International Journal for Parasitology: Parasites and Wildlife 18 (2022) 194–200

Contents lists available at ScienceDirect

International Journal for Parasitology: Parasites and Wildlife

journal homepage: www.elsevier.com/locate/ijppaw

Three new species of *Eimeria* (Apicomplexa: Eimeriidae) from the Amami rabbit, *Pentalagus furnessi* (Mammalia: Leporidae)

Toshihiro Tokiwa [a,*], Shyun Chou [a], Hina Kitazoe [a], Keiko Ito [b], Ryouta Torimoto [c], Yuki Shoshi [d], Chizu Sanjoba [d], Masami Yamamoto [e], Hisashi Yoshimura [e]

[a] *Laboratory of Veterinary Parasitology, School of Veterinary Medicine, Faculty of Veterinary Science, Nippon Veterinary and Life Science University, 1-7-1 Kyonancho, Musashino, Tokyo, 180-0023, Japan*
[b] *Amami Dog & Cat Animal Hospital, Nakagachi, Tatsugocho, Oshima, Kagoshima, 894-0106, Japan*
[c] *Yuinoshima Animal Clinic, Nazeishibashicho, Amami, Kagoshima, 894-0017, Japan*
[d] *Laboratory of Molecular Immunology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Yayoi, Bunkyo, Tokyo, 113-8657, Japan*
[e] *Laboratory of Physiological Pathology, Department of Applied Science, School of Veterinary Nursing and Technology, Nippon Veterinary and Life Science University, Kyonancho, Musashino, Tokyo, 180-8602, Japan*

ARTICLE INFO

Keywords:
Amami rabbit
Eimeria
Eimeriidae
Endangered species
Pentalagus furnessi
Leporidae

ABSTRACT

The Amami rabbit, *Pentalagus furnessi* (Mammalia: Lagomorpha: Leporidae), is a relict and endangered species endemic to the Amami-Oshima and Tokunoshima Islands, located in southwestern Japan. Here, we described three new species of *Eimeria* (Apicomplexa: Eimeriidae) parasites detected from fecal samples of wild Amami rabbits. *Eimeria furnessi* n. sp., recorded in 21 (58.3%) samples, has ellipsoidal oocysts with two walls and micropyle, 26.0 × 16.6 μm, and elongate-ovoidal sporocysts, 13.1 × 6.3 μm, with Stieda body. *Eimeria hilleri* n. sp., recorded in 9 (25.0%) samples, has ellipsoidal oocysts with two walls and micropyle, 34.7 × 21.4 μm, and elongate-fusiform to elongate-ovoidal sporocysts, 15.7 × 8.3 μm, with Stieda and substieda bodies. *Eimeria sagentae* n. sp., recorded in 13 (36.1%) samples, has ellipsoidal oocysts with two walls and micropyle, 20.9 × 14.5 μm, and elongate-ovoidal sporocysts, 10.4 × 5.0 μm, with Stieda body. The three new species can be distinguished by the size and color of their oocysts. Further studies related to the pathogenicity of these parasites can improve the breeding and propagation procedures of the Amami rabbit.

1. Introduction

Pentalagus is a lagomorph genus that is comprised of a single species the Amami rabbit, *Pentalagus furnessi*, and no extinct species are known. It is endemic on the Amami-Oshima and Tokunoshima Islands in the Ryukyu Archipelago, a string of islands in southwestern Japan located between Kyushu and Taiwan (Sugimura et al., 2000). The Amami rabbit primarily inhabits the forest on both islands; however, after deforestation in the 1970s and 1980s, it appeared in both cut-over areas and forest edges (Sugimura et al., 2003; Yamada and Cervantes, 2005). Currently, it is categorized as an endangered species by the International Union for Conservation of Nature Red List of Threatened Species because of its rapid population decline due to diminishing habitats and predation by feral and stray cats and dogs (Yamada, 2015).

Eimeria (Apicomplexa: Eimeriidae) are obligate, typically host-specific, intracellular parasite. More than 50 *Eimeria* species have been described in the family Leporidae, and each is host specific, at least to a particular genus (Duszynski and Couch, 2013). Kamiya et al. (1987) reported several *Eimeria* spp. from *P. furnessi* individuals from the Amami-Oshima Island; however, did not identify to species or provide detailed morphologic descriptions of the oocysts they recovered. In 2020, we identified by histopathological examination a coccidian infection in a wild Amami rabbit that had died following a *Staphylococcus aureus* infection. This finding led us to collect fecal samples and intestinal contents from wild Amami rabbits to determine species of coccidian present and their prevalence in the wild rabbit population.

2. Materials and methods

2.1. Ethics statement

The Amami rabbits that inhabit the Amami-Oshima and

* Corresponding author.
E-mail address: tokiwa@nvlu.ac.jp (T. Tokiwa).

https://doi.org/10.1016/j.ijppaw.2022.05.006
Received 17 April 2022; Received in revised form 25 May 2022; Accepted 26 May 2022
Available online 30 May 2022

Tokunoshima Islands are designated as a special national treasure and are protected by the Law for the Protection of Cultural Properties. Thus, the sheltering, treatment, and necropsy of rabbit was conducted by veterinarians under the permission of the Ministry of the Environment, Japan. Additionally, fecal samples of Amami rabbits were collected in the field. The sampling in the regulated area (Yamakubiri Forest Road) of the Tokunoshima Island was conducted under the research permission of the Tokunoshima Town office. No animals were harmed during sampling.

2.2. Histopathological analysis

In August 2020, one debilitated Amami rabbit was taken into the Animal clinic at the Amami-Oshima Island. *Staphylococcus aureus* was isolated from an abscess on its body; however, the rabbit died after unsuccessful treatment. A necropsy was performed at the clinic, and tissue samples of the liver, spleen, lungs, kidneys, adrenal grands, heart, and digestive tracts were fixed in 10% neutral buffered formalin and processed into paraffin blocks for routine histopathologic procession at the Nippon Veterinary and Life Science University. Paraffin-embedded tissues were sectioned at 5 μm and stained with hematoxylin and eosin (HE) for microscopic examination with BX53 microscope (Olympus, Japan). Photomicrographs were captured using a DP74 (Olympus, Japan) or NanoZoomer-SQ Digital slide scanner (Hamamatsu Photonics, Japan).

2.3. Fecal samples of Amami rabbits

From August 2020 to November 2020, 30 fecal samples of Amami rabbits were collected from the Kinsakubal Forest (28°20′ N, 129°26′ E) (n = 20) and Yamakubiri Forest Road (27°52′ N, 128°56′ E) (n = 10) on Amami-Oshima and Tokunoshima Islands, respectively (Fig. 1). Fecal mass with 10–20 wet fecal pellets was considered as feces discharged by one individual. The sampling localities and dates are listed in Table 1. Additionally, intestinal contents were collected from six Amami rabbits that died at two animal clinics from December 2020 to May 2021 on Amami-Oshima Island.

Fecal samples and intestinal contents were placed in separate vials, transported at room temperature for 3–5 days, and examined by direct smear and sucrose flotation procedures. The presence of oocysts was examined under a BX41 microscope (Olympus) with 100 × and 400 × magnification. Oocyst-positive specimens were placed in separate vials with a 2.5% (w/v) potassium dichromate ($K_2Cr_2O_7$) solution. Oocyst sporulation was induced by incubating at 23–26 °C, and the specimens were stored at 4 °C.

2.4. Morphological examination

Oocysts in $K_2Cr_2O_7$ solution were observed under a BX41 microscope (Olympus) with differential interference contrast. Photomicrographs were captured using a DP27 Photomicroscope (Olympus). Sporulated oocysts (n = 30) were measured with ImageJ software ver. 1.53 (Schneider et al., 2012) using pictures taken under an oil immersion objective (1000 × magnification). The measurements were expressed in micrometers (μm) with the ranges followed by the means in parentheses. The oocysts and sporocysts were described following the standard guidelines (Duszynski and Wilber, 1997; Wilber et al., 1998; Berto et al., 2014) including: oocyst length (L) and width (W), their ranges and ratios (L/W), micropyle (M), nucleus (N), oocyst residuum (OR), polar granule (PG), sporocyst (SP), Stieda body (SB), substieda body (SSB), parastieda body (PSB), sporocyst residuum (SR), refractile body (RB), and sporozoite (SZ).

3. Results

3.1. General findings

The histopathological examination of the deceased Amami rabbit revealed coccidial infections in the jejunum and ileum (Fig. 2). In the jejunum, few immature gamonts were found in the epithelial cells. In the ileum, numerous gametocytes, unsporulated oocysts, and few schizont-like structures were observed mixed with debris of the epithelial cells in the lumen. These parasite stages could not be traced to specific tissue sites due to postmortem changes. Nematode infections of *Lagostrongylus* and *Obeliscoides*, common nematode parasites of Amami rabbits (Fukumoto, 1986; Yamada et al., 2012), were observed in the small intestine and stomach, respectively. In addition, fungal infections were found in the esophagus, lungs, and liver.

Microscopic examination of the intestinal contents and fecal samples revealed that 72.2% (26/36, 95% CI 55.9–84.3%) of the samples were positive for *Eimeria* oocysts (Table 1). The oocysts could be classified into three types (Types A for medium, B for large, and C for small) based on their morphological characteristics, and a mixed infection was found in 63.9% (23/36) of the samples. These three types represented three new species, as described in the following sections.

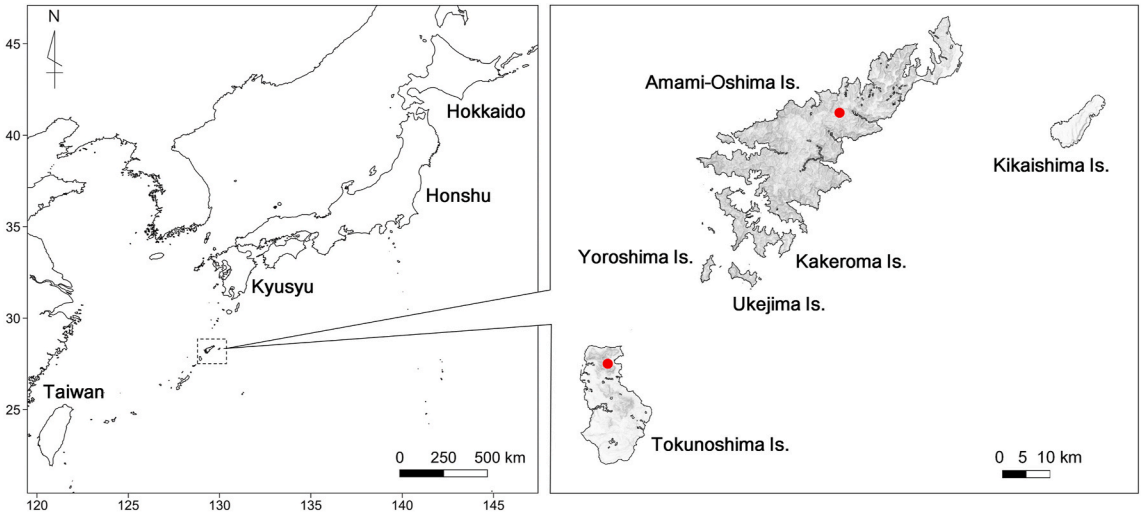

Fig. 1. Map showing the location of the Amami-Oshima and Tokunoshima Islands. Circles indicate the sites where fecal samples of Amami rabbits were collected in the field.

Table 1
Prevalence of microscopic detection of *Eimeria* species in fecal samples of the Amami rabbit.

Localities	Origin	Date of sampling	No. of samples examined	No. of oocyst-positive samples				
				Total	Type A	Type B	Type C	Mixed
Amami-Oshima Is.	Field	Nov. 2020	5	3	3	2	2	4
	Field	Jan. 2021	15	12	10	4	5	10
	Intestinal contents	Dec. 2020 to May 2021	6	3	2	0	2	2
Tokunoshima Is.	Field	Oct. 2020	10	8	6	3	4	7
		Total (%)	36	26 (72.2%)	21 (58.3%)	9 (25.0%)	13 (36.1%)	23 (63.9%)

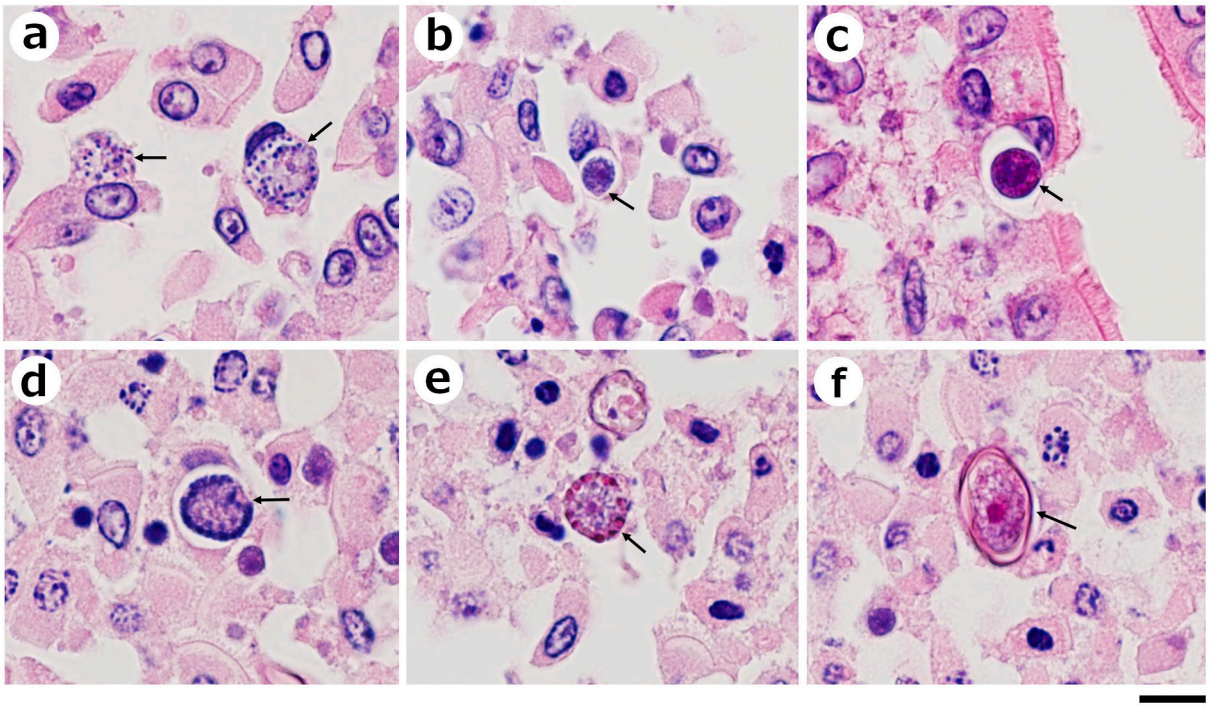

Fig. 2. Endogenous stages of coccidian parasites found in the section of the jejunum and ileum of the Amami rabbit. HE. Scale bar = 20 μm and applies to all parts. **(a)** Schizont-like structures (arrows) in detached epithelial cells. **(b)** Immature microgamont (arrow) with basophilic small nuclei. **(c)** Immature macrogamont (arrow) with eosinophilic bodies. **(d)** Microgamont (arrow) with peripherally located basophilic nuclei. **(e)** Macrogamont (arrow) with peripheral arrangement of eosinophilic wall-forming bodies. **(f)** Unsporulated oocyst (arrow) with a central nucleus and oocyst wall. High-resolution version of these slides for use with the Virtual Microscope are available as eSlide: VM06653.

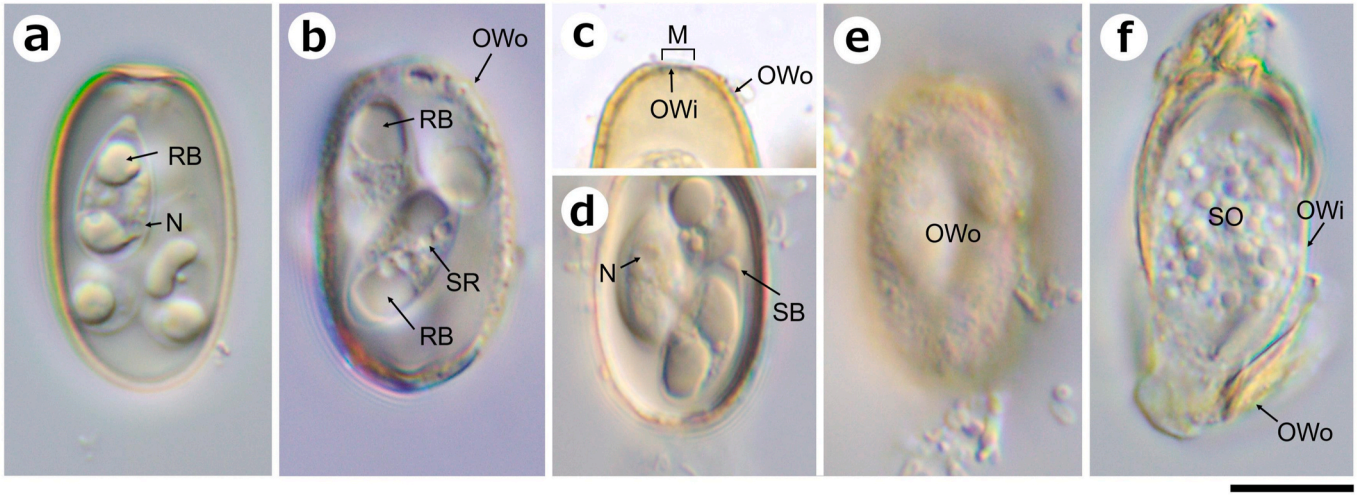

Fig. 3. Nomarski interference contrast photographs of oocysts of *Eimeria furnessi* from the Amami rabbit stored in a potassium dichromate solution. M: micropyle; N: nucleus; OWo: outer layer of the wall; OWi: inner layer of the wall; RB: refractile body; SB: Stieda body; SO: sporont; SR: sporocyst residuum. Scale bars (all in the same scale) = 10 μm.

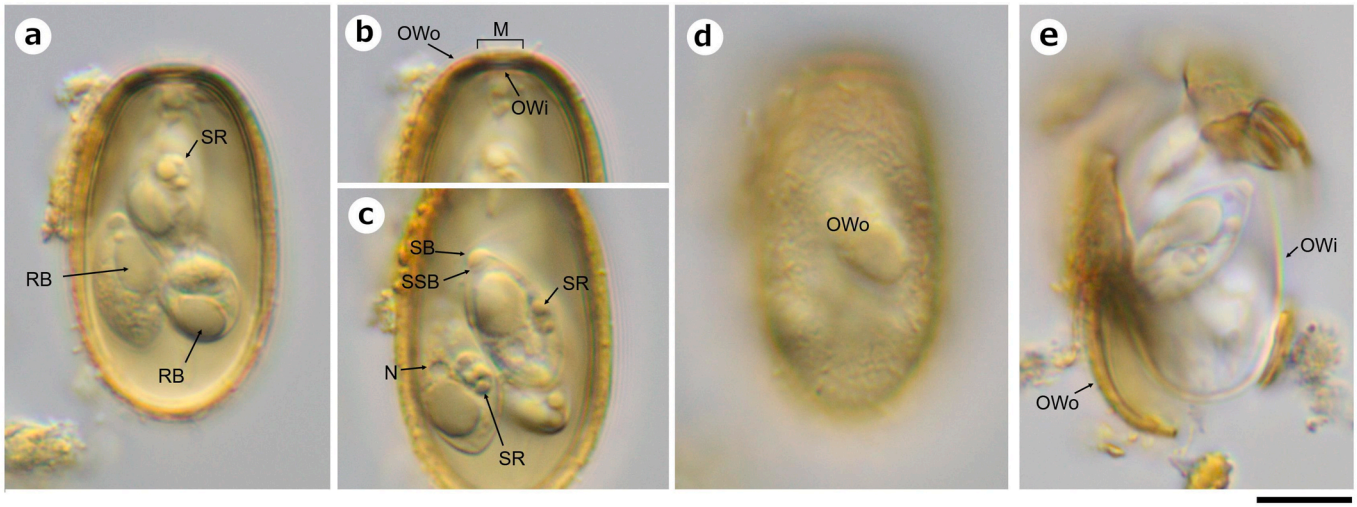

Fig. 4. Nomarski interference contrast photographs of oocysts of *Eimeria hilleri* from the Amami rabbit stored in a potassium dichromate solution. M: micropyle; N: nucleus; OWo: outer layer of the wall; OWi: inner layer of the wall; RB: refractile body; SB: Stieda body; SR: sporocyst residuum; SSB: substieda body. Scale bars (all in the same scale) = 10 μm.

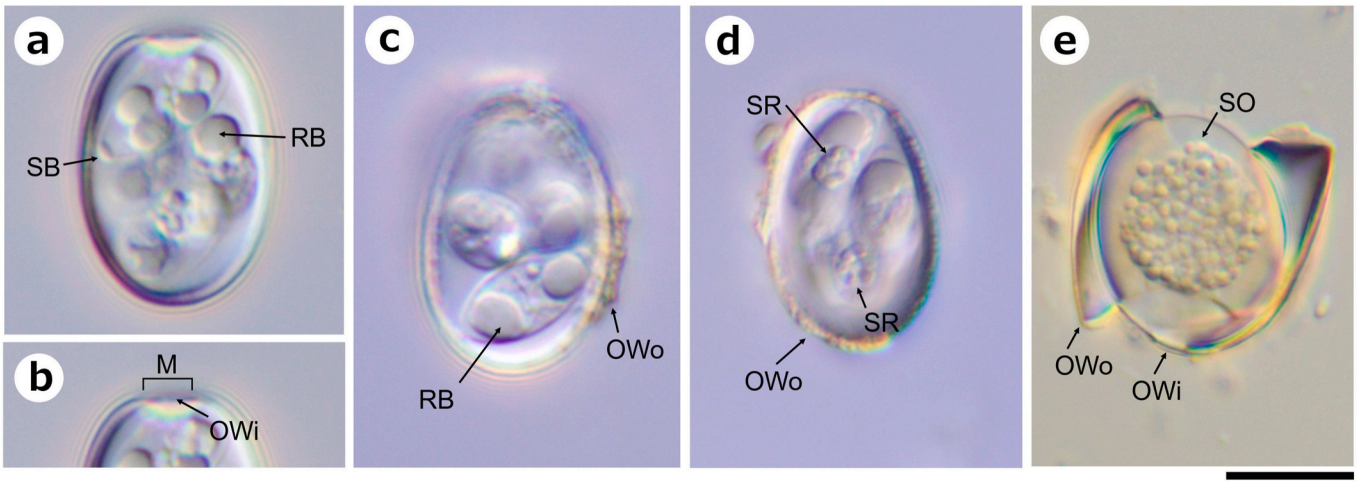

Fig. 5. Nomarski interference contrast photographs of oocysts of *Eimeria sagentae* from the Amami rabbit stored in a potassium dichromate solution. M: micropyle; N: nucleus; OWo: outer layer of the wall; OWi: inner layer of the wall; RB: refractile body; SB: Stieda body; SO: sporont; SR: sporocyst residuum. Scale bars (all in the same scale) = 10 μm.

3.2. *Eimeria furnessi n. sp.* (*Figs. 3 and 6a*)

3.2.1. *Description*

Oocyst shape: ellipsoidal; number of walls: 2; wall thickness: 1.1 (0.9–15); wall characteristics: outer is rough, yellowish, finely granular, irregular thickness, and detached readily and/or can be removed, inner is smooth and transparent; L × W: 26.0 × 16.6 (22.9–30.3 × 14.9–18.5); L/W ratio: 1.6 (1.3–1.7); M: present; M characteristics: present in the outer layer of the wall, 4.0 (3.2–5.7) wide; OR and PG: both absent.

Sporocyst shape: elongate-ovoidal; L × W: 13.1 × 6.3 (12.0–13.7 × 5.1–7.4); L/W ratio: 2.1 (1.8–2.4); SB: present; SB characteristics: knob-like; SSB and PSB: both absent; SR: present; SR characteristics: a compact mass of large granules or a few scattered granules between SZ; SZ: elongate-ovoidal and lies head-to-tail with one large RB at the wider end.

3.2.2. *Taxonomic summary*

Type host: Mammalia (Lagomorpha: Leporidae), *P. furnessi* (Stone, 1900), Amami rabbit.

Other hosts: None to date.

Type locality: Amami-Oshima and Tokunoshima Islands, Kagoshima, Japan.

Site of infection: Unknown. Oocysts were recovered from feces and intestinal contents.

Sporulation: Exogenous. The exact hours were unknown, but oocysts became fully sporulated within 5 days at 23–26 °C.

Prevalence: 58.3% (21/36, 95% CI 42.2–72.9%) of the type host.

Prepatent and patent periods: Unknown.

Materials deposited: Photosyntype (Bandoni and Duszynski, 1988) of sporulated oocysts was deposited in the Meguro Parasitological Museum, Meguro, Tokyo, Japan, under MPM Coll. No. 21857.

ZooBank LSID: The ZooBank LSID is 6E429959-1DB0-41ED-AD8B-703423E0A3FE.

Etymology: Specific epithet "*furnessi*" is adopted from the name of Dr. William Henry Furness III (1866–1920), one of the discoverers of the Amami rabbit; it is given as a noun in the genitive case, in accordance to the Article 31.1. of the International Code of Zoological Nomenclature (ICZN) (International Commission on Zoological Nomenclature, 1999).

Remarks: Sporulated oocysts of *E. furnessi* resemble, in shape, size, and the presence of two walls and micropyle, those of *Eimeria*

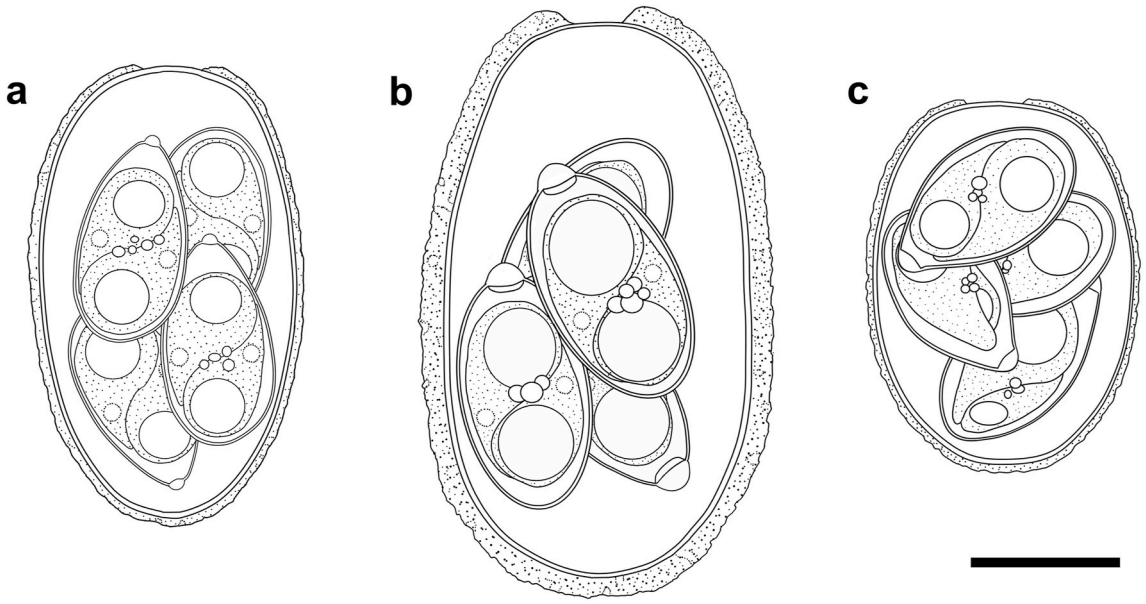

Fig. 6. Line drawings of the *Eimeria* species detected in the Amami rabbit. **(a)** *Eimeria furnessi*. **(b)** *Eimeria hilleri*. **(c)** *Eimeria sagentae*. Scale bar = 10 μ

matsubayashii [24.8 × 18.3 (22–30 × 14–22)], recorded from *Oryctolagus cuniculus* (Tsunoda, 1952), and those of *Eimeria neoirresidua* [25.7 × 17.9 (19–31 x 15–20)] (Duszynski and Marquardt, 1969) and *Eimeria poudrei* [26.0 × 18.1 (20–31 × 15–21)] (Duszynski and Marquardt, 1969), detected from *Sylvilagus audubonii*. Sporulated oocysts of *E. furnessi* differed from these three species by its rough outer layer. Furthermore, *E. matsubayashii* oocysts differed from those of *E. furnessi* by the presence of OR and by having smaller sporocysts (7 × 6). Oocysts of *E. neoirresidua* and *E. poudrei* differed from those of *E. furnessi* by having larger sporocysts (14.5 × 6.4 for *E. neoirresidua* and 14.4 × 6.4 for *E. poudrei*).

3.3. *Eimeria hilleri n. sp.* (Figs. 4 and 6b)

3.3.1. Description

Oocyst shape: ellipsoidal; number of walls: 2; wall thickness: 2.0 (1.5–2.5); wall characteristics: outer is rough, yellowish-brown, and easily detached, inner is smooth and light yellow; L × W: 34.7 × 21.4 (30.6–41.2 × 15.2–24.3); L/W ratio: 1.6 (1.5–2.0); M: present; M characteristics: in the outer layer of the wall, 3.9 (3.3–5.1) wide; OR and PG: both absent.

Sporocyst shape: elongate-fusiform to elongate-ovoidal; L x W: 15.7 × 8.3 (14.5–17.3); L/W ratio: 1.9 (1.7–2.1); SB: present; SB characteristics: nipple-like; SSB: present; SSB characteristics: narrow and rounded; PSB: absent; SR: present; SR characteristics: a compact mass of large granules or a few scattered granules between SZ; SZ: elongated with one end wider than the other, lying length-wide head-to-tail, and with a clear RB posterior to N.

3.3.2. Taxonomic summary

Type host: Mammalia (Lagomorpha: Leporidae), *P. furnessi* (Stone, 1900), Amami rabbit.

Other hosts: None to date.

Type locality: Amami-Oshima and Tokunoshima Islands, Kagoshima, Japan.

Site of infection: Unknown. Samples were obtained from feces.

Sporulation: Exogenous. The exact hours were unknown, but oocysts became fully sporulated within 5 days at 23–26 °C.

Prevalence: 25.0% (9/36, 95% CI 13.6–41.3%) of the type host.

Prepatent and patent periods: Unknown.

Materials deposited: Photosyntype of sporulated oocysts was deposited in the Meguro Parasitological Museum, Meguro, Tokyo, Japan, under MPM Coll. No. 21858.

ZooBank LSID: The ZooBank LSID is 9038A261-915C-4D4C-BF9F-7160D8A6FE40.

Etymology: Specific epithet "*hilleri*" is adopted from the name of Dr. Hiram Milliken Hiller (1867–1921), one of the discoverers of the Amami rabbit; it is given as a noun in the genitive case, in accordance to the Article 31.1. of the ICZN.

Remarks: Sporulated oocysts of *E. hilleri* resemble, in shape, the size, and the presence of two walls and micropyle, of *Eimeria gobiensis* [38.6 × 24.2 (27–49 × 19–32)] (Gardner et al., 2009), *Eimeria pierrecouderti* [35 × 24 (33–37 × 23–26] (Aoutil et al., 2005), *Eimeria vejdovskyi* [32.9 × 19.2 (30–37 × 18–21)] (Pakandl, 1988), and *Eimeria stiedai* [37 × 20 (31–42 × 17–25)] (Duszynski and Couch, 2013) detected from lagomorphs outside the *Pentalagus* genus. Oocysts of the first three species have a distinct OR, which those of *E. hilleri* lack, whereas *E. stiedai* oocysts have a few small OR, thin outer layer, and its sporocysts [18 × 10 (17–18 × 8–10)] (Duszynski and Couch, 2013) are larger than those of *E. hilleri*.

3.4. *Eimeria sagentae n. sp.* (Figs. 5 and 6c)

3.4.1. Description

Oocyst shape: ellipsoidal; number of walls: 2; wall thickness: 1.1 (0.6–1.5); wall characteristics: outer is rough, light yellowish, finely granular, and easily detached, inner is smooth and transparent; L × W: 20.9 × 14.5 (18.8–23.0 × 12.3–16.9); L/W ratio: 1.4 (1.3–1.6); M: present; M characteristics: in the outer layer of the wall, 3.9 (3.2–5.6) wide; OR and PG: both absent.

Sporocyst shape: elongate-ovoidal; L x W: 10.4 × 5.0 (8.7–11.7 × 4.3–5.9); L/W ratio: 2.1 (1.7–2.4); SB: present; SB characteristics: nipple-like; SSB and PSB: both absent; SR: present; SR characteristics: a compact mass of large granules or a few scattered granules between SZ; SZ: elongated with one end wider than the other, lying length-wide head-to-tail, and with a clear RB posterior to N.

3.4.2. Taxonomic summary

Type host: Mammalia (Lagomorpha: Leporidae), *P. furnessi* (Stone, 1900), Amami rabbit.

Other hosts: None to date.

Type locality: Amami-Oshima and Tokunoshima Islands,

Kagoshima, Japan.

Site of infection: Unknown. Samples were obtained from feces and intestinal contents.

Sporulation: Exogenous. The exact hours were unknown, but oocysts became fully sporulated within 5 days at 23–26 °C.

Prevalence: 36.1% (13/36, 95% CI 22.4–52.5%) of the type host.

Prepatent and patent periods: Unknown.

Materials deposited: Photosyntype of sporulated oocysts was deposited in the Meguro Parasitological Museum, Meguro, Tokyo, Japan, under MPM Coll. No. 21859.

ZooBank LSID: The ZooBank LSID is 82404C1B-EC2A-4C53-90BD-876D1548687C.

Etymology: Specific epithet "*sagentae*" is adopted from the name of Mr. Sagenta Nagoya (1820–1881), the author of "Nanto-Zatsuwa," an ethnography published in 1855 that describes the nature and culture of the Amami-Oshima Island; it is given as a noun in the genitive case, in accordance to Article 31.1. of the ICZN.

Remarks: Sporulated oocysts of *E. sagentae* resemble, in shape, the size, presence of two walls, and the lack of OR, of *Eimeria audubonii* [21.2 × 17.1 (15–25 × 13–20)], detected from *Sylvilagus audubonii* (Duszynski and Marquardt, 1969), and of *Eimeria rowani* [22.1 × 15.8 (18–31 × 12–18)], detected from *Lepus americanus* (Samoil and Samuel, 1977). However, *E. rowani* oocysts differ from those of *E. sagentae* by the absence of M.

4. Discussion

Coccidian parasites of the genus *Eimeria* commonly infect species of the family Leporidae. This family includes approximately 11 extant genera (*Brachylagus*, *Bunolagus*, *Caprolagus*, *Lepus*, *Nesolagus*, *Oryctolagus*, *Pentalagus*, *Poelagus*, *Pronolagus*, *Romerolagus*, and *Sylvilagus*) with over 60 distinct species; 7 of them (*Brachylagus*, *Bunolagus*, *Caprolagus*, *Oryctolagus*, *Pentalagus*, *Poelagus*, and *Romerolagus*) are monotypic (Hoffmann and Smith, 2005). Rabbit eimerians have homoxenous life cycles and to date, 57 valid *Eimeria* species are included in the family Leporidae (Pellérdy, 1974; Pakandl, 2009; Duszynski and Couch, 2013; Cui et al., 2017). Most *Eimeria* species (78.9%; 45/57) are mesostenoxenous (multiple host, but restricted to one host genus) or oioxenous (only one host species), with 29 species recorded from *Lepus*, 9 species from *Sylvilagus*, 6 species from *Oryctolagus*, and one species form *Brachylagus*. The remaining 12 species (*E. exigua*, *E. irresidua*, *E. leporis*, *E. magna*, *E. matsubayashii*, *E. media*, *E. minima*, *E. perforans*, *E. pierrecouderti*, *E. piriformis*, *E. stiedai*, and *E. sylvilagi*) are euryxenous and were recorded from or have established cross-transmission in *Lepus*, *Sylvilagus*, and/or *Oryctolagus* (Duszynski and Couch, 2013).

Eimeria infections were first reported in Amami rabbits more than 30 years (Kamiya et al., 1987). This is the first report identifying and describing *Eimeria* species present in this host. The three new *Eimeria* species described in this study can be distinguished by the size of their oocysts and sporocyst, and color: *E. furnessi* is medium and yellow; *E. hilleri* is large and yellowish-brown, and *E. sagentae* is small and light yellow. Note that the outer layers with these oocysts are easily fragile and become colorless or light yellow, making them difficult to distinguish. Although *E. furnessi* and *E. hilleri* have similar sizes after removing the outer layer, they can be distinguished by the size of their mature sporocysts.

The genus *Pentalagus* diverged from the genus *Pliopentalagus*, which once inhabited Eurasia (Tomida and Otsuka, 1993). *Pliopentalagus* became extinct from the continent, whereas *Pentalagus* survived in the Ryukyu Islands; therefore, *P. furnessi* is a relict species that is often called a living fossil. The three eimerian species described in this study were commonly found in the Amami-Oshima and Tokunoshima Islands. The rabbits do not migrate between the two islands, suggesting that the eimerian species were already endemic parasites of the Amami rabbit before the islands were split. Molecular and cross-transmission studies will help to determine their phylogenetic position within the genus *Eimeria* and their relationship with other species parasitizing rabbits that do not belong the *Pentalagus*.

The pathogenicity of the three new species to Amami rabbits is unknown. Several species, including *E. intestinalis*, *E. flavescens*, *E. piriformis*, *E. media*, *E. magna*, and *E. stiedai*, are mildly to highly pathogenic for the domestic rabbit, *O. cuniculus* (Pakandl, 2009; Pakandl and Liu, 2020). Since coccidiosis is not commonly observed in the numerous wild Amami rabbits sheltered and treated at clinics in the Amami-Oshima Island (unpublished), these coccidian parasites may not be highly pathogenic to wild individuals. Understanding the pathogenesis may improve the breeding and artificial propagation of endangered animals, as *Eimeria* species can be horizontally transmitted via oocysts, which can be problematic in closed environments. The *Eimeria* species described in this study appeared to be parasites of the intestinal tract; however, further studies are needed to determine their life cycle, pathogenicity and treatment options.

Declaration of competing interest

No conflict of interest declared by any author.

Acknowledgement

We would like to thank staff of Amami Wildlife Conservation Center (Ministry of the Environment), Shunsuke Fukui (Tokunoshima Ranger Station, Ministry of the Environment), Yuya Watari (Forestry and Forest Products Research Institute) and Sono Okada (Laboratory of Biodiversity Science, Graduate School of Agricultural and Life Sciences, The University of Tokyo) for their immense help in collecting fecal samples. We also grateful to the staff at Yuinoshima Animal Clinic for their support in collecting clinical samples. Founding for this study was provided by Japan Society for Promotion of Science (JSPS) KAKENHI Grant Number 21K06323 (TT) and by Pro Natura Foundation Japan's 32nd Pro Natura Fund (TT).

References

Aoutil, N., Bertani, S., Bordes, F., Snounou, G., Chabaud, A., Landau, I., 2005. *Eimeria* (Coccidia: Eimeriidae) of hares in France: description of new taxa. Parasite 12, 131–144.

Bandoni, S.M., Duszynski, D.W., 1988. A plea for improved presentation of type material for coccidian. J. Parasitol. 74, 519–523.

Berto, B.P., McIntosh, D., Lopes, C.W.G., 2014. Studies on coccidian oocysts (apicomplexa: eucoccidiorida). Braz. J. Vet. Parasitol. Jaboticabal 23, 1–15.

Cui, P., Liu, H., Fang, S., Gu, X., Wang, P., Liu, C., Tao, G., Liu, X., Suo, X., 2017. A new species of *Eimeria* (apicomplexa: Eimeriidae) from California rabbits in hebei province, China. Parasitol. Int. 66, 667–680.

Duszynski, D.W., Marquardt, W.C., 1969. *Eimeria* (Protozoa: Eimeriidae) of the cottontail rabbit *Sylvilagus audubonii* in northeastern Colorado, with descriptions of three new species. J. Protozool. 16, 128–137.

Duszynski, D.W., Wilber, P.G., 1997. A guideline for the preparation of species description in the Eimeriidae. J. Parasitol. 83, 333–336.

Duszynski, D.W., Couch, A.L., 2013. The Biology and Identification of the Coccidia (Apicomplexa) of Rabbits of the World. Academic Press (Elsevier), San Diego.

Fukumoto, S., 1986. A new stomach worm, *Obeliscoides pentalagi* n. sp. (Nematoda; Trichostrongyloidea) of Ryukyu rabbits, *Pentalagus furnessi* (Stone, 1900). Syst. Parasitol. 8, 267–277.

Gardner, S.L., Seggerman, N.A., Batsaikhan, N., Ganzorig, S., Tinnin, D.S., Duszynski, D. W., 2009. Coccidia (apicomplexa: Eimeriidae) from the lagomorph *Lepus tolai* in Mongolia. J. Parasitol. 95, 1451–1454.

Hoffmann, R.S., Smith, A.T., 2005. Order Lagomorpha. In: Wilson, D.E., Reeder, D.M. (Eds.), Mammal Species of the World: a Taxonomic and Geographic Reference, third ed. Johns Hopkins Univ. Press, Maryland, pp. 185–211.

International Commission on Zoological Nomenclature, 1999. International Code of Zoological Nomenclature, fourth ed. International Trust for Zoological Nomenclature, London, U.K.

Kamiya, M., Fukumoto, S., Matsuzaki, T., Suzuki, H., 1987. Capturing, rearing and parasites of the Amami rabbit *Pentalagus furnessi* (I). J. Hokkaido Vet. Med Assoc. 31, 221–228 (in Japanese).

Pakandl, M., 1988. Description of *Eimeria vejdovskyi* sp. n. and redescription of *Eimeria media* Kessel, 1929 from the rabbit. Folia Parasitol. 35, 1–9.

Pakandl, M., 2009. Coccidia of rabbit: a review. Folia Parasitol. 56, 153–166.

Pakandl, M., Liu, X., 2020. Coccidiosis in rabbits (*Oryctolagus cuniculus*). In: Dubey, J.P. (Ed.), Coccidiosis in Livestock, Poultry, Companion Animals, and Humans. CRC Press, Florida, pp. 159–168.

Pellérdy, L.P., 1974. Coccidia and Coccidiosis, second ed. Akadémiai Nyomda, Budapest, Hungary.
Samoil, H.P., Samuel, W.M., 1977. Description of nine species of *Eimeria* (Protozoa, Eimeriidae) in the snow-shoe hare, *Lepus americanus,* of central Alberta. Can. J. Zool. 55, 1671–1683.
Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH image to imageJ: 25 years of image analysis. Nat. Methods 9, 671–675.
Sugimura, K., Sato, S., Yamada, F., Abe, S., Hirakawa, H., Handa, Y., 2000. Distribution and abundance of the Amami rabbit *Pentalagus furnessi* in the Amami and Tokunoshima islands, Japan. Oryx 34, 198–206.
Sugimura, K., Yamada, F., Miyamoto, A., 2003. Population trend, habitat change and conservation of the unique wildlife species on Amami Island. Japan. Glob. Environ. Res. 7, 79–89.
Tomida, Y., Otsuka, H., 1993. First discovery of fossil Amami rabbit (*Pentalagus furnessi*) from Tokunoshima, southwestern Japan. Bull. Natn. Sci. Mus., Tokyo, Ser. C 19, 73–79.
Tsunoda, K., 1952. *Eimeria matsubayashii* sp. nov., a new species of rabbit coccidium. Bull. Natl. Inst. Anim. Health 25, 109–119 (in Japanese with English summary).
Wilber, P.G., Duszynski, D.W., Upton, S.J., Seville, R.S., Corliss, J.O., 1998. A revision of the taxonomy and nomenclature of the *Eimeria* spp. (apicomplexa: Eimeriidae) from rodents in the tribe marmotini (sciuridae). Syst. Parasitol. 39, 113–135.
Yamada, F., Cervantes, F.A., 2005. Pentalagus furnessi. Mamm. Species 782, 1–5.
Yamada, S., Yoshida, A., Yoshida, K., Kuraishi, T., Shosaku, H., Kai, C., Nagai, Y., Sakoda, T., Tatara, M., Abe, S., Fukumoto, S., 2012. Phylogenetic relationships of three species within the family Heligmonellidae (Nematoda; Heligmosomoidea) from Japanese rodents and a lagomorph based on the sequences of ribosomal DNA internal transcribed spacers, ITS-1 and ITS-2. Jpn. J. Vet. Res. 60, 15–21.
Yamada, F., 2015. *Pentalagus furnessi* (stone, 1900). In: Ohdachi, D., Ishibashi, Y., Iwasa, A., Fukui, D., Saitoh, T. (Eds.), The Wild Mammals of Japan, second ed. Shoukadoh, Kyoto, Japan, pp. 212–213.