# Description of 3 New Species of *Eimeria* (Apicomplexa: Eimeriidae) from Springbok (*Antidorcas marsupialis*) in Namibia

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ABSTRACT: We document morphological descriptions for 3 newly described *Eimeria* spp. that infect springbok (*Antidorcas marsupialis*). *Eimeria antidorcasi* n. sp. oocysts are ovoid, with average size (n = 346)  $26.2 \times 18.8$  (19.2–33.5  $\times 13.1$ –26.5) µm, a length/width ratio of 1.4 (1.2–2.0), a micropyle, and a polar granule sometimes present. Sporocysts are ovoid (n = 336),  $11.3 \times 7.3$  (6.0–14.6  $\times 5.2$ –9.8) µm, with a length/width ratio of 1.5 (1.1–2.0), a Stieda body, a prominent, ovoid sporocyst residuum, and one refractile body per sporozoite. *Eimeria versfeldi* n. sp. oocysts are ellipsoid (n = 136),  $38.9 \times 26.6$  (27.8–48.2  $\times 21.2$ –31.0) µm, with a length/width ratio of 1.5 (1.2–2.0), a micropyle cap with submicropylar material, and 2–4 polar granules. Sporocysts are elongate-ovoid (n = 132),  $18.3 \times 9.2$  (13.2–22.8  $\times 6.8$ –11.2) µm, with a length/width ratio of 2.0 (1.3–2.5), a Stieda body, a small sporocyst residuum present, and an evident nucleus and two refractile bodies per sporozoite. *Eimeria gasawayi* n. sp. oocysts are subspheroid (n = 87),  $14.8 \times 13.6$  (11.5–20.0  $\times 11.1$ –18.2) µm, with a length/width ratio of 1.1 (1.0–1.2), and no micropyle. Sporocysts are elongate-ovoid (n = 82),  $9.0 \times 4.6$  (5.5–12.5  $\times 3.6$ –6.4) µm, with a length/width ratio of 2.0 (1.3–2.4), a small Stieda body, and an irregularly shaped sporocyst residuum present. These are the first *Eimeria* spp. described from springbok.

KEY WORDS: Antidorcas marsupialis, Antilopini, Bovidae, coccidia, Eimeria, Eimeriidae, Etosha National Park, Namibia, springbok, taxonomy.

The genus Eimeria Schneider, 1875 (Apicomplexa: Eimeriidae) is a speciose group of single-celled parasitic organisms, with over 1,800 known species (Duszynski, 2011). Given that most vertebrate species examined thus far are parasitized by Eimeria, estimates put the number of described species in this genus at only 2-4% of its total diversity (Tenter et al., 2002; Duszynski, 2011). This study focuses on describing 3 new species of Eimeria observed in springbok (Antidorcas marsupialis Zimmermann, 1780). Springbok are a medium-sized, mixed-feeding herbivore in the family Bovidae, and they are a commercially important game species located in the more arid regions of southern Africa (Skinner and Chimimba, 2005). Antidorcas means "not gazelle" and is used to distinguish this species (the only member of Antidorcas) from other species in the tribe Antilopini due to its unique dentition and unusual back skin-flap (the source of the specific epithet marsupialis) (Lutley and Oldfield, 1900).

To our knowledge, no descriptions of *Eimeria* in springbok have been published, although an older abstract attributed the mortality of several springbok on a game farm in South Africa to coccidiosis caused by an unidentified *Eimeria* infection (Lopez-Rebollar et al., 1997). These authors isolated oocysts from the feces of one carcass and found lesions in the intestine that were

In general, *Eimeria* species are described as being host-specific parasites, at least to the level of host genus (Zhao and Duszynski, 2001). However, there are also examples of eimerians able to infect hosts from several closely related genera (e.g., Eimeria alabamensis, Eimeria auburnensis, Eimeria bovis, Eimeria brasiliensis, Eimeria bukidnonensis, Eimeria canadensis, Eimeria cylindrica, Eimeria ellipsoidalis, Eimeria subspherica, Eimeria wyomingensis, and Eimeria zuerinii each infect 2-4 host genera within the tribe Bovini; Levine and Ivens, 1986) or even from different host families (e.g., Eimeria dispersa and Eimeria innocua infect gallinaceous birds within the families Phasianidae and Odontophoridae; Vrba and Michal, 2015). Thus, it is important to investigate known Eimeria species from beyond the host genus when describing new species of Eimeria.

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consistent with pathology caused by Eimeria. However, no other information was provided to detail the size or shape of the oocysts. Moreover, a survey of gastrointestinal parasites conducted from 2005 to 2008 in ungulates of Etosha National Park, Namibia, showed distinct differences in the morphology of Eimeria individuals recovered from springbok feces as compared to other sympatric bovid species (Turner, 2009). Turner et al. (2012) documented the prevalence, shedding intensity, seasonality, and demographic patterns of infection for 2 springbok Eimeria morphotypes. When characterizing these morphotypes based on phenotypic descriptions of the sporulated oocysts, we discovered a third, smaller morphotype of Eimeria. Here, we provide a morphological description of these 3 springbok Eimeria morphotypes, and we discuss how they compare with known Eimeria species from related host species.

#### MATERIALS AND METHODS

#### Study area

Etosha National Park is a 22,915 km<sup>2</sup> semiarid reserve in northern Namibia between  $18^\circ 30' \text{--} 19^\circ 30' \text{S}$  and  $14^\circ 15' \text{--}$ 17°10'E. Etosha contains a 4,760 km<sup>2</sup> salt pan, a dominant geological feature that is the remnant of a paleolake (Hipondoka et al., 2006). Grasslands and dwarf shrub savanna dominate the plains habitats surrounding the Etosha salt pan (le Roux et al., 1988). These open habitats are home to the "plains ungulates," including springbok, plains zebra (Equus quagga Boddaert, 1785), gemsbok (Oryx gazella Linnaeus, 1758), and blue wildebeest (Connochaetes taurinus Burchell, 1823) (Gasaway et al., 1996). The only perennial water in Etosha comes from boreholes and artesian or contact springs (Auer, 1997), around which animals congregate in large numbers during dry conditions. Rainfall is strongly seasonal, mainly falling between November and April, with the greatest monthly rainfall in January and February (Engert, 1997). These rainfall patterns lead to strong seasonality in gastrointestinal parasite infections (Turner and Getz, 2010; Turner et al., 2012).

#### **Oocyst descriptions in springbok**

A prior study recognizing 2 morphotypes of *Eimeria* in springbok (Turner et al., 2012) showed that oocyst shedding was highest in the wet season after the year's lamb crop was born, that the intensity or prevalence of shedding decreased with age, and that 2 of the 3 morphotypes were relatively rare. To maximize the number of oocysts recovered and to increase the chances of isolating the rarer morphotypes, we sampled in the wet season, selectively collecting feces from young animals (juveniles and yearlings), and we pooled several samples into one for isolation. As a result, we were not able to determine the prevalence of these species, and we instead rely on numbers from Turner et al. (2012).

Fecal samples for morphological descriptions of oocysts were collected in April 2007, February 2008, February 2010, and April 2011, representing >100 individual hosts sampled. Samples were initially screened for oocyst presence using fecal flotation. For samples that contained oocysts, these were isolated from fecal matter via straining to remove larger debris and then processed with a series of washes, centrifugation with distilled water, suspension in saturated salt solution, recovery of the surface solution containing the oocysts, and a final wash to remove the salt solution. Oocysts were stored in a 2% (w/v) potassium dichromate ( $K_2Cr_2O_7$ ) solution at room temperature for 2–3 wk to allow sporulation and then stored in a refrigerator. All oocysts were characterized and photographed within 5 mo of sampling (the 2011 samples, which represent all photomicrographs of the new species, were analyzed within 30–58 d of sampling).

Isolated oocysts from 2007–2010 were studied at the University of Pretoria, while isolates from 2011 were studied at the Biological Imaging Facility, University of California, Berkeley, using a Zeiss AxioImager microscope. A drop of precipitated oocysts was placed on a slide, covered with a coverslip, and sealed with nail polish to prevent drying out during observations. In total, 569 oocysts were measured and characterized for this study.

Descriptions of the sporulated oocysts followed the guidelines of Duszynski and Wilber (1997) and used their abbreviations. These included oocyst shape, oocyst length (L) and width (W), length/ width ratio (L/W ratio), wall characteristics, and presence/absence of the micropyle (M), micropyle cap (MC), oocyst residuum (OR), and polar granule (PG). Description of the sporocyst included shape, length (SL), width (SW), SL/SW ratio, the presence/absence of a Stieda body (SB) and sporocyst residuum (SR), and observation of sporozoites (SP), sporozoite refractile bodies (SRB), as well as, when evident, the nucleus (N). Measurements presented are the mean, standard deviation, and range of values, and all units are in µm.

Comparisons among Eimeria oocysts from springbok and other host species were done by evaluating morphological features of Eimeria oocysts from bovids in the tribe Antilopini, and from additional species in the family Bovidae that occur in the study area. The species in Antilopini were based upon the phylogeny of Bärmann et al. (2013) and include the genera Antidorcas, Antilope, Ammodorcas, Dorcatragus, Eudorcas, Gazella, Litocranius, Madoqua, Nager, Ourebia, Procapra, Raphicerus, and Saiga. However, Eimeria species have only been described from species within Antidorcas (this study), Antilope, Gazella, Litocranius, Procapra, and Saiga. Comparisons among Eimeria spp. were based upon morphological information in the following references: Mohammed et al. (2012); Mohammed and Hussein (1992); Omer et al. (2011); Hussein and Mohammed (1992); Levine and Ivens (1986); Sugár (1981-1982); results are summarized in Table 1, which includes the taxonomic authority for each Eimeria species.

Photosyntypes were submitted to the Harold W. Manter Laboratory (HWML) of Parasitology phototype collection (accession no. P-2016-016), University of Nebraska State Museum, University of Nebraska–Lincoln, Lincoln, Nebraska, U.S.A.

#### RESULTS

### Species descriptions of *Eimeria* from springbok

Morphological examination of sporulated oocysts showed evidence of 3 different morphotypes of *Eimeria* from springbok hosts based on size, shape, and the presence or absence of a micropyle and micropyle cap. These are described here as new species.

		Oocysts					
Type host	Eimeria species	Shape	Mean size (range) (µm)	M*	MC*	OR*	PG* (no.)
Tribe Antilopini							
Antidorcas marsupialis	E. antidorcasi n. sp.	Ovoid	26.2 × 18.8 (19-34 × 13-27)	+	-	-	± (1)
Antidorcas marsupialis	E. versfeldi n. sp.	Ellipsoid	39 × 27 (28-48 × 21-31)	+	+	-	+(2-4)
Antidorcas marsupialis	E. gasawayi n. sp.	Subspheroid	$15 \times 14 (12 - 20 \times 11 - 18)$	_	-	-	_
Antilope cervicapra	E. antilocervi Ray and Mandal, 1960	Cylindrical	(28–34 × 12–16)	+	-	-	
Antilope cervicapra	E. mrigai Pande, Chauhan, Bhatia and Arora, 1972	Ovoid	49 × 30 (39–55 × 26–32)	+	+	-	
Gazella dorcas	E. dorcadis Mantovani, 1966	Cylindrical	$32 \times 19 (27 - 36 \times 16 - 24)$	+	-	-	-
Gazella gazella	E. chinkari Pande, Bhatia, Chauhan and Garg, 1970	Subspheroid	25 × 22 (24–27 × 19–26)	-	-	-	-
Gazella gazella	E. idmii Mohammed and Hussein, 1992	Ellipsoid	42 × 30 (36–48 × 27–37)	+	+		-
Gazella gazella farasani	<i>E. farasanii</i> Omer, Apio, Wronski and Mohammed, 2011	Spherical- subspheroid	21 × 20 (19–25 × 18–23)	+	-	-	-
Gazella subgutturosa	E. abenovi Svanbaev, 1979	Ovoid	$32 \times 23 (23-40 \times 19-26)$	+	-	-	-
Gazella subgutturosa	E. elegans Yakimoff, Gousseff and Rastegaieff, 1932	Elongate ovoid	(23–45 × 16–25)	+	-	-	±
Gazella subgutturosa	<i>E. gazella</i> Musaev, 1970 emend. Svanbaev, 1979	Ovoid or spherical	24 × 20 (20–28 × 17–25)	-	-		
Gazella subgutturosa marica	E. rheemi Hussein and Mohammed, 1992	Spherical or ovoid	25 × 21 (20–34 × 18–30)	+	-	-	-
Litocranius walleri	E. walleri Prasad, 1960	Roughly ovoid	$29 \times 23 (27 - 30 \times 22 - 25)$	+	_	_	_
Procapra gutturosa	E. cagandzeeri Sugár, 1982	Subspheroid	$36 \times 33(32 - 38 \times 31 - 34)$	_	_	+	
Saiga tatarica	E. ismailovi Musaev, 1970	Spherical or ovoid	$28 \times 24$ (21-33 × 18-28)	_	_	_	
Saiga tatarica	E. manafovae Musaev, 1970	Ellipsoid	$37 \times 21 (29 - 47 \times 17 - 26)$	+	_	_	+
Saiga tatarica	E. saiga Svanbaev, 1958	Spherical or short-ovoid	31 × 30 (28–34 × 27–32)	-	-	+	±
Saiga tatarica	E. sajanica Machul'skii, 1947	Ovoid or spherical	$21 \times 18 (18 - 23 \times 16 - 20)$	_	_	_	_
Saiga tatarica	E. tatarica Musaev, 1970	Ovoid	30 × 24 (25–35 × 19–30)	+	± (rarely)		
Saiga tatarica	E. tekenovi Svanbaev, 1979	Ovoid or ellipsoid	29 × 22 (23–33 × 18–24)	+	+	-	-
Other Boyidae species that	t occur in Etosha National Park	-					
Aepyceros melampus	E. impalae Prasad and Narayan, 1963	Ellipsoid	33 × 22 (30–36 × 20–24)	+	-	-	-
Aepyceros melampus	E. neitzi McCully, Basson, de Vos and de Vos, 1970	Spherical- subspheroid	32 × 30 (29–34 × 28–33)	-	-	-	±
Alcelaphus buselaphus cokii	E. talboti Prasad and Narayan, 1963	Ovoid and asymmetrical	36 × 25 (35–38 × 22–28)	-	-	-	-
Connochaetes taurinus	E. connochaetei Levine and Ivens, 1970	Ellipsoid	22 × 14 (20–27 × 13–15)	-	-	-	-
Connochaetes taurinus	E. gorgonis Prasad, 1960	Ellipsoid	23 × 17 (20-26 × 15-18)	_	_	_	+
Taurotragus oryx	E. canna Triffitt, 1924	Ovoid	$(23-24 \times 16-20)$	+	-	_	-
Taurotragus oryx	E. triffittae Yakimoff, 1934 emend. Levine and Ivens, 1970	Ellipsoid	21 × 18 (21–24 × 15–19)	-	-	-	-

 Table 1. Morphological features of described *Eimeria* species infecting hosts from the tribe Antilopini, and from other host species from the family Bovidae that occur in Etosha National Park, Namibia.

\* M, micropyle; MC, micropyle cap; OR, oocyst residuum; PG, polar granule; SB, Stieda body; SRB, sporozoite refractile bodies; SR, sporocyst residuum; +, present; -, absent;  $\pm$ , sometimes present or absent; blank cells indicate the description had no information about that character.

† The "clear globules" described in sporozoites by Levine and Ivens (1986) are interpreted to be refractile bodies based on drawings of sporulated oocysts.

### TAXONOMIC DESCRIPTIONS Eimeria antidorcasi n. sp. (Figs. 1–4, 13)

#### Description

Sporulated oocysts: Oocyst shape: ovoid; wall characteristics: smooth; L  $\times$  W (n = 346): 26.2  $\times$  18.8  $(SD = 3.2 \times 2.1;$  range  $19.2-33.5 \times 13.1-26.5);$  L/W ratio: 1.4 (SD = 1.1; 1.2-2.0); M: present at the narrow end of the oocyst; PG: sometimes present as a single PG (Fig. 2), sometimes not visible, and oocyst interior looks grainy, as if the PG disintegrated (Figs. 3, 4); MC: absent; OR: absent.

Sporulated sporocysts and sporozoites: Sporocyst shape: ovoid; SL  $\times$  SW (n = 336): 11.3  $\times$  7.3

Sporocysts								
Shape	Mean size (range) (µm)	SB*	SRB*† (no.)	SR*				
Ovoid	$11 \times 7 (6-15 \times 5-10)$	+	+(1)	+				
Elongate-ovoid	$18 \times 9 (13 - 23 \times 7 - 11)$	+	+ (2)	+				
Elongate-ovoid	$9 \times 5(6-13 \times 4-6)$	+	+ (1)	+				
Piriform	11 × 7			+				
Ellipsoid	21 × 10 (19–23 × 9–10)	+	+ (2–3)	+				
Elongate-ellipsoid	14 × 8 (12–17 × 6–9)	+	+ (2)	+				
Almond-shaped	14 × 8 (13–14 × 8–9)	+	+ (2)	+				
Elongate-ovoid	18 × 10 (10–23 × 8–13)	+	+ (2)	+				
Elongate	8 × 3 (7-10 × 2-5)	+	+ (1)	+				
Filingeid	11 7 (9, 14 ( 0)							
Ellipsoid	$11 \times / (8 - 14 \times 6 - 9)$	-	=	+				
Ovoid	$(10-14 \times 6-12)$			+				
Ovoid to broad ovoid	9 × 6 (6–11 × 5–8)			+				
Ovoid	10 × 8 (6–15 × 5–10)	+	+ (1)	+				
Broadly ovoid	(8–13 × 5–8)	+	+ (1)	+				
Ovoid	18 × 10 (17–19 × 9–11)		+ (3)					
Ovoid or spherical	$10 \times 9 (7-12 \times 7-11)$			+				
Ellipsoid to spherical	12 × 8 (11–13 × 6–11)	-		+				
Spherical or short-ovoid	10 × 8 (7–12 × 7–9)			+				
Ovoid	(5–10 × 3–5)			+				
Ovoid	10 × 8 (9–13 × 6–8)			+				
Ellipsoid	11 × 7 (9–13 × 6–9)	-		+				
Ovoid	11 × 8 (8–14 × 7–9)	±	+ (1)	-				
Ellipsoid	17 × 7 (16–19 × 6–8)	+	+ (1)	+				
Piriform	14 × 10 (12–15 × 9–10)	-	+ (1)	-				
Ovoid	4–5 × 1.5	+	+ (1)	+				
Lemon-shaped	$(12-15 \times 4-6)$	+	+ (1)	+				
Ovoid	$(12 - 13 \times 10)$ $(12 - 17 \times 5 - 7)$	+	+(1-2)	+				
Elongate	9 × 4-6		(1 2)	-				
	2							

#### Table 1. Extended.

 $(SD = 1.5 \times 0.8; 6.0-14.6 \times 5.2-9.8);$  L/W: 1.5 (SD = 0.14; 1.1-2.0). SR: present, prominent, with coarse granules, appears membrane-bound maintaining the residuum in an ovoid packet; SB: present, small; SRB: present, 1/SP, not always evident.

#### **Taxonomic summary**

Symbiotype host: Antidorcas marsupialis Zimmermann, 1780.

*Type locality:* Okaukuejo, Etosha National Park, Namibia; 19°10'S; 15°55'E.

*Material deposited:* Photosyntypes of sporulated oocysts, HWML #101984.

*Prevalence:* Unknown from this study. This species was identified in <u>Turner et al. (2012)</u> as "*Eimeria* morphotype A" and, based on fecal flotation of unsporulated oocysts, had a prevalence of 61.0% (n = 726) averaged across seasons and demographic groups (2005–2008). Infections were highly seasonal, with the monthly prevalence ranging from 10 to 96% from dry to wet season months (<u>Turner et al., 2012</u>).

*Site of infection:* Unknown. Oocysts collected from fecal specimens.

Endogenous stages: Unknown.

*Etymology:* The specific epithet *antidorcasi* is derived from the genus of the type host (the only species within this genus), because this was by far the most commonly observed oocyst in the host species. Since *Antidorcas* means "not gazelle," we extend this terminology from the host to its parasite, and in the spirit of the host's genus name, we note that *E. antidorcasi* is also not a gazelle.

#### Remarks

Based on oocyst length and width measurements, presence of M, absence of MC, and presence of SR, this species is similar to Eimeria elegans and Eimeria rheemi from Gazella subgutturosa Güldenstädt, 1780, Eimeria dorcadis from Gazella dorcas Linnaeus, 1758, and Eimeria canna from Taurotragus oryx Pallas, 1766 (Table 1). However, oocyst shape distinguishes E. antidorcasi from all 4 of these species. Eimeria dorcadis and E. elegans oocysts are nearly cylindrical (Levine and Ivens, 1986; Mohammed et al., 2012), E. rheemi oocysts are nearly spherical (Hussein and Mohammed, 1992), and, although E. canna oocysts are also ovoid, the end with the M does not narrow as it does in E. antidorcasi. In additional, E. canna oocysts have a pronounced SB, and the SR is linearly arranged along the long axis of the sporocyst (Levine and Ivens, 1970), while E. antidorcasi has a more subtle SB and an ovoid SR.

#### Eimeria versfeldi n. sp. (Figs. 5–8, 14)

#### Description

Sporulated oocysts: Oocyst shape: ovoid; wall characteristics: smooth; L × W (n = 136): 38.9 × 26.6 (SD = 4.8 × 2.2; range 27.8–48.2 × 21.2–31.0); L/W ratio: 1.5 (SD = 0.11; 1.2–2.0); M: present; MC: present and prominent, can be torn off during processing; MC depth (n = 65): 3.2 (SD = 0.59; 2.2–4.9); sub-micropylar material is evident beneath the MC (Figs. 5, 7), which is centrally placed, nongranular, nonrefractile, and irregularly shaped; PG: 2–4 present; OR: absent.

Sporulated sporocysts and sporozoites: Sporocyst shape: elongate-ovoid; SL  $\times$  SW (n = 132): 18.3  $\times$  9.2 (SD = 2.2  $\times$  0.9; 13.2–22.8  $\times$  6.8–11.2); L/W: 2.0 (SD = 0.21; 1.3–2.5); SR: present,

small, centrally located, with fine granules, and appears to be attached to the sporocyst wall (Fig. 7); SRB: 2/SP, 1 large one at either end of the sporocyst, 1 smaller one toward the middle; rarely, a second smaller SRB is visible, for a total of 3 SRB/SP; N: evident, located between the two SRBs (and intermediate in size to the SRBs; Fig. 8); SB: present, flattened.

#### Taxonomic summary

Symbiotype host: Antidorcas marsupialis Zimmermann, 1780.

*Type locality:* Okaukuejo, Etosha National Park, Namibia; 19°10'S; 15°55'E.

*Material deposited:* Photosyntypes of sporulated oocysts, HWML #101985.

*Prevalence:* Unknown from this study. This species was identified in Turner et al. (2012) as "*Eimeria* morphotype B" and, based on fecal flotation of unsporulated oocysts, had a prevalence of 16.5% (n = 726) averaged across seasons and demographic groups (2005–2008). The monthly prevalence for *E. versfeldi* ranged from 4 to 44% throughout the year, but it did not have the strong seasonality exhibited by *E. antidorcasi* (Turner et al., 2012).

*Site of infection:* Unknown. Oocysts collected from fecal specimens.

Endogenous stages: Unknown.

*Etymology:* The specific epithet *versfeldi* is for Wilferd Versfeld of the Etosha Ecological Institute, the "institutional memory" of Etosha National Park. He retired from the Ministry of Environment and Tourism after 35 y of service in March 2016. His many duties over the years included monitoring the ecology and demographics of the springbok population, which are among his favorite species.

#### Remarks

These large oocysts often ruptured under the pressure of the  $100 \times$  oil immersion objective, making them initially very difficult to measure. This problem was solved by placing two partial strands of human hair under the cover slip to elevate it slightly.

Based on the presence of MC (described as an unusual feature in *Eimeria* species from antelope and their relatives; Mohammed and Hussein, 1992), *E. versfeldi* is similar to *Eimeria idmii* from *Gazella* Pallas, 1766, *Eimeria mrigai* from *Antilope cervicapra*, and *Eimeria tekenovi* from *Saiga tatarica* Linnaeus,



Figures 1–12. Photomicrographs of sporulated oocysts. 1. *Eimeria antidorcasi*, showing the micropyle (M). 2. *E. antidorcasi*, showing the polar granule (PG; not always present) and sporocyst residuum (SR). 3. *E. antidorcasi*, showing the granular interior of the oocyst (and no PG). 4. *E. antidorcasi*, showing a single refractile body per sporozoite (SRB), Stieda body (SB), and SR. 5. *Eimeria versfeldi*, showing the oocyst with prominent micropyle cap (MC). 6. *E. versfeldi*, showing two PGs, the flattened SB, and in a sporozoite, the nucleus (N) and the large posterior SRB. 7. *E. versfeldi* sporocyst, showing the SR and the large SRB. 8. *E. versfeldi* sporocyst, showing the large and small SRBs and the N. 9–12. *Eimeria gasawayi* oocysts, showing a SB and SR.

1766. *Eimeria tekenovi* is smaller than *E. versfeldi*, while *E. idmii* is similarly sized (Table 1); however, *E. idmii* and *E. tekenovi* both lack a PG (Levine and Ivens, 1986; Mohammed and Hussein, 1992), and *E. versfeldi* has 2–4 present. *Eimeria mrigai* shares with *E. versfeldi* the presence of sub-micropylar material, but *E. mrigai* oocysts are larger, and the SR is dispersed throughout the sporocyst (Levine and Ivens, 1986), whereas the SR of *E. versfeldi* is centrally placed in the sporocyst.

## *Eimeria gasawayi* n. sp. (Figs. 9–12, 15)

#### Description

Sporulated oocysts: Oocyst shape: subspheroid; wall characteristics: smooth; L × W (n = 87): 14.8 × 13.6 (SD = 2.5 × 2.1; range 11.5–20.0 × 11.1–18.2); L/W: 1.1 (SD = 0.05; 1.0–1.2); M: absent; MC: absent; PG: absent; OR: absent.



Figures 13–15. Composite line drawings of the sporulated oocysts. 13. Eimeria antidorcasi. 14. Eimeria versfeldi. 15. Eimeria gasawayi.

Sporulated sporocysts and sporozoites: Sporocyst shape: elongate-ovoid; SL × SW (n = 82): 9.0 × 4.6 (SD = 1.5 × 0.6; 5.5–12.5 × 3.6–6.4); SL/SW ratio: 2.0 (SD = 0.22; 1.3–2.4); SR: present, granular, generally located off-center to the long axis of the sporocyst and of variable shape, from elongate-ovoid to irregular; SB: present, small; individual SP often not evident; SRB: absent.

#### Taxonomic summary

Symbiotype host: Antidorcas marsupialis Zimmermann, 1780.

*Type locality:* Okaukuejo, Etosha National Park, Namibia; 19°10'S; 15°55'E.

*Material deposited:* Photosyntypes of sporulated oocysts, HWML #101986.

*Prevalence:* Unknown, described from pooled fecal samples; rarer than *E. antidorcasi* and *E. versfeldi*.

*Site of infection:* Unknown. Oocysts collected from fecal specimens.

Endogenous stages: Unknown.

*Etymology:* The specific epithet *gasawayi* is named after the late William C. Gasaway, who studied springbok and other plains herbivores in Etosha National Park from the late 1970s to the early 1990s, and who obtained funds from the U.S. Agency for International Development (USAID) and the Zoological Society of San Diego to build Okaukuejo's research camp, the William Gasaway Memorial Research Camp.

#### Remarks

*Eimeria gasawayi* has similar features (oocyst shape; absence of M, MC, OR, and PG; sporocyst shape; presence of SB and SR) to *Eimeria sajanica* in *S. tatarica*, *Eimeria chinkari* in *G. gazella*, *Eimeria neitzi* in *Aepyceros melampus* Lichtenstein, 1812, and *Eimeria*  *gazella* in *G. subgutturosa* (Levine and Ivens, 1986). However, *E. gasawayi* is smaller than all of these species, with the mean oocyst length and width outside the ranges reported for all 4 of these species (Table 1).

#### *Eimeria* of sympatric hosts in Etosha National Park

Turner (2009) did a survey of gastrointestinal parasites of 13 different ungulate species in Etosha National Park. Of these species, Eimeria species were observed only from species in the family Bovidae, including springbok, black-faced impala (Aepyceros melampus petersi Bocage, 1879), red hartebeest (Alcelaphus buselaphus caama É. Geoffroy Saint-Hilaire, 1803), blue wildebeest (C. taurinus), gemsbok (O. gazella), and greater kudu (Tragelaphus strepsiceros Pallas, 1766). These parasites were only examined as unsporulated oocysts, but the size, shape, and M characteristics suggest they all differ from the springbok Eimeria species (Turner, 2009). Among the bovid species from Etosha that were also infected with Eimeria, the following species are recognized: in hartebeest (from Alcelaphus buselaphus cokii, not A. b. caama), Eimeria talboti; in impala (from common, not black-faced impala), Eimeria impalae and E. neitzi; and in wildebeest, Eimeria connochaetei and Eimeria gorgonis (Levine and Ivens, 1986). It is interesting to note that no Eimeria samples were recovered from the feces of nonbovid ungulate species examined, including Loxodonta africana Blumenbach, 1797 (n = 100), Diceros bicornis Linnaeus, 1758 (n = 71), Eq. quagga (n = 403), Equus zebra hartmannae Matschie, 1898 (n = 30), Phacochoerus africanus Gmelin, 1788 (n = 15), or Giraffa camelopardalis Linnaeus, 1758 (n = 55) (Turner, 2009; Turner and Getz, 2010).

In 2010, we further examined a few pooled fecal samples from blue wildebeest and from gemsbok to observe the diversity of *Eimeria* species recovered in these species, which share the grassland habitat with springbok. No Eimeria species have been described from gemsbok, and 2 have been described for blue wildebeest, E. gorgonis, Prasad 1960 and E. connochaetei (Prasad, 1960) Levine and Ivens, 1970, although the description and history of E. connochaetei are somewhat unclear. These 2 wildebeest Eimeria species were identified by Prasad (1960), who described E. gorgonis as a new species and labeled the other observed morphotype as E. ellipsoidalis Becker and Frye, 1929, based on morphological similarities to this species, which was originally described from Bos taurus (Levine and Ivens, 1986). Prasad (1960) erroneously labeled the host of these Eimeria



**Figures 16–21.** *Eimeria* observed in 2 bovid species sympatric with springbok in Etosha National Park, Namibia. **16–17.** Two examples of a smaller morphotype found in blue wildebeest, *Connochaetes taurinus*. These oocysts have a micropyle and average size of 21.4 × 18.1 (19.7–23.5 × 17.9–18.3) µm (n = 3). **18–19.** Two examples of a larger morphotype found in *C. taurinus*. These oocysts have a asymmetrically placed micropyle cap and average size of 31.2 × 23.2 (27.8–34.5 × 21.5–25.4) µm (n = 6). *Co*–21. Two examples of the morphotype found in gemsbok, *Oryx gazella*. These oocysts have a micropyle cap and an average size of 44.0 × 32.4 (38.8–52.3 × 29.1–34.9) µm (n = 6).

as black wildebeest (*Connochaetes gnou* Zimmermann, 1780), a species that only occurs in southern Africa, yet stated that the samples were collected from wildebeest in East Africa. Using the data published in <u>Prasad (1960)</u>, Levine and <u>Ivens (1970)</u> described this morphotype as a new species, *E. connochaetei* (correcting the host record to *C. taurinus*), because the sporocysts were smaller than *E. ellipsoidalis* and because the phylogenetic distance between host species would suggest the 2 *Eimeria* morphotypes are different.

We observed 2 morphotypes of *Eimeria* from blue wildebeest (Figs. 16–17, 18–19), and 1 morphotype of *Eimeria* from gemsbok (Figs. 20–21). Our smaller wildebeest morphotype (Figs. 16–17) resembles *E. connochaetei* in size, shape, and other features, except it was described in Levine and Ivens, (1970) as lacking a M, which is evident in our morphotype. The larger wildebeest morphotype (Figs. 18–19) has a MC and hence differs from both known species (Levine and Ivens, 1986). Thus, these morphotypes may each represent novel species. However, since only a few oocysts were examined (3–6 per morphotype), we do not

attempt a description, but we rather use them as a comparison to springbok *Eimeria*.

The smaller morphotype from wildebeest does not look like the springbok Eimeria species based on its shape and size (Figs. 16-17), while the larger wildebeest morphotype and the gemsbok morphotype both superficially resemble E. versfeldi, with the presence of a MC (Figs. 18-21). Eimeria versfeldi is smaller than the gemsbok morphotype, with an average oocyst size of  $38.9 \times 26.6$  versus  $44.0 \times 32.4$ , respectively. The gemsbok morphotype also has 2 large SRBs centrally located in the sporocyst, giving it the appearance of eyes in an elongate face, whereas E. versfeldi has large SRBs at the ends of the sporocysts. Compared with the bigger of the 2 wildebeest morphotypes (Figs. 18-19), E. versfeldi has a broader, more symmetrical MC and is a larger oocyst, with an average size of  $38.9 \times 26.6$  versus  $31.2 \times$ 23.2, respectively. Thus, the Eimeria species described from springbok also seem unique when compared with Eimeria species infecting sympatric, if more distantly related, bovid species. Our results from this study are consistent with the pattern suggested by Zhao and Duszynski (2001), i.e., that Eimeria infecting artiodactyl hosts lack an OR (although we note that Eimeria saiga and Eimeria cagandzeeri both have an OR; Table 1).

#### DISCUSSION

Springbok are the only extant species in the genus Antidorcas, and there have been no Eimeria species described from this species or its closest relative, Ammodorcas clarkei Thomas, 1891 (Clarke's gazelle) from Ethiopia and Somalia. Within the tribe Antilopini, which consists of 13 genera (Bärmann et al., 2013), Eimeria have been described from only 7 species: Antilope cervicapra Linnaeus, 1758 (Eimeria antilocervi and E. mrigai), G. dorcas (E. dorcadis), G. gazella (E. chinkari, E. idmii, Eimeria farasanii), G. subgutturosa (Eimeria abenoui, E. elegans, E. gazella, and E. rheemi), Litocranius walleri Brooke, 1879 (Eimeria walleri), Procapra gutturosa Pallas, 1777 (E. cagandzeeri), and S. tatarica (Eimeria ismailovi, Eimeria manafovae, E. saiga, E. sajanica, E. tatarica, and E. tekenovi) (Levine and Ivens, 1986; Hussein and Mohammed, 1992; Mohammed and Hussein, 1992; Mohammed et al., 2012; Omer et al., 2011; Sugár, 1981-1982). None of these host species shares a geographic distribution with springbok, and they are located in East Africa (L. walleri), North Africa (G. dorcas), the Middle East (G. gazella), South and Central Asia (G. subgutturosa), Central Asia (S. tatarica and P. gutturosa), and the

Indian subcontinent (*A. cervicapra*). Based on morphology, the springbok *Eimeria* species differ from all of these known species (Table 1).

We broadened the taxonomic group of hosts considered beyond Antilopini to include other members of the family Bovidae that co-occur with springbok in Etosha National Park for comparison. Of these additional host species, *Eimeria* species have been described in 4 species: A. melampus (E. impalae and E. neitzi), A. buselaphus (E. talboti), C. taurinus (E. connochaetei and E. gorgonis), and T. oryx (E. canna and Eimeria triffittae). Again, based on morphology, the Eimeria species from springbok differ from each of these species (Table 1). We further assessed if the springbok Eimeria species seem locally species specific by evaluating oocysts infecting hosts that share the grassland habitats with springbok (Figs. 16-21) and found the Eimeria spp. infecting C. taurinus and O. gazella are clearly different from the species infecting springbok.

In summary, none of the known *Eimeria* spp. considered shares all the features of the *Eimeria* described here from springbok. In addition, the 3 *Eimeria* spp. described here are clearly different from each other in the size, shape, and phenotypic characteristics of the oocysts. Therefore, we feel confident that *E. antidorcasi*, *E. versfeldi*, and *E. gasawayi* represent 3 distinct species, each of which is new to science.

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