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### Polymorphism of Eimerian Oocysts: A Dilemma Posed by Working with Some Naturally Infected Hosts

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# RESEARCH NOTES

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## Polymorphism of Eimerian Oocysts: A Dilemma Posed by Working with Some Naturally Infected Hosts

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Morphological variation of sporulated oocysts within individual eimerian species is well documented (Joyner, 1982. *In* Biology of the coccidia, P. L. Long (ed.). University Park Press, Baltimore, pp. 35-62). In some cases, oocysts of a single eimerian species are known to vary in size by as much as 40% during patency (Duszynski, 1971, *Journal of Parasitology* 57: 948-952). During a survey to determine the prevalence of coccidiosis in sandhill cranes (*Grus canadensis*) wintering in New Mexico (Parker and Duszynski, 1986, *Journal of Wildlife Diseases* 21: 25-35), marked polymorphism was observed among sporulated oocysts of *Eimeria reichenowi*. Oocysts were obtained from intestinal contents of cranes necropsied in the field and processed for study by routine sporulation and flotation techniques (Duszynski et al., 1982, *Journal of Parasitology* 68: 1146-1148). Initially, oocysts were categorized into 5 groups based on obvious qualitative/quantitative features including oocyst wall texture, appearance of the sporocyst residuum, and the number of polar bodies (Table I, Figs. 1-6). Fifty-seven of 118 (48%) fecal samples with *E. reichenowi* contained 2 or more morphological types of oocysts. Oocysts were measured under oil immersion (100× Neofluar objective, Zeiss Universal Photomicroscope) and differences between the 5 oocyst groups were tested for significance ( $P \leq 0.05$ ) using the Student-Newman-Keuls procedure.

Group 1 oocysts had a rough outer oocyst wall (Fig. 1). Oocysts in groups 2 and 4 had 1 polar body (Figs. 2, 4). Oocysts in groups 1, 3, and 5 contained 2 or more (up to 8) polar bodies (Figs. 1, 3, 5). The sporocyst residuum varied in appearance from compact and coarsely granular (groups 1, 2; Figs. 1, 2) to diffuse and either coarsely granular (groups 3, 4; Figs. 3, 4) or finely granular, often obscuring the sporozoites (group 5; Figs. 5, 6).

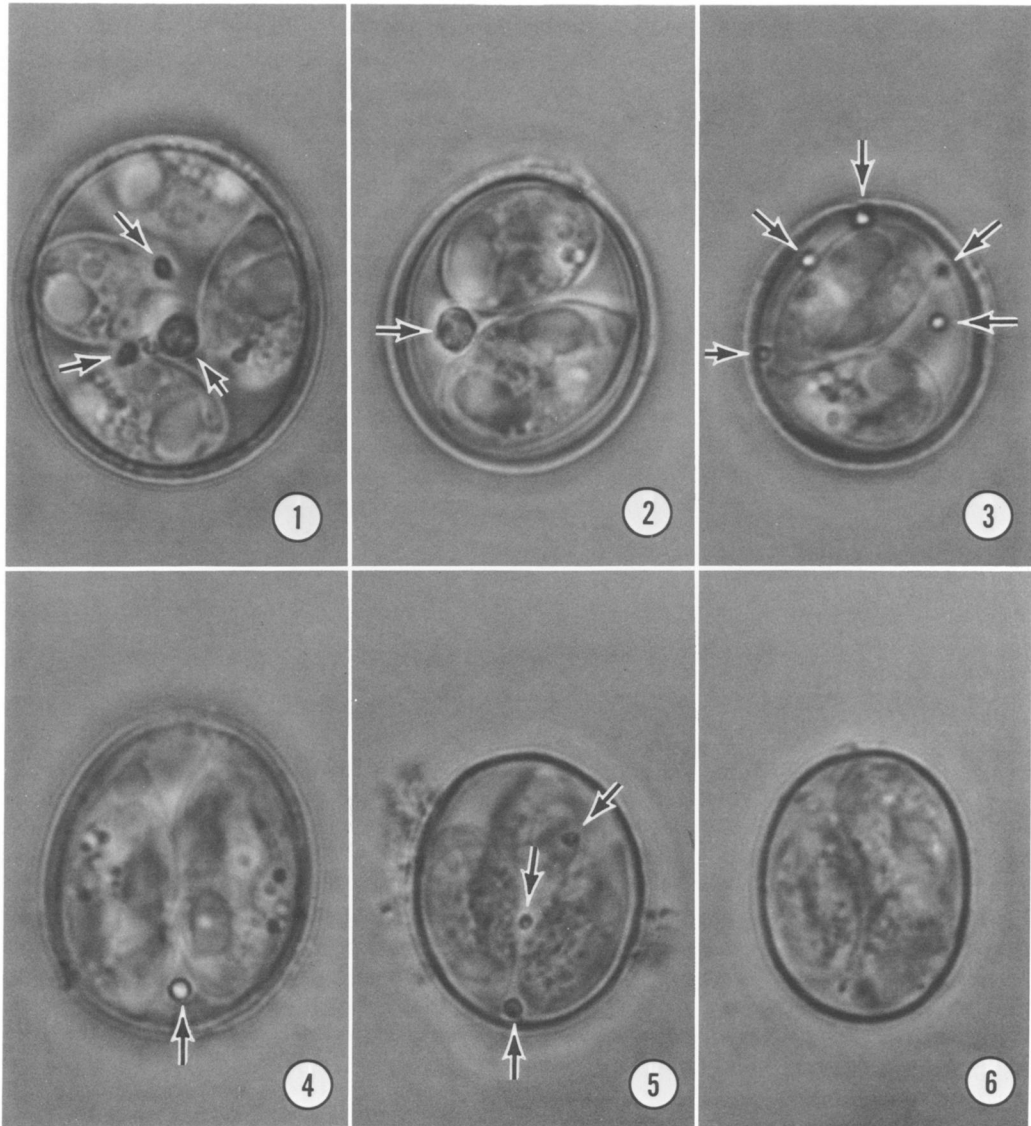
Significant differences in oocyst and sporocyst

sizes and L:W ratios were observed between the 5 groups (Tables II, III). Group 5 oocysts and sporocysts were the smallest, had the largest sporocyst L:W ratio (Table II) and were significantly different from other groups in all measurements except oocyst L:W ratio (Table III). Group 4 oocysts had distinct oocyst width, sporocyst width, and L:W values, and a significantly larger oocyst L:W ratio. Group 1 oocysts had significantly larger sporocyst length and width measurements. Oocysts in groups 2 and 3 were not significantly different from each other in size measurements (Table III).

Polymorphism among sporulated oocysts of *E. reichenowi* has been reported by others, who noted differences in texture of the oocyst wall and number of polar bodies (Courtney et al., 1975, *Journal of Parasitology* 61: 695-699) and a wide range in oocyst size and shape (Pellérdy, 1974. *Coccidia and coccidiosis*, 2nd ed. Verlag Paul Parey, Berlin, pp. 329-330). Here we quantify and document these differences, but what does it all mean? The question remains whether or not the oocysts we measured represent one, or more than one, species. From the data presented here it is clear that *E. reichenowi* is either highly polymorphic in oocyst morphology or the oocysts examined represent multiple species within an *E. reichenowi* complex. Further studies involving experimental infection of sandhill

TABLE I. Five categories of oocysts of *E. reichenowi* demonstrating the variation observed in oocyst wall texture, number of polar bodies, and characteristics of the sporocyst residuum.

Group	Oocyst wall texture	Polar bodies	Sporocyst residuum
1	Rough	2 or more	Compact, coarsely granular
2	Smooth	1	Compact, coarsely granular
3	Smooth	2 or more	Diffuse, coarsely granular
4	Smooth	1	Diffuse, coarsely granular
5	Smooth	2 or more	Diffuse, finely granular



FIGURES 1-6. Sporulated oocysts of *E. reichenowi*. Note the difference in the number of polar bodies (arrows) among the different morphological types.  $\times 2,100$ . 1. Group 1 oocyst; note large size of oocyst, rough oocyst wall, and coarsely granular sporocyst residuum. 2. Group 2 oocyst; oocyst is similar to Figure 1 oocyst, but wall is smooth and only single polar body is present. 3. Group 3 oocyst; oocyst is similar to Figure 2 oocyst, but multiple polar bodies are present. 4. Group 4 oocyst; note sporocyst L:W is visibly larger, and coarse sporocyst residuum is more diffuse than oocysts in Figures 1-3. 5, 6. Group 5 oocysts; note small oocyst size, large sporocyst L:W ratio, and finely granular sporocyst residuum, which obscures sporozoites. Polar body in Figure 6 not in focal plane.

cranes with each morphological type of oocyst may clarify this problem. However, a fundamental problem in working with some wild hosts, as with cranes, is that they are difficult or impossible to maintain in captivity for such experiments.

For those who work, or plan to work, on the coccidia of wild animals, a final statement should be made on whether or not such studies provide fruitful results, or whether they result in hopeless taxonomic nightmares. As we measure and identify oocysts of hundreds of coccidian species from

TABLE II. *Quantitative morphological variation among 5 groups of Eimeria reichenowi oocysts.\**

Group	n	Oocyst length	Oocyst width	Oocyst L:W ratio	Sporocyst length	Sporocyst width	Sporocyst L:W ratio
1	75	19.6 (17.5–22.2)	17.0 (14.3–18.3)	1.16 (1.04–1.32)	13.4 (11.9–14.3)	7.6 (6.8–8.3)	1.75 (1.61–1.88)
2	25	19.0 (16.3–22.2)	16.6 (13.5–18.3)	1.16 (1.04–1.64)	12.9 (11.1–14.3)	7.3 (6.4–7.9)	1.76 (1.65–1.88)
3	33	18.8 (16.7–21.4)	16.3 (14.3–19.1)	1.16 (1.00–1.39)	12.8 (11.9–14.3)	7.5 (6.4–7.9)	1.72 (1.51–1.99)
4	55	19.4 (16.7–21.4)	15.3 (13.5–17.5)	1.27 (1.11–1.42)	12.9 (11.5–15.1)	7.0 (6.0–7.9)	1.85 (1.65–2.11)
5	58	16.8 (15.1–18.3)	14.7 (13.5–15.9)	1.15 (1.00–1.30)	12.0 (11.1–12.7)	6.3 (5.6–7.2)	1.89 (1.59–2.13)

\* Values represent group means (ranges) in micrometers.

thousands of host specimens (birds, reptiles, fish, mammals) from many geographic areas (Canada, Japan, Mexico, North and South America), we note that oocysts from wild hosts present unique problems of identification that seem to be dependent upon the host group in which they are found. These can be separated into 4 problem areas: 1) oocysts presumed to be a single parasite species may be highly polymorphic within the same or closely related host species (e.g., *E. reichenowi*, present study); 2) oocysts from different parasite species may be similar in structure with-

in a closely related host group (e.g., chicken eimerians, see Joyner, 1982. *In The biology of the coccidia*, P. L. Long (ed.). University Park Press, Baltimore, pp. 35–62); 3) oocysts presumed to be a single species may be highly monomorphic in many unrelated host species (e.g., *Isospora lacazei*, see Levine, 1982, *Transactions of the American Microscopical Society* **101**: 66–74; *Eimeria tamiasciuri*, see Hill and Duszynski, 1986, *Journal of Protozoology* **32**: 282–288); or 4) oocysts of many different coccidian species may be highly distinctive within and between host groups (see Duszynski, 1985, *Journal of Protozoology* **32**: 577–580, for groups surveyed to date). Each of these problems presents a special challenge to the taxonomist who works with populations of wild hosts, and solutions to such problems should engage the creative energies of parasitologists for years to come.

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TABLE III. *Homogeneous subsets (underlined) of groups of Eimeria reichenowi oocysts.\**

Characteristic	Group				
	5	<u>3</u>	<u>2</u>	<u>4</u>	<u>1</u>
Oocyst length	5	<u>3</u>	<u>2</u>	<u>4</u>	<u>1</u>
Oocyst width	5	4	<u>3</u>	<u>2</u>	1
Oocyst L:W ratio	<u>5</u>	<u>3</u>	<u>2</u>	<u>1</u>	4
Sporocyst length	5	<u>3</u>	<u>4</u>	<u>2</u>	1
Sporocyst width	5	4	<u>2</u>	<u>3</u>	1
Sporocyst L:W ratio	<u>3</u>	<u>1</u>	<u>2</u>	4	5

\* Determined by Student-Newman-Keuls procedure with significance at  $P \leq 0.05$ .