The Journal of Veterinary Medical Science

NOTE

Wildlife Science

Phylogenetic characterization of *Isospora jaracimrmani* oocysts from a veiled chameleon (family Chamaeleonidae; *Chamaeleo calyptratus*) reared at a zoo in Ishikawa, Japan

Fitrine EKAWASTI[1,2)#], Kazuya KITAGAWA[3)#], Hiroshi DOMAE[3)#], April Hari WARDHANA[1,2,4)#], Junki NAGASAWA[2)#], Tomoyuki SHIBAHARA[2,5)#], Masaharu TOKORO[6)#], Kazumi SASAI[2,7)#] and Makoto MATSUBAYASHI[2,4,7)*#]

[1)]Indonesian Research Center for Veterinary Science, Bogor 16114, Indonesia
[2)]Department of Veterinary Science, Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Osaka 598-8531, Japan
[3)]Ishikawa Zoo, Nomi, Ishikawa 923-1222, Japan
[4)]Department of Veterinary Parasitology, Faculty of Veterinary Medicine, Airlangga University, Surabaya 60115, Indonesia
[5)]Division of Pathology and Pathophysiology, National Institute of Animal Health, NARO, Tsukuba, Ibaraki 305-0856, Japan
[6)]Department of Parasitology, Graduate School of Medical Sciences, Kanazawa University, Kanazawa 920-8640, Japan
[7)]Asian Health Science Research Institute, Osaka Prefecture University, Osaka 598-8531, Japan

J. Vet. Med. Sci.
83(8): 1240–1243, 2021
doi: 10.1292/jvms.21-0152

Received: 12 March 2021
Accepted: 31 May 2021
Advanced Epub:
17 June 2021

ABSTRACT. Oocysts of *Isospora* sp. were detected in the feces of a veiled chameleon (family Chamaeleonidae; *Chamaeleo calyptratus*) kept at a zoo in Ishikawa, Japan. Phylogenetic analysis placed the sequence in the cluster of *Isospora* spp. isolated from reptiles. Based on a comparison of morphological data of ten previously reported *Isospora* species from the Chamaeleonidae family, this isolate was morphologically similar to *I. jaracimrmani*, which has been considered to be a virulent species. This case study suggests the possibility that species of *Isospora* might not always cause disease because the animal that shed these oocysts showed no symptoms for more than two months.

KEY WORDS: *Isospora*, Japan, oocyst, veiled chameleon

The veiled chameleon (family Chamaeleonidae; *Chamaeleo calyptratus*) is endemic to the southwestern area of the Arabian Peninsula and is one of the most popular chameleon species in the world. They prefer humid coastal lowlands, coastal slopes, and high plateaus and generally feed on insects such as locusts, grasshoppers, and crickets, by capturing them with their sticky tongues. Chameleons sometimes consume the leaves of plants as a source of water, especially during the dry season [9].

To date, ten species of protozoan coccidian parasites, *Isospora*, have been isolated and described from seven members of the Chamaeleonidae from four geographic areas, Africa, the Republic of Madagascar, the Seychelles, and the Republic of Yemen (summarized by McAllister) [7] (see Table 1). Among them, *I. jaracimrmani* has been reported to cause serious health problems such as weight loss and weakness in infected hosts [11, 12]. In this study, isosporan oocysts were isolated from a veiled chameleon reared at a zoo in Japan. We compared the morphology of the isolates with that of previously reported isolates and analyzed the genetics to determine the species and phylogenetic position.

A veiled chameleon (1-year-old) kept in captivity at a zoo in Ishikawa Prefecture, Japan, since its birth in September 2017, was periodically screened for parasites as a routine examination before exhibition based on examination of feces by the sucrose centrifugal flotation method [19]. The chameleon did not show any clinical symptoms when fecal samples were collected. Oocysts of *Isospora* sp. were detected on November 8, 2018, and January 27, 2019 (Fig. 1), and an anti-coccidiostat, 5 mg of toltrazuril (0.1 ml) (Bayer

*Correspondence to: Matsubayashi, M.: matsubayashi@vet.osakafu-u.ac.jp

#All the authors contributed equally to this work.

©2021 The Japanese Society of Veterinary Science

This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial No Derivatives (by-nc-nd) License. (CC-BY-NC-ND 4.0: https://creativecommons.org/licenses/by-nc-nd/4.0/)

Table 1. Comparison of morphology of *Isospora* spp. in the present study and in the ones isolated from the Chamaeleonidae

Species	Oocysts					Sporocysts					Host	Locality	References
	Mean length × width (range) (μm)	Mean L/W (range)	Mi	OR	PG	Mean length × width (range) (μm)	Mean L/W (range)	SB	SSB	SR			
This study	**35.5 (28.2–42.4) × 23.4 (19.4–27.4)**	**1.5 (1.14–1.99)**	**-**	**-**	**-**	**14.0 (12.6–16.1) × 11.3 (10.0–13.0)**	**1.27 (1.11–1.56)**	**+**	**+**		***Chamaeleo calyptratus***	**Japan**	
Isospora brygooi	20.7 (17–25) × 19.3 (16–23)	1.1	-	-	+	12.2 (12–13) × 8.1 (8–9)	1.5	+	+	+	*Furcifer pardalis*	Madagascar	[10]
Isospora freedi	23.7 (21–26) × 21.2 (18–24)	1.1 (1.1–1.2)	-	-	+/–	13.9 (13–14) × 10.3 (9–11)	1.34 (1.3–1.4)	+	+	+	*Chamaeleo dilepis*	Namibia	[7]
Isospora jaracimrmani	38.4 (35.2–42.8) × 25.6 (23.8–27.0)	1.5	-	-	-	15.9 (14.8–17.0) × 11.2 (10.4–12.0)	1.4	+	+	+	*Chamaeleo calyptratus*	Yemen	[11]
Isospora mandelai	36.9 (34–39) × 31.0 (26–35)	1.2 (1.1–1.5)	-	-	-	15.3 (14–16) × 11.1 (10–12)	1.37 (1.2–1.5)	+	+	+	*Chamaeleo dilepis Leach*	Namibia	[7]
Isospora mesnili	30 (diam)					16 × 10	1.6				*Chamaeleo chameleon*	Algeria	[2, 16]
Isospora muriyu	23.6 (21.5–25) × 21.9 (21–23)	1.08 (1–1.1)	-	-	-	12.4 (12–13) × 8.7 (8–10)	1.4 (1.2–1.6)	+	+	+	*Triceros jacksoni*	Kenya	[14]
Isospora necasi	26.6 (21–30) × 24.3 (20–27)	1.1 (1.05–1.16)	-	-	-	12.8 (12–14) × 9.8 (9–10)	1.31 (1.20–1.44)	+	+	+	*Triceros melleri*	Tanzania	[14]
Isospora taizii	28 × 22	1.3				13 × 9	1.4				*Chamaeleo calyptratus*	Yemen	[1]
Isospora tigris	22.5 (19–24) × 18 (16–20)	1.25 (1.15–1.35)	-	-	-	13.6 (12–15) × 7 (6–8)	1.9 (1.6–2.2)	+	+	+	*Calumma tigris*	Republic of the Seychelles	[13]
Isospora wildi	25 22–28) × 21 (18–24)	1.17 (1.09–1.33)	-	-	-	12.3 (12–13) × 9.7 (9–10)	1.28 (1.2–1.33)	+	+	+	*Calima dilepis*	Tanzania	[14]

*Blank: data not available, Mi: micropyle, OR: oocyst residuum, PG: polar granules, SB: stieda body, SSB: sub-stieda body, SR: sporocyst residuum.

AG, Leverkusen, Germany) was orally administered on February 7, 2019 based on previous reports [15, 20]. After treatment, no oocysts were found in the feces on February 22 and March 6, 2019. This animal was previously bred with other veiled chameleons, one of which had shed oocysts in its feces on August 26, 2018 (although without clinical symptoms) and was subsequently cured with toltrazuril.

For identification of *Isospora* sp., feces were collected for several weeks before treatment. Oocysts were purified from the feces by the sucrose flotation method and allowed to sporulate in 2.5% (w/v) potassium dichromate solution at 26°C for approximately 1 week, as previously reported [11]. After sporulation, the resulting cells were stored at 4°C until further characterization. Sporulated oocysts were observed under light or differential interference contrast microscopy at 1,000× magnification. The internal structures of the isolates were analyzed by examination of 50 oocysts.

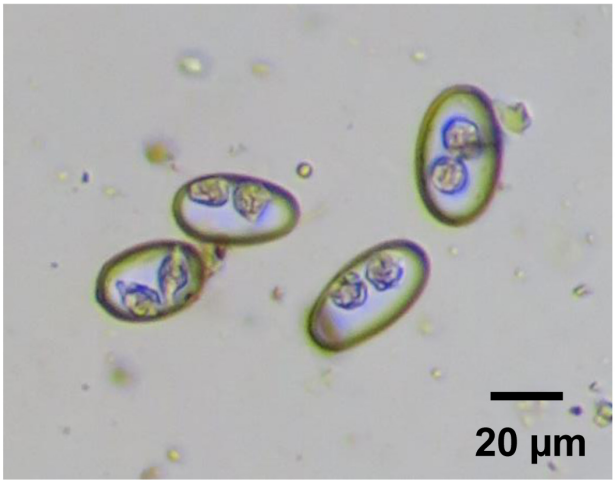

Fig. 1. *Isospora* oocysts detected in the feces of a veiled chameleon.

Twenty-eight single oocysts of the purified *Isospora* sp. isolate were obtained using disposable glass capillary micropipettes and processed as templates for polymerase chain reaction (PCR) based on previously reported methods [4]. Molecular identification of *Isospora* sp. was performed by PCR and sequencing using previously reported primer pairs targeting the 18*S* ribosomal RNA (rRNA) gene [6]. Phylogenetic trees were constructed as described previously [4]. Briefly, sequences were aligned using Clustal X (Version 2.0) [5], and all gaps were deleted. Maximum likelihood analyses with 500 bootstrap replicates were performed using the MEGA software package (version 10.0) [18], and a phylogenetic tree was constructed using the substitution model with optional parameters of the Tamura-Nei model with (G+I) distribution [17].

The oocysts isolated from the veiled chameleon were ovoidal to ellipsoidal in shape without polar granules, oocyst residuum, or micropyle (Table 1). The mean dimensions of the oocysts were 35.5 (range, 28.2–42.4) μm × 23.4 (19.4–27.4) μm with a mean length/width (L/W) ratio of 1.5 (1.14–1.99). Although all oocysts were not clearly sporulated after incubation at 26°C, the sporocysts were 14.0 (12.6–16.1) μm × 11.3 (10.0–13.0) μm with a mean L/W ratio of 1.27 (1.11–1.56). A stieda body and sub-stieda body were observed in the sporocysts. Based on the comparison of the ten species of *Isospora* previously detected in Chamaeleonidae, as summarized in Table 1, this isolate was most similar to an isolate of *I. jaracimrmani*.

Six samples of the 28 single oocysts with primers targeting 18S rRNA were successfully amplified in the PCR analyses, and no differences were observed in the sequences among these samples (Accession No. LC617200). BLAST searches of the GenBank database revealed a nucleotide identity of 99.9% with *I. takydromi* (Accession No. KU180238) isolated from *Takydromus*

sexlineatus (family Lacertidae) and 99.8% from *I. abdallahi* (Accession No. KU180240) isolated from *Acanthodactylus boskianus* (family Lacertidae). We then constructed a phylogenetic tree using the 18S rRNA gene sequence obtained in the present study and published the sequences of related parasites. The sequence obtained in the present study was placed in a clade with the closely related *Isospora* spp. from reptiles (Fig. 2).

Isospora spp. that infect lizards are thought to show a high degree of host specificity [3], and more than 100 species of *Isospora* spp. have been described from reptiles, mainly based on the morphological data of oocysts and the host animal species [8]. The isolate in the present study was morphologically similar to *I. jaracimrmani*, which has previously been suggested to show pathogenicity [11, 12]. Although oocysts were not collected from the previously treated chameleon, both the previous case and the veiled chameleon in the present study did not show severe symptoms before administration of coccidiostats. Although these animals may have been lightly infected, this species of *Isospora* might not always cause the disease. One of the possible transmission routes might be breeding environments, including soils contaminated with oocysts. Although no sequence data of *Isospora* spp. from members of the Chamaeleonidae are available, the sequence from the isolate was placed in the cluster of *Isospora* spp. from reptiles of other families. However, compared to data available for other species of Coccidia (e.g., *Eimeria* spp.), the sequence data of *Isospora* spp. are largely lacking and, thus, there is a necessity of molecular analysis of the isolates and of other gene loci for understanding the classification or identification of parasites and for further evaluation of pathogenicity.

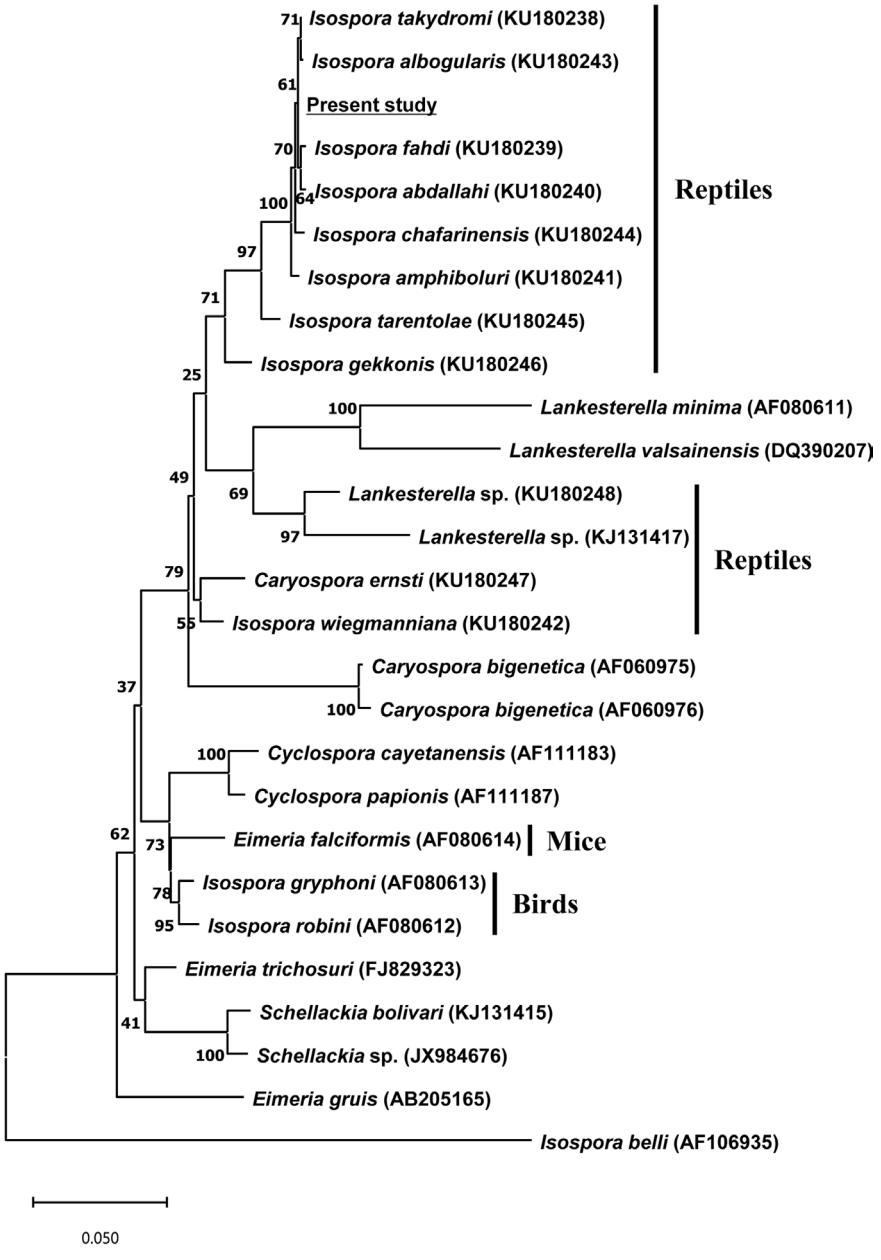

Fig. 2. Phylogenetic tree based on partial 18S rRNA gene sequences (approximately 1,600 bp) from *Isospora* sp. isolated from a veiled chameleon in the present study and related parasites based on the maximum likelihood method. The GenBank accession number for each isolate is shown in parentheses, and the known host of each parasite is indicated in the right side of the figure. The scale bar represents the number of substitutions per nucleotide, and the numbers below the branches indicate bootstrap values (>50% from 500 pseudo-replicates). Substitution model and optional parameters: TN93+G+I.

POTENTIAL CONFLICTS OF INTEREST. The authors declare that they have no conflicts of interest.

ACKNOWLEDGMENTS. The authors gratefully acknowledge Rika Sekiguchi and Noriko Asama (Osaka Prefecture University) for their help with fecal and molecular examinations. Financial support for this study was provided by a Grant-in-Aid from the Japan Society for the Promotion of Science (JSPS) (Grant No. 19H04319).

REFERENCES

1. Abdel-Wasae, B. M. 2004. *Isospora taizii* (Apicomplexa: Eimeriidae), a new coccidian parasite from the Yemen chameleon (*Chameleon calyptratus*) (Sauria: Chamaele-onidae) in Taiz City, Yemen Republic. *Assiut Univ. Bull. Environ. Res.* **7**: 29–34.
2. Brygoo, E. R. 1963. Contribution a la connaissance de laparasitologie des cameleons malgaches. *Ann. Parasitol. Hum. Comp.* **38**: 525–739. [Medline] [CrossRef]
3. Duszynski, D. W., Upton, S. J. and Couch, L. 2008. Coccidia (*Eimeria* and *Isospora*) of Sauria. In: The Coccidia of the world. http://biology.unm.edu/coccidian/sauria.html [accessed on March 12, 2021].
4. Ekawasti, F., Kitagawa, K., Domae, H., Wardhana, A. H., Shibahara, T., Uni, S., Tokoro, M., Sasai, K. and Matsubayashi, M. 2020. Molecular identification of *Eimeria hestermani* and *Eimeria prionotemni* from a red-necked wallaby (Macropodidae; *Macropus rufogriseus*) in Japan. *Parasitol. Res.* **119**: 1271–1279. [Medline] [CrossRef]
5. Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., Valentin, F., Wallace, I. M., Wilm, A., Lopez, R., Thompson, J. D., Gibson, T. J. and Higgins, D. G. 2007. Clustal W and Clustal X version 2.0. *Bioinformatics* **23**: 2947–2948. [Medline] [CrossRef]
6. Matsubayashi, M., Carreno, R. A., Tani, H., Yoshiuchi, R., Kanai, T., Kimata, I., Uni, S., Furuya, M. and Sasai, K. 2011. Phylogenetic identification of *Cystoisospora* spp. from dogs, cats, and raccoon dogs in Japan. *Vet. Parasitol.* **176**: 270–274. [Medline] [CrossRef]
7. McAllister, C. T. 2012. Two new species of *Isospora* Schneider, 1881 (Apicomplexa: Eimeriidae) from the flap-necked chameleon *Chamaeleo dilepis* (Sauria: Chamaeleonidae) in the Republic of Namibia. *Syst. Parasitol.* **83**: 15–20. [Medline] [CrossRef]
8. Megía-Palma, R., Martínez, J., Nasri, I., Cuervo, J. J., Martín, J., Acevedo, I., Belliure, J., Ortega, J., García-Roa, R., Selmi, S. and Merino, S. 2016. Phylogenetic relationships of *Isospora, Lankesterella*, and *Caryospora* species (Apicomplexa: Eimeriidae) infecting lizards. *Org. Divers. Evol.* **16**: 275–288. [CrossRef]
9. Melero, A., Novellas, R., Mallol, C., Ríos, J., Silvestre, A. M. and Martorell, J. 2020. Ultrasonographic appearance of the coelomic cavity organs in healthy veiled chameleons (*Chamaeleo calyptratus*) and panther chameleons (*Furcifer pardalis*). *Vet. Radiol. Ultrasound* **61**: 58–66. [Medline] [CrossRef]
10. Modrý, D., Daszak, P., Volf, J., Veselý, M., Ball, S. J. and Koudela, B. 2001. Five new species of coccidia (Apicomplexa: Eimeriidae) from Madagascan chameleons (Sauria: Chamaeleonidae). *Syst. Parasitol.* **48**: 117–123. [Medline] [CrossRef]
11. Modrý, D. and Koudela, B. 1995. Description of *Isospora jaracimrmani* sp. n. (Apicomplexa: Eimeriidae) from the Yemen chameleon *Chamaeleo calyptratus* (Sauria: Chamaeleonidae). *Folia Parasitol. (Praha)* **42**: 313–316.
12. Modrý, D. and Koudela, B. 1998. Isosporan infections of *Chamaeleo calyptratus* represent growing problem for its breeding in captivity. *Reptile Amphibian Mag* **54**: 38–41.
13. Modrý, D., Koudela, B. and Volf, J. 1997. Four new species of *Isospora* Schneider, 1881 (Apicomplexa: Eimeriidae) from reptiles from the islands of Seychelles. *Syst. Parasitol.* **37**: 73–78. [CrossRef]
14. Modrý, D., Slapeta, J. R. and Koudela, B. 2000. Six new species of coccidia (Apicomplexa: Eimeriidae) from East African chameleons (Sauria: Chamaeleonidae). *J. Parasitol.* **86**: 373–379. [Medline] [CrossRef]
15. Modrý, D. and Sloboda, M. 2004. Control of coccidiosis in chameleons using toltrazuril -results of an experimental trial-. pp. 93–99. In: Proceedings of the 7th International Symposium on Pathology and Medicine in Reptiles and amphibians (Seybold, J. and Mutschmann F. eds.), Edition Chimaira, Berlin.
16. Sergent, M. E. 1902. Sur une coccidie nouvelle, parasite du cameleon vulgaire. *C. R. Sci. Soc. Biol.* **54**: 1260–1261 (Paris).
17. Tamura, K. and Nei, M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol. Biol. Evol.* **10**: 512–526. [Medline]
18. Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.* **30**: 2725–2729. [Medline] [CrossRef]
19. Uga, S., Matsuo, J., Kono, E., Kimura, K., Inoue, M., Rai, S. K. and Ono, K. 2000. Prevalence of *Cryptosporidium parvum* infection and pattern of oocyst shedding in calves in Japan. *Vet. Parasitol.* **94**: 27–32. [Medline] [CrossRef]
20. Walden, M. R. Characterizing the epidemiology of *Isospora amphiboluri* in captive bearded dragons (*Pogona Vitticeps*). 2009. Ph.D. thesis Louisiana State University. LSU Doctoral Dissertations, Baton Rouge, etd-05292009–214931.