrara G, Maiorino L, Delfino M, Pedde L, Kotasik T. The role of post-natal ontogeny in the evolution of phenotypic diversity in _Podarcis sicula_ lizards. J Evol Biol. 2011;24:2705–20.
5. Kaplasa G, Gavrilidil I, Adamopoulot C, Fopoulous J, Pafilos P. Effective thermoregulation in a newly established population of _Podarcis sicula_ in Greece: a possible advantage for a successful invader. Acta Herpetol. 2016;11:111–8.
6. Podnar M, Mayer W, Tvrkovič N. Phylogeography of the Italian wall lizard, _Podarcis sicula_, as revealed by mitochondrial DNA sequences. Mol Ecol. 2005;14:575–88.
7. Senczuč G, Colangelo P, De Simone E, Aloise G, Castiglia R. A combination of long term fragmentation and glacial persistence drove the evolutionary history of the Italian wall lizard _Podarcis sicula_. BMC Evol Biol. 2017;17:6.
8. De Falco M, Seillieti A, Sciarillo R, Capalda A, Valante S, Iachetta G, et al. Nonylphenol effects on the HPA axis of the bioindicator vertebrate, _Podarcis sicula_ lizards. Chemosphere. 2014;104:190–6.
9. Lanza B, Capolongo D. Die blaue Ruineidechse der tyrrhenischen Insel Liscia (Salerno). Salamandra. 1972;821–6.
10. Adler GH, Levins R. The island syndrome in rodent populations. Q Rev Biol. 1994;69:473–90.
11. Meiri S. Size evolution in island lizards. Glob Ecol Biogeogr. 2007;16:702–8.
12. Raia P, Carotenuto F, Meiri S. One size does not fit all: no evidence for an optimal body size on islands. Glob Ecol Biogeogr. 2010;19:475–84.
13. Raia P, Guarino FM, Turano M, Polese G, Rippa D, Carotenuto F. The blue lizard spandrel and the island syndrome. BMC Evol Biol. 2010;10:289.
14. Novosolov M, Raia P, Meiri S. The island syndrome in lizard. Glob Ecol Biogeogr. 2013;22:184–19.
15. Cooper WE, Dimopoulos I, Pafilos P. Sex, age, and population density affect aggressive behaviors in island lizards promoting cannibalism. Ethology. 2015;121:260–9.
16. Ducrest AL, Keller L, Roulin A. Pleiotropy in the melanotan system, colouration and behavioural syndromes. Trends Ecol Evol. 2008;23:502–10.
17. Emaresi G, Ducrest AL, Bize P, Richter H, Simon C, Roulin A. Pleiotropy in the melanotan system: expression levels of this system are associated with melanogenesis and pigmentation in the tawny owl (Strix aluco). Mol Ecol. 2013;22:4915–30.
18. Kim SY, Fargallo JA, Vergara P, Martinez-Padilla J. Multivariate heredity of melanin-based coloration, body mass and immunity. Heredity. 2013;111:139.
19. Monti DM, Raia P, Vroonen J, Maselli V, Van Damme R, Fulgione D. Physiological change in an insular lizard population confirms the reversed island syndrome. Biol J Linn Soc Lond. 2013;108:144–50.
20. Casanova JC, Milazzo C, Ribas A, Cagnin M. Pleiotropy in the melanotan system: expression levels of this system are associated with melanogenesis and pigmentation in the tawny owl (Strix aluco). Mol Ecol. 2013;22:4915–30.
21. Lhermitte N, Bain O, Virga A. _Podarcis sicula_ (Nematoda: Pharyngodonidae) parasite of _Podarcis sicula_ (Lacertidae) from Italy. J Parasitol. 2003;89:577–8.
22. Lhermitte N, Bain O, Virga A. _Podarcis sicula_ (Nematoda: Pharyngodonidae) parasite of _Podarcis sicula_ (Lacertidae) from Italy. J Parasitol. 2003;89:577–8.
24. Rinaldi L, Mihalca AD, Cirillo R, Maurelli MP, Montesano M, Capasso M, et al. FLOTAC can detect parasitic and pseudoparasitic elements in reptiles. Exp Parasitol. 2012;130:282–4.
25. Labbé A. Recherches zoologiques et biologiques sur les parasites endoglobulaires du sang des vertébrés. Arch Zool Exp Gen. 1894;2:55–258.
26. Jacobson ER. Immobilization, blood sampling, necropsy techniques and diseases of crocodilians: a review. J Zoo Anim Med. 1984;15:38–45.
27. Joyner P, Brown JD, Holladay S, Sleeman JM. Characterization of the bacterial microflora of the tympanic cavity of eastern box turtles with and without aural abscesses. J Wildl Dis. 2006;42:859–64.
28. Meyer H, Frank W. Bacteriological investigations on reptiles and amphibians (author’s transl). Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene. Erste Abteilung Originale. Zentralbl Bakteriol Orig A. 1974;229:470.
29. Foti M, Giacopello C, Bottari T, Fischella V, Rinaldo D, Mammina C. Antibiotic resistance of gram negatives isolates from loggerhead sea turtles (Caretta caretta) in the Central Mediterranean Sea. Mar Pollut Bull. 2009;58:1363–6.
30. Gargiulo A, Rinaldi L, D’Angelo L, Dipineto L, Borrelli L, Fioretti A, et al. Survey of Campylobacter jejuni in stray cats in southern Italy. Lett Appl Microbiol. 2008;46:267–70.
31. Cringoli G, Rinaldi L, Maurelli MP, Utzinger J. FLOTAC: new multivalent techniques for qualitative and quantitative copromicroscopic diagnosis of parasites in animals and humans. Nat Protoc. 2010;5:503–15.
32. Jacobson ER. Reptile necropsy protocol. J Zoo Anim Med. 1978;9:7–13.
33. Clinical and laboratory standards institute (CLSI): Performance Standards for Antimicrobial Disk Susceptibility Tests. In: Approved Standard M02-A11. 7th ed. PA, USA: CLSI: Wayne; 2012.
34. Clinical and laboratory standards institute (CLSI): Performance Standards for Antimicrobial Disk Susceptibility Tests. In: Approved Standard M100-S24. 24th ed. PA, USA: CLSI: Wayne; 2014.