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ORIGINAL PAPER

Two new *Eimeria* species (Protozoa: Eimeriidae) from wild rock ptarmigans, *Lagopus muta islandorum*, in Iceland

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Abstract One hundred rock ptarmigans, *Lagopus muta islandorum* (Faber, 1822), were collected in early October 2006 in northeastern Iceland and examined for coccidian parasites. Two *Eimeria* species were identified, and each is described as a new species. Sporulated oocysts of one species are ellipsoidal, 24.9×16.6 (19.5–30×14.5–19) μm. Oocysts have a small micropyle and a two-layered, smooth wall ~1.0 μm thick. An oocyst residuum is absent, but one to three polar granules are present. Sporocysts have a rounded end opposite a nipple-like Stieda body and are 14.3×6.3 (12–16.5×5.5–7) μm. Sporocysts contain one refractile body and a diffuse granular residuum; the entire contents of each sporocyst is enclosed by a thin membrane. Sporulated oocysts of the second eimerian are subspherical, 24.7×22.2 (20–28×18–24.5) μm. The oocysts are without a micropyle but with a two-layered wall, which is ~1.5 μm thick, with the outer layer having a rough surface texture. Oocyst residuum is absent, but one to two polar granules are present. Sporocysts have a rounded end opposite the nipple-like Stieda body atop a prominent sub-Stieda body and are 14.4×8.0 (12–15.5×6.5–9) μm. Sporocysts contain a diffuse granular residuum, and each sporozoite has two different-sized refractile bodies.

Introduction

Only five species of *Eimeria* are known from the feces of the rock ptarmigan *Lagopus muta* (Montin, 1781). The descriptions originate from three distinct geographic areas of the world. From Europe, Galli-Valerio (1929) briefly described *Eimeria lagopodi* from *L. m. helveticus* in Switzerland. The description, however, omits most of the morphological characters needed to recognize the form as a distinct species. In North America, Levine (1953) described *E. brinkmanni* and *E. fanthami* from *L. m. rupestris* from the Perry River region of the Canadian Arctic. These descriptions, however, are based on studies of five and ten sporulated oocysts from single birds, respectively. In Japan, Kamimura and Kodama (1981) described *E. uekii* from *L. m. japonicus*, and recently, Ishihara et al. (2006) further examined *E. uekii* and also gave a description of a still unnamed eimerian that they refer to as Type B.

Coccidiosis, caused by eimeriids of an unknown taxonomic status, was already reported in the 1920s from three other subspecies of the rock ptarmigan: *L. m. islandorum* from Iceland (Kloster 1923), *L. m. muta* in Norway, and *L. m. hyperboreus* from Spitzbergen (Brinkmann 1926). Recent surveys have shown that eimeriids are common in rock ptarmigans in Norway (Holmstad 2004; Holmstad et al. 2005) and Iceland (Skirnisson 1998). In this paper, we describe two *Eimeria* species found in rock ptarmigans in Iceland.

Materials and methods

During October 2–5, 2006, 100 ptarmigans (20 adults and 30 sub-adults of both sexes) were sampled in northeastern Iceland (65°47′N, 17°10′W). The rectum was cut open, and all feces were removed and weighed. Each fecal sample was transferred to a 20-ml plastic container with a screw cap and diluted and mixed with the same volume (1 g diluted with 1 ml) of 3% (*w*/*v*) aqueous potassium dichromate ($K_2Cr_2O_7$) solution. The samples were kept

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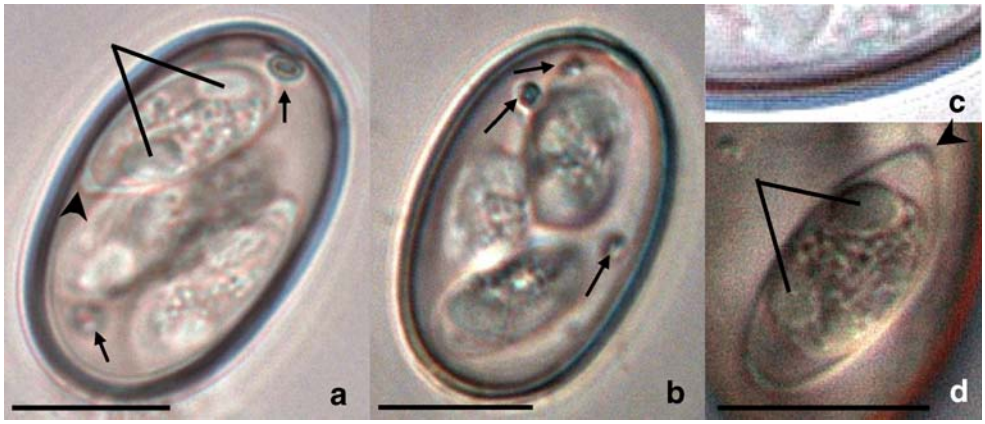

Fig. 1 Nomarski interference contrast photomicrographs of sporulated oocysts and a sporocyst of *E. muta* from *L. m. islandorum*. Bar= 10 μm. **a**, **b** M visible at top end of the oocyst, medial in **a**, slightly displaced to one side in **b**; two large, elongate PGs (*arrows*) in **a**, three smaller PGs (*arrows*) in **b**. **c** Smooth, bicolored outer wall and dark inner wall. **d** Nipple-like SB (*arrowhead*) at the pointed end of sporocysts; one elongate RB (*lines*) visible in each SP and a number of large SR granules in each SP; membrane encloses the contents (SP, SR) of the sporocyst (all features also seen in **a**)

for a week at room temperature (23–25°C) for sporulation, and after that refrigerated at 3°C. To detect coccidian oocysts, samples were examined using the McMaster method (Anonymous 1987). After centrifugation of 14 ml of the suspension, the supernatant was decanted and refilled with Parasitosol (specific density 1.27 g/ml, Meku®, DK 7171, Denmark). Oocysts per gram (opg) was estimated by counting oocyst numbers in four McMaster counting chambers at 125× and the average count multiplied by a constant to obtain the estimate of opg. For microscopy, oocysts were isolated in Parasitosol solution and examined using a Leica DMLB microscope equipped for differential interference contrast (DIC) microscopy (Nomarski). Ten sporulated oocysts from each of six heavily infected birds were examined and measured. Several dozens of oocysts of each species were photographed with a Leica DC 300 digital camera. Measurements are in micrometers [mean± SD (μm)] with the range and the number (*n*) of stages measured in parentheses. Abbreviations used in the species descriptions are as suggested by Duszynski and Wilber (1997) and Wilber et al. (1998). Oocyst characters include length (*L*), width (*W*), and their ranges and ratio (*L*/*W*), micropyle (M), residuum (OR), and polar granule (PG). Sporocyst characters include length (*L*), width (*W*), and their ranges and ratio (*L*/*W*), Stieda body (SB), sub-Stieda body (SSB), para-Stieda body (PSB), residuum (SR), sporozoites (SP), refractile bodies (RB), and nucleus (N) in SP. Photosyntypes and photoneosyntypes of sporulated oocysts (see Duszynski 1999) are deposited in the U.S. National Parasite Collection (USNPC, Beltsville, Maryland).

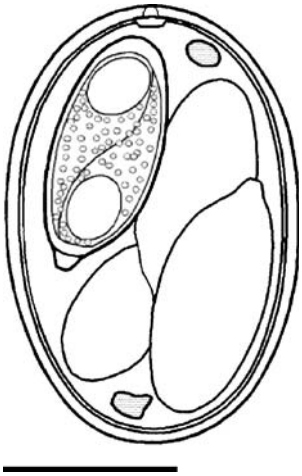

Fig. 2 Line drawing of a sporulated oocyst of *E. muta* from a wild *L. m. islandorum*. Bar= 10 μm

Results

Two *Eimeria* species were found. After examination, measurements and photomicrographs of the oocysts we found were compared to the five *Eimeria* species already described from *L. muta* (Galli-Valerio 1929; Ishihara et al. 2006; Kamimura and Kodama 1981; Levine 1953; Pellérdy 1974), as well as to dozens of *Eimeria* species described from other bird species in the family Phasianidae (Gassal 2003; Hospes 1996; Levine 1961; Pellérdy 1974; Rommel 2000; Smith et al. 2003; Stabler et al. 1979; Todd and Hammond 1971). These comparisons convinced us that both of the forms we saw were new species, which we describe below.

Description

E. muta n. sp. (Figs. 1 and 2)

Description of sporulated oocyst Oocyst shape: ellipsoidal; number of walls: 2; wall thickness: 1.0 (0.9–1.1); wall characteristics: outer wall smooth, ~4/5 of total thickness

and appears bicolored, outermost portion light blue, innermost portion yellow to red-brown, inner wall dark. M usually visible, ~1.5 wide, usually medial, but sometimes slightly displaced to one side, or sometimes indistinct or absent. $L \times W$ (n=60), 24.9 (±2.2)×16.6 (±0.8), (19.5–30×14.5–19); L/W ratio, 1.5 (1.2–1.8); OR absent. One to three PGs present, 1 (in 62.7% of examined oocysts), either ellipsoidal in shape, 1.6×2.5 (2–3×1–2.5) or round, ~2.4 (2–3), 2 (in 29.4% of the oocysts), almost round, ~1.6 (1–2), or 3 (in 7.9% of the oocysts), round, ~1.1 (1–1.5). Distinctive features of oocyst: M usually visible, one to three prominent PGs, thin bicolored outer wall, with light blue outer portion and yellow to red-brown inner portion, and a dark inner wall.

Description of sporocyst and sporozoites Sporocyst: rounded at end opposite SB; $L \times W$ (n=60), 14.3 (±0.9)×6.3 (±0.4), (12–16.5×5.5–7); L/W ratio, 2.3 (1.9–2.7); SB, present, nipple-like; SSB and PSB, absent; SR, present; SR characteristics: several dozens of dispersed granules; SP have one posterior RB, subspheroid 3.9×2.9 (3–5×2–4); a thin membrane encloses the contents of each sporocyst. Distinctive features of sporocyst: nipple-like SB, granular SR, one large RB in each SP, and contents enclosed within a thin membrane.

Taxonomic summary Type host: *Lagopus muta islandorum* (Faber, 1822), rock ptarmigan.

Type locality: Þingeyjarsýsla (65°47′N, 17°10′W), northeast Iceland.

Prevalence: in 92/100 (92%); average opg, 7,900 (range 50–238,000).

Prepatent and patent periods: unknown.

Site of infection: Oocysts recovered from fecal material in the small intestine and cecum.

Material deposited: photosyntypes (see Duszynski 1999) of sporulated oocysts deposited in the USNPC 099802.

Etymology: the nomen triviale is derived from the scientific name of the host.

Remarks The ellipsoidal oocyst shape of *E. muta* resembles *E. brinkmanni*, *E. fanthami*, and *E. uekii*. All have a similar L/W ratio, 1.5. *E. muta* and *E. uekii* oocysts, however, have a M (sometimes indistinct), which *E. brinkmanni* and *E. fanthami* lack. Also, sporocysts of the latter two species lack a SR that is prominent in *E. muta* and *E. uekii*. Various morphological characters distinguish *E. muta* and *E. uekii*: the wall thickness (1.0 and 0.6, respectively); the number of wall layers (2 layers vs 1, respectively); the wall color (bicolored outer wall and a dark inner wall in *E. muta*, yellowish in *E. uekii*); relatively larger oocyst and sporocyst sizes in *E. muta* than in *E. uekii*; different sporocyst $L \times W$ ratios (2.3 in *E. muta*, 1.8–2.0 in *E. uekii*); different RB numbers [1 in *E. muta*, 1–2 (Ishihara et al. 2006) or 2 (Kamimura and Kodama 1981) in *E. uekii*]. The remaining eimeriids that have been described from rock ptarmigans can easily be distinguished from *E. muta* on their oocyst shape (subspherical in *E. rjupa* and *Eimeria* Type B, ovoidal to ellipsoidal in *E. lagopodi*). Furthermore, *E. lagopodi* sporocysts are spherical (~12). This sporocyst form also distinguishes the species from all the remaining rock ptarmigan eimeriids that have elongate (pyriform, lemon-shaped, elongate–ovoid, fusiform, or spindle-formed) sporocysts (Galli-Valerio 1929; Ishihara et al. 2006; Kamimura and Kodama 1981; Levine 1953; Pellérdy 1974). In the case of *E. muta*, the M was usually more visible on unsporulated than sporulated oocysts. On sporulated oocysts, the M was often slightly displaced to the side rather than exactly medially on the end of the oocyst.

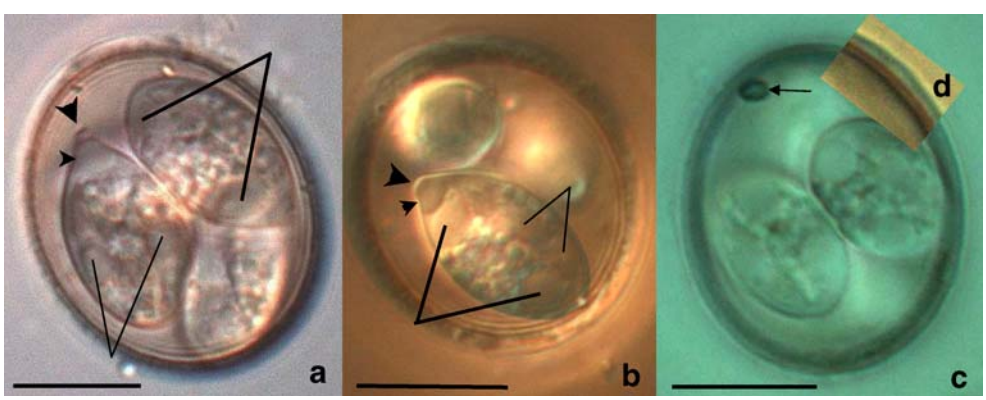

Fig. 3 Nomarski interference contrast photomicrograph of sporulated oocysts of *E. rjupa* from *L. m. islandorum*. Bar=10 μm. **a**, **b** Rough surface of the oocyst wall; nipple-like SB (*large arrowheads*) and SSB (*small arrowheads*) are visible at the pointed end of the ovoidal sporocysts; one elongate RB (*thick lines*) and smaller, usually round RB (*thin lines*) are visible in each SP; a number of large, scattered SR granules are visible in each SP that obscure SP detail. **c** PG (*arrow*) is visible at the one end of the subspherical oocyst. **d** Bicolored outer wall (light blue and brown) and dark inner wall

In our material, 25 birds were infected with both species, but 67 had single-species infection.

Description

Eimeria rjupa n. sp. (Figs. 3 and 4)

Description of sporulated oocyst Oocyst shape: subspherical; number of walls: 2; wall thickness: 1.5 (1.4–1.6); wall characteristics: outer wall rough, ~2/3 of total thickness and appears bicolored, outermost portion light blue, innermost portion red-brown, inner wall dark. M absent. $L \times W$ (n=60), 24.7 (±2.1)×22.2 (±1.5), (20–28×18–24.5); L/W ratio, 1.1 (1.0–1.3); OR absent. One to two PG present, elongate in shape, either 1 (in 86.2% of the oocysts), 1.7×1.2 (3–1×2–0.8) or 2 (in 13.8% of the oocysts), 1.2×1.1 (1.5–1×1.4–0.8). Distinctive features of oocyst: M absent, usually one, rarely two, prominent PGs, rough, bicolored outer wall, with light blue outer portion and red-brown inner portion, and a dark inner wall.

Description of sporocyst and sporozoites Sporocyst: ovoidal, rounded at end opposite SB; $L \times W$ (n=60), 14.4 (±0.6)×8.0 (±0.6), (12–15.5×6.5–9); L/W ratio, 1.8 (1.6–2.2); SB, present, nipple-like; SSB, present; PSB, absent; SR, present; SR characteristics: large, scattered granules, often more than 40, obscure SP detail; SP have two RBs, one ellipsoidal 3.9×2.9 (3–5×2–4) and one round, ~2.5, often difficult to see under the dense SR granules. Distinctive features of sporocyst: nipple-like SB, prominent SSB, large, granular SR and two RBs, 1 large, ellipsoidal, another smaller, more or less round, in each SP.

Taxonomic summary Type host: *Lagopus muta islandorum* (Faber, 1822), rock ptarmigan.

Type locality: Þingeyjarsýsla (65°47′N, 17°10′W), northeast Iceland.

Prevalence: in 26/100 (26%); average opg, 8,200 (range 50–67,600).

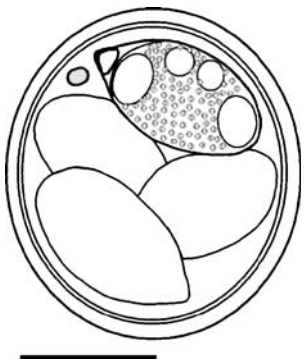

Fig. 4 Line drawing of a sporulated oocyst of *E. rjupa* from a wild *L. m. islandorum*. Bar=10 μm

Prepatent and patent periods: unknown.

Site of infection: Oocysts recovered from fecal material in the small intestine and cecum.

Material deposited: photosyntypes (see Duszynski 1999) of sporulated oocysts deposited in the USNPC 099803.

Etymology: the nomen triviale is derived from the Icelandic name of the host, rjúpa.

Remarks The subspherical oocysts of *E. rjupa* resemble *Eimeria* Type B (Ishihara et al. 2006). Both coccidians have the same L/W ratio (1.1). Various morphological characters, however, distinguish these coccidians; the wall color is different (bicolored outer wall with a dark inner wall in *E. rjupa*, colorless wall in *Eimeria* Type B); oocyst sizes are different (24.7×22.0 in *E. rjupa*, 21.3×19.1 in *Eimeria* Type B); sporocyst sizes are different (14.4×8.0 and 12.1×7.4, respectively); the sporocyst form is different (ovoidal in *E. rjupa*, fusiform in *Eimeria* Type B); and a prominent SSB is present in *E. rjupa* but is absent in *Eimeria* Type B (Ishihara et al. 2006). Information on the surface texture and the number of wall layers of *Eimeria* Type B is lacking. The ellipsoidal oocysts of *E. brinkmanni*, *E. fanthami*, *E. uekii*, and *E. muta*, as well as the spherical sporocysts (~12) in *E. lagopodi*, easily distinguish these species from *E. rjupa* (Galli-Valerio 1929; Ishihara et al. 2006; Kamimura and Kodama 1981; Levine 1953; Pellérdy 1974).

In our material, 25 birds were infected with both species, but only one had a single-species infection.

Discussion

The first to mention coccidians from the rock ptarmigan was Kloster (1923) who reported coccidian infections from *L. m. islandorum* that had been shot in northern Iceland in April 1923 and immediately shipped to Bergen in Norway for parasitological examinations. Coccidians were found in jejunum and cecum of all nine birds he examined. In one of the ptarmigans, coccidians were also found in the duodenum. A few years later, Brinkmann (1926) also described coccidian infections in rock ptarmigans, both in birds that had been collected in Norway (*L. m. mutus*) and in Spitzbergen (*L. m. hyperboreus*). Kloster (1923) and Brinkmann (1926) used the unacceptable name "*E. avium*" to refer to the species present in their materials. The taxonomic status of the eimeriids present in ptarmigans in Norway and Spitzbergen has not yet been resolved. Similarly, the coccidians that are known to occur in the subspecies *L. m. millaisi* in Scotland (Watson 1965) have not yet been named.

So far, *E. lagopodi* has been found in *L. m. helveticus* in Switzerland (Galli-Valerio 1929), *E. brinkmanni* and *E.*

fanthami in *L. m. rupestris* in the Canadian Arctic (Levine 1953), and *E. uekii* as well as an unnamed eimeriid (Type B) in *L. m. japonicus* in Japan (Ishihara et al. 2006; Kamimura and Kodama 1981). If *E. muta* and *E. rjupa* are added to this list, seven *Eimeria* species have now been found in only 4 of the 26–28 rock ptarmigan subspecies (Holder and Montgomerie 1993). These data suggest that some *Eimeria* species are still to be described from rock ptarmigan populations around the northern hemisphere.

It is generally accepted that bird coccidia in the genus *Eimeria* are host specific, not only under natural conditions (Hiepe and Jungmann 1983) but also in farmed birds (Rommel 2000). This was recently reviewed for pheasants (*Phasianus colchicus*) that are infected with seven host-specific *Eimeria* species (Gassal 2003; Hospes 1996). Whether or not this is also the case in sympatric species in the genus *Lagopus* (Brinkmann 1923, 1926; Holmstad et al. 2005; Holt 1952; Hudson 1992; Willumsen 1916) is unknown.

In general, little information is available on eimeriids that infect wild game birds such as grouse and ptarmigans. This regards not only the taxonomy of the species involved but also the consequences of infections by the usually different pathogenic species on the health status of the hosts, both on individual and population levels. And almost nothing is known on the seasonality of coccidia infections in galliform game birds. However, knowing how severe disease and high mortality can be caused in galliform birds in captivity (Rommel 2000), future research on game bird population dynamics should not overlook protozoan infections when doing parasitological examinations.

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